Polymorphisms of TCF7L2 and HHEX genes in Chinese women with polycystic ovary syndrome

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Received: 21 May 2009 / Accepted: 3 December 2009 / Published online: 30 December 2009
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Abstract

Purpose This study was to evaluate whether polymorphisms of TCF7L2 (rs7903146) and HHEX (rs1111875) genes responsible for insulin secretion are associated with the polycystic ovary syndrome (PCOS) in Chinese people.

Methods 326 PCOS patients and 290 healthy individuals as controls were studied. Blood samples were obtained for DNA analyses and hormone measurements. Genotyping of the TCF7L2 (rs7903146) and HHEX (rs1111875) genes was carried out by the polymerase chain reaction–restriction fragment length polymorphism method.

Results We did not find statistically significant differences in the distribution of the TCF7L2 rs7903146 and HHEX

rs1111875 polymorphisms between the Chinese women with PCOS and the controls. Levels of hormones such as insulin, FSH, LH, LH/FSH, P, T and E2 were also similar between the different genotypes of the genes TCF7L2 and HHEX, respectively, which was confirmed within either the PCOS subjects or controls.

Conclusions There was no association of either of the two variants, rs7903146 of TCF7L2 and rs1111875 of HHEX, with the occurrence of PCOS in the Chinese population.

Keywords HHEX · Polycystic ovary syndrome · Single nucleotide polymorphisms · TCF7L2

Capsule We did not find statistically significant differences in the distribution of the TCF7L2 rs7903146 and HHEX rs1111875 polymorphisms between Chinese women with PCOS and the controls. Levels of hormones such as insulin, FSH, LH, LH/FSH, P, T and E2 were also similar between the different genotypes of the genes TCF7L2 and HHEX, respectively. This conclusion was reached within either the PCOS subjects or controls. The results suggested that these genetic mutations did not affect the susceptibility to PCOS.

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Introduction

Polycystic ovary syndrome (PCOS) is a complex gynecological endocrinopathic disease which is one of the most common causes of hyperandrogenism, hyperinsulinemia and chronic oligo-anovulation. PCOS is caused by a combination of genetic susceptibility and environmental exposures. Although we now know that multiple genes are involved in its onset and the development, the pathophysiological basis of PCOS remains unclear despite its growing global importance.

To date, a number of genes have been reported to be associated with PCOS. Most of the genes are investigated because they are presumed to be relevant to the pathogenesis of PCOS based on their functions. However, because the overall picture of the pathogenesis of PCOS is still lacking, this ‘candidate-gene approach’ is limited in power to detect novel disease-susceptibility genes.

Insulin resistance (IR) is a notable characteristic of PCOS, although it’s not considered as a parameter for diagnosis. About 50–70% women with PCOS also develop IR, and vice versa [1]. IR and its accompanying hyperinsulinaemia may

play a key role in the etiology of PCOS, as weight loss and insulin sensitizing drugs improve the clinical manifestations such as hyperandrogenaemia and restoring ovulation [2]. The PCOS patients who risk developing IR can also be obese and may also have impaired glucose tolerance. If the patients are not treated, they will likely develop type 2 diabetes (T2DM) in the future.

T2DM is a disease characterized by impaired insulin sensitivity. Candidate gene mapping and positional cloning have suggested many putative susceptibility variants, but only a few genetic variants leading to T2DM have been clearly identified including transcription-factor-7-like 2 (TCF7L2) and hematopoietically expressed homeobox protein (HHEX) [3, 4].

Recently, the TCF7L2 gene on chromosome 10q25.2 has been found to contribute substantially to the risk of T2DM [5]. A study on T2DM progression also suggests that TCF7L2 might be associated with beta-cell dysfunction on insulin secretion but not with insulin resistance [6]. The TCF7L2 gene product is a high mobility group (HMG) box-containing transcription factor implicated in blood glucose homeostasis. Yi et al [7] suggests that TCF7L2 acts on regulation of proglucagon through repression of the proglucagon gene in enteroendocrine cells via the Wnt signaling pathway. Now it has been definitively identified as the most important T2DM susceptibility gene [5, 8]. The single nucleotide polymorphism (SNP) rs7903146 is in strong linkage disequilibrium with the microsatellite and strongly associated with an increased risk of T2DM [9–14], and the T allele is identified as the variant which most strongly determines the T2DM [15]. Thus, genotyping the SNP rs7903146 is probably the best way to evaluate the risk effect of the TCF7L2 on PCOS.

The HHEX gene on chromosome 10q23.33, which also contains the insulin-degrading enzyme (IDE) and kinesin family member 11 (KIF11), encodes a 270 amino acid protein. Morgutti et al. [16] have found that the HHEX gene contains 4 exons and spans 5.7 kb of genomic DNA. A genome-wide scan for association (GWSA) shows evidence ($OR=1.13$, $P=5.7 \times 10^{-10}$ for rs1111875) in the all-data meta-analysis that HHEX is an excellent candidate susceptibility gene for T2DM [17]. In addition, its effect is independent of adiposity, suggesting that it affects insulin sensitivity or secretion [18]. SNP rs1111875 is located in a region including HHEX, which is critical for the development of the ventral pancreas. The HHEX gene represents a second strongest biological candidate given postulated effects on both insulin signaling and islet function. Therefore, we chose to investigate the replication at the SNP rs1111875 representing a powerful biological candidate.

Polycystic ovary syndrome (PCOS) is strongly associated with hyperinsulinemia and type 2 diabetes mellitus (T2DM).

Since there is strong evidence implicating the roles of TCF7L2 (rs7903146) and HHEX (rs1111875) genes on insulin secretion in T2DM, and IR is the notable characteristic both of PCOS and T2DM, the functional impact of these two gene mutations by SNP was investigated [19]. Given the phenotypic overlap between PCOS and T2DM, we tried to investigate if these SNPs which are risk factors for T2DM also influence susceptibility to PCOS who are often vulnerable to develop IR in the Chinese population.

Materials and methods

Patients

We studied 616 women among whom 326 were PCOS patients and 290 were non-PCOS women who had normal menstrual cycles (<32 days) with no obesity, hirsutism, acne, or overmuch sebum. The participants were enrolled consecutively at Department of Obstetrics and Gynecology, the Hospital affiliated with Medical School of Nanjing University, or enrolled consecutively at Department of Obstetrics and Gynecology, Anhui Medical University. All the subjects included in the study were of Chinese Han ethnicity.

The diagnosis of PCOS was based on the 2003 Rotterdam ESHRE/ASRM-Sponsored Symposium on PCOS [20]. The criteria used included clinical and/or biochemical signs of hyperandrogenism, chronic anovulation and polycystic ovaries, as well as the exclusion of other etiologies such as congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing's syndrome, etc.

Dates and hormone assays

We recorded the menarche age and calculated the body mass index ($BMI = \text{body weight in kilograms} / \text{square of height in meters}$) and fasting insulin levels to assess obesity. Peripheral blood was obtained by a single venipuncture during menstrual cycle between 8 AM and 9 AM after a 12-hour overnight fast. Samples were immediately centrifuged and serum was separated and frozen at -80°C until assayed. Serum hormone levels including total testosterone (T), follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P), estradiol (E2) and prolactin (PRL) were determined by RIA. We failed to assay the hormone levels in a very small number of women with or without PCOS. None of the participants had been taking hormonal medications, including contraceptive pills, for the previous 3 months. The study was approved by Medical School of Nanjing University, and informed consent was obtained from all participants.

DNA isolation and analysis

Genomic DNA was extracted from leukocytes using ChelexR-100 as a medium in accordance with the manufacturer's instruction (Promega, Madison, WI, USA).

Genotyping of the SNP rs7903146 of TCF7L2 was performed with polymerase chain reaction–restriction fragment length polymorphism. The primers were designed based on the published sequences of the human TCF7L2 gene: forward, 5'-AATTAGAGAGCTAAGCACTTTT TAGGTA-3', reverse, 5'-CAAGCTTCTCAGTCACAC AGG-3'. The reaction mixture in 25 μ L contained 50 ng of genomic DNA; 2.5 pM of each primer; 2.5 μ L STR (short tandem repeat) and 0.5 U of Taq DNA polymerase (Promega, Madison, WI, USA). The PCR was performed in a PTC-100 (MJ ResearchTM, Inc.) thermocycler as follows: 30 cycles consisting of 1 min of denaturation at 94°C, 1 min of annealing at 60°C, and 1 min of extension at 72°C. An initial denaturation step of 5 min at 94°C and a final extension of 10 min at 72°C were used. The 176-bp length PCR products were then digested with Rsa I at 37°C for 16 hs, which cleaves the C allele to generate DNA fragments of 27 and 149 bp, respectively, in size. The DNA fragments were separated by electrophoresis on a 3% agarose gel, and visualized by staining with ethidium bromide. 5 μ L PCR products were mixed with 1 μ L STR 6 $\times$ loading solution (Promega, Madison, WI, USA) and were run for 48 min.

The SNP rs1111875 of the HHEX gene was also detected by PCR-RFLP analysis. The 161-bp length product encompassing the polymorphic site was amplified by PCR with the sense primer 5'-CATCATAACTTCTCACTCCCTTCC-3' and the antisense primer 5'-GCTGCTTATGGA AACTGCATTACT-3'. The amplification condition was the same as above. Thereafter, the products were digested with Xba I, which cleaves the A allele to generate two DNA fragments of 50 and 111 bp, respectively. The DNA fragments and the PCR products were then electrophoresed on a 2% agarose gel and also stained with ethidium bromide.

Statistical analysis

Genotype frequencies of the TCF7L2 (rs7903146) and the HHEX (rs1111875) genes were checked for Hardy–Weinberg equilibrium of genotypes in both the PCOS and control groups. Fisher's Exact Test was used to compare the genotype distribution in the case-control study. The analysis was performed using the SAS system software (SAS Institute Inc., Cary, USA). The results of serum hormone levels were reported as means $\pm$ SD. Differences in serum hormone levels among different genotypic individuals were assessed using analysis of covariance (ANCOVA) to correct for age and BMI. $P < 0.05$ was considered statistically significant.

Results

The 326 women with PCOS had a mean ($\pm$ SD) age of 26.1 ± 4.0 years, and a mean ($\pm$ SD) BMI of 22.5 ± 4.0 kg/m². The 290 healthy women had a mean ($\pm$ SD) age of 29.1 ± 5.1 years, a mean ($\pm$ SD) BMI of 21.4 ± 2.2 kg/m².

In all the 616 study subjects the frequencies of genotypes were as follows: 0.795 CC, 0.190 CT and 0.010 TT, for rs7903146 in the TCF7L2 gene and 0.203 AA, 0.558 AG and 0.239 GG for rs1111875 in the HHEX gene. Genotypic distributions of the SNP rs7903146 (CC, CT and TT) in women with PCOS (0.801, 0.187, and 0.013, respectively) did not differ from those in controls (0.800, 0.193, and 0.007, respectively). Genotypic distributions of the SNP rs1111875 (AA, AG and GG) in women with PCOS (0.215, 0.555, and 0.230, respectively) showed no difference from that in controls (0.190, 0.562, and 0.248, respectively) either. All genotypic distributions were in Hardy–Weinberg Equilibrium. The results were summarized in Table 1.

Additionally, no statistical differences were observed among the different genotypes either in the PCOS group or the control group in terms of these measurements: BMI, age at menarche and basal hormone levels including insulin, FSH, LH, LH/FSH, P, T and E2 (Tables 2 and 3).

Table 1 Haplotype analysis for the rs7903146 and rs1111875 loci and risk for PCOS

Haplotype	Total (frequency)	PCOS (frequency)	Control (frequency)	<i>P</i> -value
rs7903146				
CC	490(0.795)	261(0.801)	232(0.800)	0.715
CT	117(0.190)	61(0.187)	56(0.193)	
TT	6(0.010)	4(0.012)	2(0.007)	
rs1111875				
AA	125(0.203)	70(0.215)	55(0.190)	0.704
AG	344(0.558)	181(0.555)	163(0.562)	
GG	147(0.239)	75(0.230)	72(0.248)	

Table 2 Anthropometric characteristics and serum hormone concentrations in PCOS and control subjects with different genotypes

rs7903146 SNP/genotype	PCOS		<i>P</i> -value	Control		<i>P</i> -value
	CC	XT		CC	XT	
Age (years)	26.16±4.10	27.33±4.09	0.1341	30.01±3.43	30.27±3.65	0.6090
Menarche (years)	14.37±1.61	14.44±2.08	0.8282	14.44±1.52	14.50±1.21	0.9718
BMI (kg/m ²)	22.62±4.15	22.28±2.65	0.6419	21.41±2.24	21.37±2.14	0.4423
Fasting Insulin (mU/L)	16.68±4.43	14.60±5.69	0.6089	12.50±6.07	10.30±5.45	0.7931
FSH (IU/mL)	8.00±4.77	7.49±3.74	0.5706	6.95±2.03	6.86±1.43	0.5826
LH (IU/mL)	21.94±20.41	20.82±22.40	0.6639	4.55±2.12	4.20±2.02	0.3799
LH/FSH	2.94±1.39	2.68±1.20	0.3168	0.68±0.35	0.63±0.33	0.5445
PRL (ug/L)	20.69±19.09	14.14±10.85	0.0780	15.64±8.38	26.62±46.12	0.0687
T (nMol/L)	4.70±4.08	3.44±2.68	0.0910	1.12±0.70	0.84±0.49	0.3214
E2 (pMol/l)	251.44±121.67	216.09±125.99	0.1749	170.89±115.18	134.16±106.30	0.1199

Discussion

Polycystic ovary syndrome (PCOS), the most common reproductive endocrine disorder of premenopausal women, is strongly associated with IR and T2DM.

To our best knowledge, this was the first study on the association between PCOS and T2DM-associated variants of TCF7L2 and HHEX in a Chinese population. We tested the relationship between the polymorphisms of TCF7L2 (rs7903146) and HHEX (rs1111875), respectively, and the clinical findings such as insulin secretion and PCOS. Our results indicated that there was no evidence that these variations were associated with the occurrence of PCOS in case-controlled analyses. Our observations supported the notion that TCF7L2 rs7903146 and HHEX rs1111875 cannot be regarded as fundamental candidate genes for PCOS susceptibility.

Our finding showed that there were no convincing evidence on the association between the polymorphism of

TCF7L2 and PCOS ($P=0.725$), which is in full agreement with Barber's study [19]. We additionally examined the relationship between the TCF7L2 polymorphism and clinical and metabolic features in Chinese women with PCOS. We speculated that rs7903146 of TCF7L2 might affect the secretion of insulin, by stimulating the insulin composition or causing IR, and that the increased insulin levels may lead to elevated secretion of testosterone. However, in our study, the polymorphism of TCF7L2 showed no effect on insulin secretion, i.e. no difference in insulin and T levels in different genotypes of patients with PCOS. Additionally, we found that the T allele frequency was lower than the reports studying other ethnic groups [13, 19, 21–25]. Several groups, found that the CC haplotype of SNP rs7903146 is associated with lower plasma glucose and insulin levels, whereas the XT haplotype is associated with higher levels [21, 23, 25]. Although Schäfer et al. [24] and Christopoulos [26] detected a slight increase in fasting plasma glucose and insulin in carriers of the CC haplotype

Table 3 Anthropometric characteristics and serum hormone concentrations in PCOS and control subjects with different genotypes

rs1111875 SNP/genotype	PCOS			<i>P</i> -value	Control			<i>P</i> -value
	AA	AG	GG		AA	AG	GG	
Age (years)	26.46±3.81	26.65±3.80	26.36±4.58	0.9137	30.08±3.68	30.76±3.11	30.20±3.36	0.5866
Menarche (years)	14.46±2.20	14.36±1.65	14.66±1.61	0.6346	14.38±1.26	14.37±1.16	14.91±3.21	0.5409
BMI (kg/m ²)	23.06±4.86	22.53±3.61	22.32±3.87	0.7302	22.16±2.63	21.72±2.22	20.96±2.00	0.1988
Fasting Insulin (mU/L)	15.90±7.19	14.88±4.78	14.73±5.63	0.9547	13.50±4.69	10.25±3.16	11.45±4.36	0.9219
FSH (IU/mL)	8.95±4.16	7.58±4.11	7.74±5.43	0.7258	6.88±1.48	6.78±2.00	7.40±2.56	0.3553
LH (IU/mL)	24.33±14.61	22.66±26.47	20.83±18.72	0.8147	3.78±1.59	4.28±2.15	4.93±2.20	0.1447
LH/FSH	2.74±0.96	2.94±1.42	2.84±1.39	0.8726	0.55±0.21	0.65±0.37	0.71±0.34	0.4220
PRL (ug/L)	22.39±14.63	17.69±16.92	16.09±11.81	0.2309	17.55±7.73	16.56±8.39	16.57±8.62	0.6689
T (nMol/L)	5.04±4.64	4.21±2.89	4.32±4.59	0.6004	1.33±0.65	1.03±0.68	1.12±0.53	0.2510
E2 (pMol/l)	244.18±99.98	231.14±123.10	248.46±132.82	0.7500	204.81±163.43	163.64±139.76	157.09±142.72	0.9788

formed by SNP rs7903146 when compared with carriers of the XT haplotype in German and Dutch populations and a Greek population, respectively. However, in our study plasma glucose was not measured due to economic reasons. We only observed that the patients with PCOS with the XT genotype of SNP rs7903146 had lower fasting plasma insulin levels than those with the CC genotype, but the difference did not reach statistical significance. This discrepancy is possibly due to population stratification.

Recently, many studies have demonstrated that common variants of TCF7L2 most likely influence IR susceptibility through impairment of insulin secretion [27]. Cauchi S et al have reported that a large-scale meta-analysis revealed associations between the rs7903146 and IR with p values between 10–80 and ~10–140 [12]. Biyasheva et al. [28] has found evidence of associations with two independent TCF7L2 loci in a PCOS cohort: association between the proinsulin: insulin molar ratio and the T2DM locus, and association between reproductive PCOS phenotype and a novel locus. This study suggests that variation in different regions of TCF7L2 contributes to distinct phenotypes of PCOS. Our results are to a certain degree similar with the recent study by Wang and colleagues [29] which has not found association between PCOS and insulin-degrading enzymes in relationship with IR. They also reach the conclusion that the single nucleotide polymorphism in the human IR gene is associated with metabolic features of PCOS women in a Chinese population.

With respect to the HHEX polymorphism, we found no evidence on the relationship between the SNP rs1111875 and susceptibility to PCOS or other relevant clinic manifestations. Our results showed that the G allele frequency in Han population was higher than in other ethnic groups. The G and A allele frequencies in a German population was 58.3% and 41.7%, respectively.

However, a considerable number of women with IR did not develop PCOS and lean PCOS patients with IR were also reported [30]. A study on sisters of PCOS patients demonstrated that, on the same genetic basis, hyperandrogenaemia and/or anovulation develop mostly in those sisters who are obese. Previous studies suggest that insulin can stimulate ovarian steroidogenesis synthesis disorder. With the use of antibodies to the insulin receptor, insulin is demonstrated to stimulate steroidogenesis in theca cells of PCOS patients via its own receptors. We speculated that SNPs rs 7903146 and rs1111875 were correlated to the insulin sensitivity or secretion, which, as risk factors of IR, may be candidate genes to the etiology of PCOS. However, the present study failed to substantiate the association between the variations of the TCF7L2 and HHEX genes and the PCOS pathogenesis.

In conclusion, our findings supported the notion that, in contrast to T2DM, genetic variation in rs7903146 of

TCF7L2 and rs1111875 of HHEX which influence beta cell sensibility and secretion, may not be the main determining factors of PCOS pathogenesis in Chinese women.

Acknowledgements This study has been supported by the National Natural Science Foundation of China (30672228) and the National Basic Research Program of China (973 programme 2010CB945103).

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