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GENETICS

Association study between FSHR Ala307Thr and Ser680Asn variants and polycystic ovary syndrome (PCOS) in Northern Chinese Han women

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Abstract
Background Polycystic ovary syndrome (PCOS) is a common complex genetic endocrinopathy. It has high heritability, and twin studies indicate that it is a complex polygenic disorder. Searching for major genes of PCOS is crucial to clarify its molecular pathogenesis. A previous genome-wide association study in Chinese women with PCOS identified a region on chromosome 2p16.3 that encodes the follicle-stimulating hormone receptor (FSHR) genes as a reproducible PCOS susceptibility locus. In the present study, we performed a replication analysis of the association between two common variants of the FSHR gene and PCOS in Northern Chinese Han women.
Results We recruited 384 unrelated PCOS patients and 768 healthy individuals from the Shaanxi province in the northern part of China. Two polymorphisms (Ala307Thr and Ser680Asn) of the FSHR gene and the clinical characteristics of the study subjects were analyzed in the case-control sample. The frequency of FSHR Ala307Thr and Ser680Asn variants along with the haplotype was not significantly different between the PCOS patients and the controls; however, the Ser680 variants may be associated with high levels of FSH and low E2 levels.
Conclusion The variant of Ser680 was not associated with PCOS but it may be related to high FSH levels. The present study suggests that the two variants of the FSHR gene are not a causative factor of PCOS in Northern Chinese Han women.

Keywords Polycystic ovary syndrome · Follicle-stimulating hormone receptor · Single nucleotide polymorphisms

Capsule This is a large size sample study of FSHR gene polymorphisms and clinical characteristics in women who suffer from PCOS.

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Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder that affects 4–12 % of women of reproductive age [1]. PCOS, which is characterized by hyperandrogenism, menstrual irregularity and polycystic ovarian morphology [2], is the leading cause of anovulatory infertility and hirsutism. The pathogenesis of PCOS is still not fully understood. Epidemiological studies have shown that PCOS is closely associated with genetic factors, and the results of twin studies and studies of familial segregation patterns suggest that PCOS is a multifactorial disease [3].

Follicle-stimulating hormone (FSH) is a pituitary glycoprotein that plays an important role during folliculogenesis by promoting the proliferation and differentiation of granulosa cells and the maturation and development of follicles. The effect of FSH is mediated by binding of the hormone with a specific receptor (i.e., FSHR) that is specifically situated on the granulosa cells of the ovary. Through the cascade effect, FSHR transfers the biological signals of FSH to the downstream network.

Because of the important role of FSHR in the signaling transmission of FSH, FSHR genes may be an important candidate gene for PCOS. Indeed, a previous genome-wide association study in Chinese women with PCOS identified a region on chromosome 2p16.3 that encoded the FSH receptor (FSHR) genes as a reproducible PCOS susceptibility locus [4]. A well-known combination of two polymorphisms in exon 10 may be involved in the pathogenesis of PCOS [5]. One of these polymorphisms codes for the amino acid residue at position 307, which may be occupied by either Ala(GCT) or Thr (ACT), and the other polymorphism codes for the amino acid residue at position 680, which may be occupied by either Asn (AAT) or Ser (AGT).

Previously, we examined the relationship of these two SNPs with PCOS and POF [6] and found that the two sites may be associated with PCOS; however, the study had a relatively small sample size.

On the basis of the above evidence, we performed a case-control association study on a large sample size to further investigate whether the two polymorphisms in the FSHR gene are associated with PCOS.

Subjects

A total of 384 unrelated PCOS patients [mean age (SEM): 28.3 (2.2)] were recruited from the outpatient clinic of the fourth Xi'an hospital and the Shaanxi Maternal and Child Care Service Center. Recruitment was based on the revised Rotterdam diagnostic criteria [2]: 1. Oligo- or anovulation, 2. Clinical and/or biochemical signs of hyperandrogenism and 3. Polycystic ovaries and exclusion of other etiologies. Women who met at least two of these criteria were defined as having PCOS. No patients had taken hormonal medication, including oral contraceptives, for at least 3 months prior to starting the study.

In addition, 768 healthy nondiabetic female volunteers [mean age (SEM): 27.7 (1.8)] with regular menstrual cycles and a normal menstrual record were recruited for the control group. All members of the control group were fertile, and none had any endocrine disorder related to PCOS. Healthy statuses were determined by their medical history, physical and pelvic examination and complete blood chemistry. All case and control subjects were unrelated Han Chinese from the Shaanxi Province, and each subject donated 5 ml of venous blood for genomic DNA extraction. The hormone and glucose levels, which included plasma FSH, LH, E2 and PRL, were estimated and analyzed to determine the differences between the samples obtained from the patients and the normal controls.

All participants gave informed consent to the use of their samples for research purposes. The study was approved by our local Medical Ethical Committee.

Genotyping

The genotypes of the FSHR gene were determined using DNA sequencing. All enrolled subjects were screened for FSHR gene variations using genomic DNA that was isolated from blood leukocytes with the QIA-amp DNA Blood Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Genotyping was performed without knowledge of the subjects' clinical status.

Regions encompassing the Ala307Thr and Ser680Asn polymorphisms of exon 10 within the FSHR gene were amplified by PCR, which was performed in a 20 μl reaction mixture containing 10 ng of DNA, 10 pmol of each primer, 2.5 mM $MgCl_2$, 0.2 mM dNTP and 0.25 U Taq DNA polymerase. All reagents were purchased from Takara (Takara, Tokyo, Japan). The PCR primer sequences were FSHR exon 10A forward and reverse were 5′-CTAAACTGTGATGCCCTAC-3′ and 5′-CTTTTCAGAGCCTTCCTA-3′ (for nucleotide 919 genotyping; codon 307, rs6165), respectively, and the PCR primer sequences for FSHR exon 10B forward and reverse were 5′-GCAGCATTTAGTCCTTGTGA-3′ and 5′-TCCATACCCCTAGTTGTACG-3′ (for nucleotide 2,039 genotyping; codon 680, rs6165), respectively. All reactions had an initial denaturation step of 3 min at 94 °C followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s and 72 °C for 1 min, and a final elongation step at 72 °C for 10 min on a Gene Amp PCR system 9,700 (Applied Biosystems, Foster City, CA).

The sequences were analyzed in an ABI PRISM model 3,100 DNA Sequencer (PE Applied Biosystems, Perkin-Elmer) to determine the genotype variation at the same position on both the forward and reverse sequences. Any discrepancies were resolved by re-genotyping the samples (with an overall error rate <0.05 %).

Statistics

Genotype and allele frequencies for the case and control groups were compared using the χ^2 test, which was performed using SPSS (version 19.0). Linkage disequilibrium statistics were computed using Haploview [7] and expressed as D' and r^2. The odds ratios (ORs) and their respective 95 % confidence intervals (95 % CIs) were calculated to estimate the allele effects. The Hardy-Weinberg equilibrium test was performed using

Table 1 Allele and genotype frequencies of the FSHR genetic variants in women with PCOS (n=384) and healthy controls (n=768). H-W test = Hardy-Weinberg equilibrium test

ALLELE			P	OR (95 % CI)	GENOTYPE			P	H-W test
307	Thr	Ala			Thr/Thr	Thr/Ala	Ala/Ala		
case	540(0.703)	228(0.297)	0.39	1.09 (0.90~1.31)	192(0.500)	156(0.406)	36(0.094)	0.66	0.60
control	1053(0.686)	483(0.314)			362(0.471)	329(0.428)	77(0.100)		0.86
680	Asn	Ser			Asn/Asn	Asn/Ser	Ser/Ser		
case	536(0.698)	232(0.302)	0.45	1.08 (0.89~1.30)	187(0.487)	162(0.422)	35(0.091)	0.75	0.99
control	1048(0.682)	488(0.318)			357(0.465)	334(0.435)	77(0.100)		0.93

STATA (version 10.0). Haplotype frequencies were estimated using SHEsis [8] (http://analysis.bio-x.cn/myAnalysis.php). A value of $P<0.05$ was considered statistically significant.

Results

In the present study, the two common genetic variants of the FSHR gene were screened among 1,152 individuals (384 PCOS cases and 768 healthy controls).

The genotype and haplotype frequencies of the two polymorphisms were in accordance with the Hardy-Weinberg equilibrium in both the patient and the control groups. The distribution of the genotypes and their ORs for association with PCOS risk are shown in Table 1. The genotype and haplotype frequencies were not found to be significantly different between the patients and controls for either of the two SNPs. As shown in Table 1, the haplotype Thr307/Asn680 and the genotype 307Thr/Thr were more frequent in the PCOS patients compared with the controls, but the difference was not statistically significant. To calculate the extent of LD in pairwise combinations of the SNPs, we calculated D' and r^2. The pairwise D' values were 0.95 for both sites, which are situated in exon 10 within only hundreds of bases of each other.

Table 2 shows the clinical characteristics of both the patients and the control groups within their respective rs6166 genotypes. We can see that the values of BMI, LH and PRL of the patient group are higher than the control group, whereas the FSH value is the opposite. This is consistent with the PCOS hormone level characteristics that have been previously described [9]. The FSH value for the patient group was significantly higher than the control group, whereas the E2 value of the patient group is lower than the control group. There were no significant differences in BMI, LH and PRL.

Discussion

PCOS is a complex genetic endocrinopathy that is attributed to both environmental factors and genetic factors. The clinical manifestations of PCOS are complex. Indeed, they are heterogeneous and differ between racial groups [10]. PCOS has high heritability, and twin studies have indicated that it is a complex polygenic disorder. Searching for major genes of PCOS is the key to clarifying its molecular pathogenesis. Because the symptoms associated with PCOS vary due to obesity, insulin resistance, glucose intolerance and dyslipidemia, a number of studies have been performed on various SNPs, including SNPs

Table 2 Clinical characteristics of each rs6166 genotype. The data represent the mean ± SEM. BMI indicates body mass index. The FSH value of the patient group was significantly higher than the control group, whereas the E2 value of the patient group is lower than the control group. There were no significant differences in BMI, LH and PRL

	Control			PCOS patient			P
GENOTYPE	Asn/Asn	Asn/Ser	Ser/Ser	Asn/Asn	Asn/Ser	Ser/Ser	
BMI(kg/m^2)	20.6±0.4	20.3±0.6	20.8±0.4	21.1±0.4	20.8±0.5	22.2±0.6	<0.05
LH (MIU/ml)	4.49±0.42	3.89±0.52	4.28±0.72	3.74±0.40	3.48±0.69	4.08±0.26	<0.05
FSH (MIU/ml)	6.22±0.52	6.38±0.31	6.82±0.38	5.98±0.46	6.16±0.36	6.64±0.29	<0.05
PRL (ng/ml)	24.89±1.46	25.84±2.28	25.32±2.33	25.72±2.37	25.54±1.51	25.36±2.50	<0.05
E2 (pg/ml)	46.12±4.22	41.57±3.90	39.72±2.28	44.81±2.69	42.43±3.37	40.16±3.16	0.19

in GnRH [11], the insulin receptor [12] and CYP19 [13], to identify the pathogenesis of PCOS. Based on the importance of FSH in the regulation of the reproductive process, the FSH receptor may be a potential candidate gene of PCOS.

Since the first mutation of the FSHR gene was reported in 1995 [14], nearly twenty mutations related to reproductive disorders have been described. Inactivating mutations and activating mutations have been identified in the extracellular domain, the transmembrane domain and the intracellular domain. Most of the gene variants have a very low heterozygosity, and two SNPs (Ala307Thr and Ser680Asn) have a considerable high frequency in the general population. Protein structure studies have shown that Ala307Thr is linked to the binding of ligands and Ser680Asn is linked to the coupling of G protein. Therefore, it is necessary to detect whether they are associated with PCOS.

Although some researchers are focusing on the roles of the two variants of FSHR in PCOS, the current conclusions are either inconsistent or contradictory. Gu [15] have reported that Ser680 may be associated with PCOS in Korean populations, whereas Ala307 was not. Mohiyiddeen [16], however, did not find an association between the Ser680Asn site of the FSHR gene and PCOS. In addition, Valkenburg examined the genotype of FSHR in 518 Caucasian PCOS women and 2,996 unselected controls and found that FSHR variants were strongly associated with the severity of PCOS clinical features, but not with disease risk [11].

In the present study, we did not find an association between PCOS and the two SNPs or the haplotype of FSHR. A study of PCOS clinical characteristics revealed that genotype affects FSH and E2 levels, whereas other hormone levels revealed no effects. In the signaling pathway, FSH is the upstream hormone of FSHR, and E2 is the downstream hormone. Our study provides little evidence that FSHR polymorphisms exert any significant influence on PCOS risk; however, the FSHR genotype may affect the FSH signaling pathway.

The present results conflict with a study we conducted previously on a small sample size of the Shanghai population. The inconsistency could be explained by the different sample sizes and ethnic populations that were used in the studies. Our previous samples were recruited from Shanghai, the southern part of China, whereas the samples in the present study were collected from Xi'an, the northern part of China. Many studies found that a cryptic borderline was observable between the Southern Han and the Northern Han samples [17–19]. Thus, population regions may influence the data. The population stratification arising from the interplay of different geographic areas may influence the polymorphism pattern. Therefore, further study on a large sample size of the Shanghai population is needed to help clarify whether FSHR is related to PCOS susceptibility.

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