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Original Article

Association of common variants of FTO in women with polycystic ovary syndrome

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Abstract: Background: Polycystic ovary syndrome (PCOS) is a common and complex multisystemic genetic disease. Previous genome-wide association study (GWAS) of PCOS has found several potentially causative single nucleotide polymorphisms (SNPs) in Han Chinese population. The goal of present investigation was to assess the potential association between rs1121980, rs1421085, rs1558902, rs8050136 SNPs and PCOS. In order to make a better elucidation of this disease, further investigations of association between SNPs susceptibility and PCOS become necessary. Methods: In the present study, we enrolled 212 patients with PCOS and 198 control subjects. Four polymorphisms of FTO gene (rs1121980, rs1421085, rs1558902, rs8050136) were genotyped by Taqman-MGB method, and their relationship with PCOS was speculated. Results: The allele frequency has no significant difference between the PCOS group and the controls. Genotype frequencies of the four SNPs in the additive, dominant and recessive models showed no significant difference between PCOS cases and controls. Conclusions: Our results demonstrate that FTO gene has little association in PCOS development.

Keywords: FTO, genome-wide association study (GWAS), polycystic ovary syndrome (PCOS)

Introduction

Polycystic ovary syndrome (PCOS) is the most frequent heterogeneous endocrine-metabolic disorder in women of reproductive age, with a prevalence of 5-10% of Chinese women aged 19~45 years [1]. The clinical manifestations of PCOS are diverse, characterized by menstrual irregularities, infertility, acne, anovulation and hirsutism, which can lead to a significant decrease in quality of life, mood disorders and sexual dysfunction [2, 3]. Affected PCOS patients are also at higher risk of developing various clinical implications, including type 2 diabetes mellitus (T2DM), cardiovascular disease (e.g. obesity, insulin resistance and atherosclerosis), endometrial carcinoma. Familial aggregation analysis and clinical traits demonstrate the pathogenesis of PCOS is associated with multiple genetic and environmental factors [4]. It is widely accepted that the etiology of PCOS is strongly heritable, and genetic approaches are rapidly uncovering new regions of the genome that appear to confer risk for PCOS. Recent genome-wide association study (GWAS) identified several genetic loci that were independently associated with PCOS in Han Chinese women, among which rs9939609 is the most extensively studied and is the only successfully replicated SNP in Han Chinese populations [5]. The fat mass and obesity associated gene (FTO) have been implicated as obesity-susceptibility locus in children and adolescents, located in chromosome region 16q12.2. FTO is widely expressed throughout the body and the brain, such as the hypothalamic arcuate (ARC), ventromedial (VMH) nuclei, and paraventricular, dorsomedial (DMH), and also highly expressed in skeletal muscle and adipose tissue [6]. In the present study, we performed a meta-analysis to investigate the association between FTO gene variants (rs1121980, rs1421085, rs1558902, rs8050136) and the risk of PCOS.

Materials and methods

Subjects

A total of 212 PCOS cases and 198 unrelated controls of Han Chinese women were recruited consecutively during May 2006 to February 2007 from Reproductive Hospital Affiliated to Shandong University. PCOS cases patients were 23 to 43 years old, with an average age of 29.32 years old, and controls patients were 24 to 40 years old, with an average age of 31.43 years old. Recruitment of PCOS patients was based on European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) diagnostic criteria [7], meeting at least two of the following three features: oligo and/or anovulation; clinical and/or biochemical hyperandrogenism; and the presence of 12 or more follicles with a diameter of 2~9 mm in either side of the ovary, and/or total ovary volume ≥10 mL on ultra-sound. Patients with other diseases such as androgen-secreting tumor, congenital adrenal hyperplasia and Cushing syndrome were excluded. The controls were healthy women exhibited regular menstrual cycles. The inclusion criteria for these participants include male factor (aspermia, hypospermia and asthenozoospermia), and participants with hyperandrogenism and polycystic ovaries morphology were excluded. All individuals who were receiving hormonotherapy during last 3 months were excluded. The study has been approved by the Institutional Review Board of the Reproductive Hospital Affiliated to Shandong University. All the participant subjects of this study have given written informed consent the procedures of using their biological data and materials.

Sample collection, hormonal and biochemical measures

A blood test was performed on the 3rd-5th day of the menstrual cycle. For patients of without regular menstrual cycle and dominant follicle while receiving transvaginal ultrasound examination, hormonal and biochemistry detection was performed after an overnight fast. Fasting blood samples of all subjects were collected during the follicular phase of one menstrual cycle. Body mass index (BMI) was calculated as body weight (kg) divided by body height squared (m^2). Waist hip ratio (WHR) was calculated as hipline (cm) divided by waistline (cm). Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T), androstenedione (A) and insulin concentrations were determined by chemiluminescence immunoassay (AU640 automatic biochemistry analyzer; Olympus Company, Hamburg, Germany). 75 g oral glucose tolerance test (OGTT) was carried out for PCOS patients. The glucose levels and insulin levels at 0 min and 120 min were evaluated. Glucose levels, total cholesterol (TC) and triglycerides (TG) were determined by oxidase methods. High-density lipoprotein (HDL-C) by synthetic polymer/detergent HDL-C assay and low-density lipoprotein (LDL-C) by surfactant LDL-C assay. The homeostasis model assessment (Homa-IR) method was applied to determine insulin resistance according to the formula: fasting glucose (mmol/L)*fasting insulin (m IU/L)/22.5.

Genotyping

Genomic DNA was extracted from EDTA anticoagulated blood using the standard salting out method (2). Taqman-MGB fluorescence quantitative polymerase chain reaction (PCR) were carried out on the Light Cycle system (Roche480) as followings: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 40 s. The primer sequences of *FTO were as follows:* rs1121980 forward: 5'-AACCAAAAGCCAGATAAGGAGAC-3'; reverse: 5'-GGAAGGCACAATAAGAGGAGAT-3'; rs1421085 forward: 5'-CAAAAGCAGGAGATGACACACA-3'; reverse: 5'-GTAGACTAAACAGGGCTAAGGA-3'; rs1558902 forward: 5'-GTCTTGAGTTAGCTGAAGTTCTCTT-3'; reverse: 5'-TATCAAGTTAGGGTACGTTGCA-3'; rs8050136 forward: 5'-CAACCAAGGTCCTTATAGGAAGAGC-3'; reverse: 5'-TGGTCATGTCTGATCTAGAGTACCC-3'. PCR products were analyzed using 2% agarose gel electrophoresis stained with ethidium bromide.

DNA sequencing

The FTO PCR products (212 PCOS cases and 198 unrelated controls) were sequenced in both directions. All were done by automatic genotype sequencing instrument (ABI Prism 3100-Avant) in Peking Huada Gene Company.

Statistical analysis

General clinical characteristics of cases and controls were expressed as means ± SD. To

Table 1. Clinical characteristics in PCOS cases and controls

Clinical characteristics	Control (N = 198)	PCOS (N = 212)	t	P	P adj
BMI (kg/m^2)	22.32±3.64	27.51±3.75	0.042	0.047	0.231
Age (year)	36.1±5.33	28.2±4.72	0.257	0.039	0.342
HC (cm)	96.34±8.18	95.60±7.73	0.513	0.574	0.281
WC (cm)	86.90±12.44	86.30±10.16	0.350	0.701	0.640
WHR	0.88±0.12	0.86±0.06	0.152	0.684	0.534
FSH (IU/L)	6.48±1.72	21.29±1.76	1.100	0.324	0.186
LH (IU/L)	11.72±6.51	10.32±3.10	1.238	0.260	0.143
T (ng/dl)	46.78±11.64	39.06±14.53	3.221	0.351	0.244
LH/FSH	1.84±0.74	1.62±0.81	1.606	0.143	0.156
FPG (mmol/L)	5.62±1.37	5.64±1.21	0.114	0.871	0.803
2hPG (mmol/L)	6.97±2.47	6.66±2.51	0.719	0.384	0.334
FINS (mIU/L)	12.67±5.83	13.37±4.31	0.256	0.787	0.716
2hINS (mIU/L)	78.13±26.04	70.10±36.08	0.648	0.450	0.380
HOMA-IR	3.407±2.873	3.419±2.604	0.031	0.921	0.766
CHOL (mmol/L)	4.85±0.88	4.61±0.92	1.319	0.204	0.147
TG (mmol/L)	1.41±0.93	1.36±1.15	0.347	0.763	0.679
HDL-C (mmol/L)	1.36±0.38	1.34±0.31	0.196	0.824	0.735
LDL-C (mmol/L)	3.38±0.89	3.40±0.95	0.248	0.608	0.595

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 frequencies in PCOS cases and controls

SNPs	Allele	PCOS	Control	OR	P
rs1121980	T/C	74/350	70/326	0.985	0.324
rs1421085	C/T	52/376	43/353	1.135	0.486
rs1558902	A/T	61/363	47/349	1.248	0.127
rs8050136	A/C	56/368	44/352	1.217	0.249

OR: odds ratio. PCOS, Polycystic ovary syndrome; Allele, the data of rs1121980, rs1421085, rs1558902 and rs8050136 are presented as T/C, C/T, A/T and A/C, Adjustment study, adjusted by body mass index in logistic regression. 95% CI, 95% Confidence Interval.

evaluate the relationship between each SNPs, pairwise linkage-disequilibrium (LD) (D9 and correlation coefficients r2) were calculated by Haploview. Chi-square test was used to compare allele frequencies of rs1121980, rs1421085, rs1558902 and rs8050136. Data was presented as odds ratio (OR) and 95% confidence interval (95% CI). Genotypes of each SNPs were analyzed by additive (+/+ vs. +/- vs. -/-), dominant (+/+ plus +/- vs. -/-) and recessive (+/+ vs. +/- plus -/-). Genotype-phenotype correlation of PCOS was analyzed by independent sample T test. In phenotype analysis, Chi-square test, independent T test were analyzed, and logistic regression analysis used for age and BMI adjustment by SPSS17.0 software (SPSS Inc., Chicago, IL, USA). Statistic significant level was defined as $P<0.05$.

Results

The clinical characteristics in PCOS patients and control subjects were summarized in **Table 1**. The PCOS group was younger than the control group ($P<0.001$). BMI in PCOS group was higher than that in the control group ($P<0.001$). Therefore, we adjusted BMI and age in the subsequent analysis. Through Haploview, Hardy-Weinberg equilibrium test, no deviation of allele frequencies of the four SNPs was found in both PCOS cases and controls (Not provided). There were little linkage between rs1121980 and rs1421085 (D9 = 0.235, r2 = 0.037), rs1121980 and rs1558902 (D9 = 0.174, r2 = 0.126), rs1121980 and rs8050136 (D9 = 0.573, r2 = 0.405). rs1421085 and rs1558902 (D9 = 0.315, r2 = 0.059), rs1421085 and rs8050136 (D9 = 0.381, r2 = 0.533), rs1558902 and rs1558902 (D9 = 0.425, r2 = 0.276). The allele frequencies of rs1121980, rs1421085, rs1558902 and rs8050136 were shown in **Table 2**. In the PCOS group, allele frequency of rs1121980 has no statistically significance compared with the control group (*P* = 0.324, OR = 0.985, 95% CI = 1.073-1.241), even adjustment for age and BMI (*P* = 0.582). Moreover, statistical difference of allele frequency was not found in rs1421085 (P = 0.486, OR = 1.135, 95% CI = 0.861-1.109), rs1558902 (*P* = 0.127, OR = 1.248, 95% CI = 0.684-1.153) and rs8050136 (*P* = 0.249, OR = 1.217, 95% CI = 0.973-1.115). Genotype frequency of the four SNPs was further analyzed by chi-square test under additive, dominant and recessive models (**Table 3**). In the additive model, no significant difference

Table 3. Genotype frequencies in PCOS cases and controls

SNP	Genotype	PCOS	Control	x^2	Padd	Pdom	Prec
rs1121980	TT/TC/CC	8/58/146	8/54/136	7.714	0.990	0.746	0.531
rs1421085	CC/CT/TT	5/42/165	2/39/157	4.726	0.560	0.521	0.496
rs1558902	AA/AT/TT	8/45/159	7/33/158	5.183	0.487	0.436	0.708
rs8050136	AA/AC/CC	6/44/162	6/32/160	6.054	0.489	0.371	0.182

Padd: *P* value of the additive genotype model (rs1121980: TT/TC/CC; rs1421085: CC/CT/TT; rs1558902: AA/AT/TT; rs8050136 AA/AC/CC) in the two groups. Pdom: *P* value of the dominant genotype model (rs1121980: TT+TC/CC; rs1421085: CC+CT/TT; rs1558902: AA+AT/TT; rs8050136 AA+AC/CC) in the two groups. Prec: *P* value of the recessive genotype model (rs1121980: TT/TC+CC; rs1421085: CC/CT+TT; rs1558902: AA/AT+TT; rs8050136 AA/AC+CC) in the two groups.

was found in rs1121980 (P = 0.990), rs1421085 (P = 0.560), rs1558902 (P = 0.487) and rs8050136 (P = 0.489). In dominant model, no significant difference was found in all four SNPs rs1121980 (P = 0.746), rs1421085 (P = 0.521), rs1558902 (P = 0.436) and rs8050136 (P = 0.371). Moreover, there was also no significant difference in recessive model.

Discussion

Recent replication analyses and genome-wide association studies reported that FTO gene variants have an association with PCOS, mostly in Asians [8]. In the present study, we identified the common FTO SNPs rs1121980, rs1421085, rs1558902 and rs8050136 have no association with PCOS in a cohort of Women from Shandong Province. Furthermore, Allele frequencies and genotype frequencies of the four FTO SNPs showed no significant difference in PCOS cased and controls, indicating that FTO SNPs rs1121980, rs1421085, rs1558902 and rs8050136 were not candidates in PCOS development. Moreover, the risk alleles of FTO SNPs (rs1121980, rs1421085, rs1558902, rs8050136) was associated with testosterone. Whereas, no correlation was found to the other clinical characteristics.

Bioinformatics analysis have uncovered that FTO is one of the members of the AlkB family of non-heme Fe (II)/2-OG-dependent oxidative DNA/RNA demethylases [9]. FTO may participates in CREB signaling pathway through interacting with CaMKII and finally evokes NPY, which resulting in regulation of food intake and energy homeostasis by acting through NPY1R and BDNF.

CaMKII, a serine/threonine protein kinase family, which was encoded by genes named α, β, γ and δ in mammals [10]. CaMKII was regulated through multiple phosphorylation sites, therefore altering the enzymatic activity and interactions with other proteins [11]. CaMKII phosphorylates have a large range of substrates that are involved in a number of physiological pathways, such as cell development, proliferation, transport, neuronal function and so on [12-14]. CaMKII has also been demonstrated to regulate hepatic glucose homeostasis in obesity [15].

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Disclosure of conflict of interest

None.

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