Association study of *HNF1A* in women with polycystic ovary syndrome

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Abstract

Purpose This study aims to ascertain whether an association exists between *hepatocyte nuclear factor 1 alpha* (*HNF1A*) and polycystic ovary syndrome (PCOS).

Methods One thousand one hundred thirty-eight PCOS and 1125 healthy control Han Chinese women were recruited from Reproductive Hospital Affiliated to Shandong University. Serum hormone, blood lipid level, and genomic DNA were obtained from the peripheral blood for this research. Two single-nucleotide polymorphisms (SNPs)—rs2393791 and rs7305618—located in *HNF1A* were genotyped using the Sequenom MassARRAY system.

Results The allele frequencies of SNP rs7305618 had significant differences between PCOS patients and controls after adjusting for age and BMI ($p = 0.023$). Besides, PCOS patients carrying the rs7305618 CC genotype shown a higher

testosterone level than the patients with CT + TT genotypes after being adjusted by age and BMI ($p = 0.019$).

Conclusions A SNP located in the *HNF1A* gene is associated with PCOS among Han Chinese women. This suggested that variations in *HNF1A* might confer risk for PCOS.

Keywords Polycystic ovary syndrome · *HNF1A* · Genetic association study · Single-nucleotide polymorphisms Yue Lv and Changfa Sun contributed equally to this work.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common female endocrinopathies and affects 5–8% of reproductive-aged women worldwide [1, 2]. PCOS is characterized by two of the following three criteria: clinical and/or biochemical androgen excess, oligo-ovulation and/or anovulation, and polycystic ovaries [3]. In addition, women with PCOS often have an increased risk of suffering from metabolic syndrome, dyslipidemia, type 2 diabetes mellitus (T2DM), and cardiovascular disease [4]. For example, PCOS patients have a threefold increased prevalence of metabolic syndrome [5] and a fourfold increased prevalence of T2DM [6]. In addition, nearly 70% of PCOS patients suffer from dyslipidemia [7]. Currently, several susceptibility genes of these disorders have been proven to be related to PCOS, including *THADA* [8], *TCF7L2* [9], *YAP1* [10], and *MTNRI B* [11].

The *hepatocyte nuclear factor 1 alpha* gene (*HNF1A*) is located on human chromosome 12q24 and contains nine exons. *HNF1A* expression is enriched in the liver, and it is also expressed in many other tissues such as the pancreas, kidneys, ovaries, and testes [12, 13]. *HNF1A* encodes a transcription factor that belongs to the homeobox protein family, and *HNF1A* binds to the promoters of a variety of genes that are expressed predominantly in the liver [14]. In addition,

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HNF1A is essential for the expression of several target genes (e.g., *DYRK1B* and *SLC2A2*) involved in lipoprotein metabolism and glucose-stimulated insulin secretion [15, 16]. Recently, it has been confirmed that defects in the *HNF1A* gene are a cause of maturity-onset diabetes of the young type 3 [17] and are related to the development of T2DM [18]. Furthermore, there is a study that has shown that *HNF1A* is a common susceptibility gene for several diseases, such as coronary artery disease (CAD), hypertension, and dyslipidemia [16] and is associated with the concentration of C-reactive protein (CRP) in the plasma [19]. All of these results suggest that the *HNF1A* gene might be involved in the etiology of PCOS.

Previous studies have shown that rs2393791, located in intron 1 of *HNF1A*, is linked with changes in CRP [19, 20] and is involved in hypertension and hypertriglyceridemia [16]. SNP rs7305618, which is located at the 5' flank of *HNF1A*, has been shown to be associated with the risk of T2DM in a Chinese population [21]. In another genome-wide association study, rs7305618 was found to be strongly associated with plasma CRP concentration [19]. Therefore, to determine any correlation between the *HNF1A* gene and PCOS, we selected the above two SNPs (rs2393791 A/G and rs7305618 C/T) for analysis.

Materials and methods

Subjects

We recruited 1138 Chinese women with PCOS and 1125 controls from the Reproductive Hospital Affiliated to Shandong University from June 2011 to April 2013. The diagnosis of PCOS patients was based on the 2003 Rotterdam criteria and included at least two of the following three criteria: oligomenorrhea or amenorrhea, clinical or biochemical hyperandrogenism, and polycystic ovaries on ultrasound. Women with other related diseases, such as adrenal congenital hyperplasia, Cushing syndrome, and androgen-secreting tumors, were excluded. The controls were women who had normal menstrual cycles without hyperandrogenism and polycystic ovaries. Each participant signed a written informed consent, and the study was approved by the Institutional Review Board of Reproductive Medicine of Shandong University.

Serum hormone, blood lipid level, and genomic DNA were obtained from the peripheral blood. Fasting blood samples were collected on days 3–5 of the menstrual cycle. Follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) were measured with a chemiluminescence analyzer (Beckman Coulter, USA). The concentrations of serum cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured, and an oral glucose tolerance test (OGTT) was performed in all participants using an AU640

automatic biochemistry analyzer (Olympus Inc., Germany). During OGTT, serum glucose and insulin levels were evaluated at 0 min and 120 min, and the homeostasis model assessment index to estimate insulin resistance (HOMA-IR) was calculated by the formula: fasting glucose (mmol/L) $\times$ fasting insulin (mIU/L) / 22.5.

Genotyping

We used a DNA blood kit (Qiagen, USA) following the manufacturer's protocol to extract the genomic DNA from the whole blood samples. The genotyping of the two SNPs was performed by Bioyong Technologies Inc. (Beijing, China).

Statistical analysis

Tests of Hardy–Weinberg equilibrium and linkage disequilibrium analysis were performed with the HaploView software. The clinical data are presented as the mean $\pm$ SD. The allele frequencies and genotype frequencies of the two SNPs were tested with the chi-square test and adjusted by logistic regression analysis. The genotypes of the two SNPs were analyzed by three different models: the additive model (+/+ vs. +/- vs. /-), the dominant model (+/+ plus +/- vs. /-), and the recessive model (+/+ vs. +/- plus /-). The analysis of the association between clinical characteristics and genotypes was performed with the independent sample *t* test.

Genotype differences and other statistical analyses, including chi-square test, independent *t* test, and logistic regression analysis, were performed using the SPSS software version 20.0 (SPSS, Chicago, IL, USA), and the data were expressed as means $\pm$ SD. Associations with a two-tailed *p* value <0.05 were considered statistically significant.

Results

The allele frequency of the two SNPs (rs2393791 and rs7305618) had no deviation from Hardy–Weinberg

Table 1 The clinical characteristics of PCOS patients and controls

	PCOS	Control	<i>t</i>	<i>p</i> value
<i>N</i>	1138	1125		
Age (years)	27.68 $\pm$ 3.89	31.27 $\pm$ 5.38	-18.423	<0.001
BMI (kg/m ²)	24.85 $\pm$ 4.63	22.67 $\pm$ 3.25	12.804	<0.001
FSH (IU/L)	6.34 $\pm$ 1.74	7.61 $\pm$ 2.91	-11.684	<0.001
LH (IU/L)	11.91 $\pm$ 41.84	4.88 $\pm$ 2.24	5.528	<0.001
T (ng/dl)	41.93 $\pm$ 17.58	15.00 $\pm$ 5.83	27.069	<0.001

Data are presented as mean $\pm$ SD

BMI body mass index, FSH follicle stimulating hormone, LH luteinizing hormone, T testosterone

Table 2 Allele frequencies of SNPs rs2393791 and rs7305618

SNP	Allele	PCOS	Control	OR	<i>p</i> value	<i>p</i> _{adjusted}
rs2393791	A/G	0.365	0.335	0.918	0.162	0.090
rs7305618	C/T	0.488	0.462	1.111	0.078	0.023

*p*_{adjusted} adjusted by age and BMI using logistic regression analysis

equilibrium in the PCOS and control groups ($p > 0.05$). There was no link between rs2393791 and rs7305618 ($D' = 0.885$, $r^2 = 0.621$). The genetics power was calculated by Genetic Power Calculator (<http://pnu.mgh.harvard.edu/purcell/gpc/>). And the power reached 0.8 at $p = 0.05$.

The clinical characteristics of women with PCOS and the control group are shown in Table 1. The average age of the PCOS patients was younger than the control group ($p < 0.001$). BMI, LH, and T were significantly higher in the PCOS group compared to the control group ($p < 0.001$), while the FSH level was significantly lower in the PCOS group ($p < 0.001$).

Analysis of the allele frequencies is presented in Table 2. Before adjustment, no significant difference was observed in allele frequencies of rs2393791 ($p = 0.162$, OR = 0.918) or rs7305618 ($p = 0.07$, OR = 1.111). However, after adjusting for age and BMI by logistic regression, the allele frequency of SNP rs7305618 showed a significant *p*_{adjusted} value of 0.023, while rs2393791 had a *p*_{adjusted} value of 0.09.

As presented in Table 3, the genotypes of the two SNPs were analyzed by the chi-square test (Table 3). Three different models—the additive model, dominant model, and recessive model—were tested, but there were no significant differences among the different genotypes.

We next analyzed the clinical and metabolic characteristics in PCOS patients. As shown in Table 4, the level of T was slightly higher in patients with PCOS carrying the rs7305618 CC genotype compared to patients with the CT + TT genotype ($p = 0.045$), and an increased difference was shown after being adjusted for the potential covariates of age and BMI (p _{adjusted} = 0.019). No significant differences were observed in the clinical or metabolic characteristics related to the SNP rs2393791 genotypes in PCOS (Supplemental Table 1).

We further analyzed the genotype frequencies of PCOS patients who have diabetes mellitus, dyslipidemia, obesity,

or not. However, there is no difference between the genotypes of two SNPs in PCOS patients who have disorders and who do not (Supplemental Tables 2, 3, 4).

Discussion

PCOS is a heterogeneous disorder, and its occurrence and development are affected by both heredity and environment. In recent years, a number of genes and SNPs related to PCOS have been identified by genome-wide association studies [22–25]. However, there are likely many unidentified genes that are related to the relevant disorders of PCOS and might play important roles in the pathogenesis of PCOS. In light of this, we identified *HNFI*A, which is involved in lipoprotein metabolism, as a new susceptibility gene for PCOS.

Genotype analysis of the two selected SNPs showed that the *HNFI*A gene does not have a direct effect on the PCOS phenotype, but the allele frequency analysis showed that SNP rs7305618 might contribute to the occurrence of PCOS. Because the frequency of the risk allele C at the rs7305618 locus was significantly higher in PCOS cases compared to control groups after adjusting for age and BMI, we further analyzed the relationship between the rs7305618 genotypes and the clinical characteristics of the PCOS patients. Interestingly, we found that the PCOS patients with the CC genotype had a higher T level than the patients with CT + TT genotypes. The results remained significant after being adjusted for age and BMI, and this suggested that the rs7305618 genotypes are associated with hyperandrogenism. However, these studies need to be repeated to confirm this result.

The *HNFI*A gene is an essential regulator of lipoprotein metabolism, and the ways that *HNFI*A regulates lipoprotein metabolism have been partly revealed by *HNFI*A $-/-$ mice [15]. The *HNFI*A $-/-$ mice have defects in bile acid synthesis and transport, and HDL-C metabolism is impaired in these mice. In addition, circulating LDL-C is significantly reduced after liver-specific knockdown of *HNFI*A in normolipidemic mice [26]. By regulating lipoprotein metabolism, *HNFI*A plays an important role in dyslipidemia. Dyslipidemia is the most common metabolic abnormality in PCOS, and previous research has shown that PCOS patients have 4–5 times greater risk of suffering from dyslipidemia than healthy women [27,

Table 3 Genotype frequencies in PCOS patients and controls

SNPs	Genotype	PCOS	Control	χ^2	<i>P</i> _{add}	<i>P</i> _{dom}	<i>P</i> _{rec}
rs2393791	AA/AG/GG	382/538/206	393/529/176	2.236	0.327	0.157	0.356
rs7305618	CC/CT/TT	271/565/298	233/562/318	3.326	0.190	0.223	0.092

*P*_{add} *P* value of additive model

*P*_{dom} *P* value of dominant model

*P*_{rec} *P* value of recessive model

Table 4 Clinical and metabolic characteristics of PCOS patients according to SNP rs7305618 genotype

Characteristics	Allele CC (<i>N</i> = 271)	Allele CT + TT (<i>N</i> = 859)	<i>t</i>	<i>p</i> value	<i>P</i> _{adjusted}
Age (years)	27.92 ± 4.481	27.59 ± 3.805	1.189	0.235	
BMI (kg/m ²)	24.59 ± 4.561	24.59 ± 4.659	−0.952	0.342	
FSH (IU/L)	6.44 ± 2.103	6.32 ± 1.662	0.933	0.351	0.431
LH (IU/L)	10.37 ± 6.171	12.45 ± 48.078	−0.697	0.486	0.589
T (ng/dl)	43.81 ± 18.325	41.30 ± 17.373	2.010	0.045	0.019
F-GLU (mmol/L)	5.46 ± 0.982	5.45 ± 0.956	0.132	0.895	0.456
Glucose 2 h (mmol/L)	7.00 ± 2.285	6.78 ± 2.280	1.336	0.182	0.126
F-INS (mIU/L)	13.33 ± 8.242	13.89 ± 8.973	−0.908	0.364	0.708
Insulin 2 h (mIU/L)	81.49 ± 75.465	76.61 ± 71.985	0.948	0.343	0.571
HOMA-IR	3.30 ± 2.693	3.31 ± 2.760	−0.094	0.925	0.582
CHOL (mmol/L)	4.48 ± 0.861	4.40 ± 0.892	1.348	0.178	0.126
TG (mmol/L)	1.58 ± 1.739	1.72 ± 5.593	−0.395	0.693	0.779
HDL-C (mmol/L)	1.11 ± 0.356	1.11 ± 0.326	−0.043	0.966	0.875
LDL-C (mmol/L)	3.10 ± 0.954	3.08 ± 0.941	0.360	0.719	0.488

F-GLU fasting glucose, *F-INS* fasting insulin, *HOMA-IR* homeostasis model assessment of insulin resistance, *CHOL* cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

28]. All of these studies have indicated that *HNF1A* might regulate lipoprotein metabolism and participate in the metabolic disorder seen in women with PCOS.

HNF1A can bind to two sites in the promoter region of the *CRP* gene and activate gene expression [29]. Several studies have shown that the *HNF1A* gene is associated with the plasma concentrations of CRP, which is a biomarker of inflammation and CAD and is an independent risk factor for T2DM [19, 30–34]. PCOS is also accompanied by chronic low-grade inflammation, and many researchers have shown that CRP increases significantly in subjects with PCOS compared to healthy women [35, 36], and that CRP levels might be a reliable marker of low-grade chronic inflammation in PCOS patients [37]. Because *HNF1A* can directly regulate the expression of the *CRP* gene, any abnormalities in the regulation of *HNF1A* might lead to the overexpression of the *CRP* gene and contribute to the pathogenesis of PCOS. In this study, we have no the data on the serum CRP level, but according to previous studies [38, 39] part of the pathogenesis of PCOS might be attributable to the role that *HNF1A* plays in the inflammatory response.

In conclusion, our study shows that SNP rs7305618 located in the *HNF1A* gene is associated with PCOS among Han Chinese women, and this suggests that rs7305618 might be related to the occurrence of hyperandrogenism. Thus, *HNF1A* might play a role in the pathogenesis of PCOS, and the function of this gene requires further study.

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Compliance with ethical standards Each participant signed a written informed consent, and the study was approved by the Institutional Review Board of Center for Reproductive Medicine of Shandong University.

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Conflict of interest The authors declare that they have no conflict of interest.

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