SHORT REPORT

Identification of YAP1 as a novel susceptibility gene for polycystic ovary syndrome

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ABSTRACT

Background A previously reported genome-wide association study (GWAS) of polycystic ovary syndrome (PCOS) in Han Chinese found that several loci of p value around 10e-5 warrant investigation. Replication of the GWAS was applied in this study to determine whether gene YAP1 (yeast associated protein 1) is associated with PCOS.

Methods An independent set of 1115 PCOS patients and 1137 controls were recruited; single nucleotide polymorphisms (SNPs) rs11225138, rs11225161, and rs11225166 from YAP1 were selected for the replication study. Real-time quantitative PCR was applied for genotyping by TaqMan-MGB probe assay.

Results Meta-analysis showed that the allele frequency of rs11225161 (A/G) was significantly different between PCOS and controls at a GWA significance (Pmeta =3.98e-09). Genotype–phenotype correlation study found 30 min and 60 min glucose of the oral glucose tolerance test were higher in PCOS patients with rs11225161 risk allele A. The G allele of SNP rs11225138 (G/C) was a further risk factor for higher luteinising hormone level in PCOS patients (p=0.041).

Conclusion YAP1 appears to be a new susceptibility gene for PCOS in Han Chinese women.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrinopathy, affecting 6–8% of women of reproductive age.1 Complications include dyslipidaemia, atherosclerosis, hyperinsulinaemia, and type 2 diabetes. The risk for certain carcinomas such as endometrial and ovarian cancers is increased in PCOS patients.2

The characteristic of familial aggregation makes PCOS worthy of genetic investigation.3 In order to identify susceptibility genes, we previously conducted a genome-wide association study (GWAS) in a Chinese Han population. Follow-up studies involving single nucleotide polymorphisms (SNPs) with p value <10e-6 were replicated, with three susceptibility loci confirmed.4 However, other loci with p value around 10e-5 remain intriguing and may also signal potential risks for PCOS.

Several interesting SNPs with p values ranging from 10e-3 to 10e-5 aggregated in the gene YAP1 on chromosome 11q13 (supplemental table 1). YAP1, carrying the Src homology domain (SH domain) and WW domain, has been shown to bind to a number of signalling proteins.5 DNA damage caused by oxidative stress would accumulate YAP1 and influence target gene expression and its participation in pro-apoptosis.5 YAP1 is also a pivotal transcriptional co-activator of the Salvador–Warts–Hippo pathway, which governs organ volume and plays an important role in tumours such as breast, lung, and ovarian cancers.7–10

Combining our GWAS data and previous information on YAP1, we speculate YAP1 may be a susceptible candidate for PCOS. To determine this, three SNPs (rs11225138, rs11225161, and rs11225166) in YAP1 were genotyped in an additional independent sample of 1115 PCOS patients and 1137 controls for replication study. Meta-analysis was applied to combine our GWAS result and current replication data; genotype–phenotype correlation between the SNPs and PCOS was analysed as well.

SUBJECTS AND METHODS

Subjects A total of 2252 northern Han Chinese women from the Shandong province of China were recruited consecutively at the Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, from June 2009 to May 2011. The 1115 women with PCOS were diagnosed according to the Rotterdam PCOS consensus criteria, which is satisfied by at least two of the three phenotypic criteria after exclusion of other known causes of hyper-androgenaemia and ovulatory dysfunction (including 21-hydroxylase deficiency, congenital adrenal hyperplasia, Cush- ing’s syndrome, androgen-secreting tumours, thyroid disease, and hyperprolactinaemia). The 1137 controls had normal menstrual cycles, and neither hyperandrogenaemia nor polycystic ovaries. Subjects taking oral contraceptives during the past 3 months were excluded.

Measures Follicle stimulating hormone (FSH), luteinising hormone (LH), prolactin, testosterone (T), and oestradiol (E2) values of all the subjects were measured by a chemiluminescent analyser (Beckman Access Health Company, Chaska, Minnesota, USA). A 75 g oral glucose tolerance test (OGTT) was performed for PCOS patients (AU640 automatic biochemistry analyser; Olympus Company, Hamburg, Germany). Insulin resistance was assessed using the homeostasis model (HOMA-IR= fasting glucose [FBG mmol/l] × fasting insulin [FINS mIU/l]/22.5).

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SNP selection
*YAP1* contains nine exons and is 122963 bp long. The selected SNPs of the GWAS were integrated to the HapMap database (taken from HapMap, CHB; [http://snp.cshl.org/](http://snp.cshl.org/)) [figure 1](#). SNPs in *YAP1* were selected for replication according to the following criteria: presence in the SNP 6.0 chip, typically representing a block; and minor allele frequency >10% in the Han Chinese population; SNPs of risk allele (OR >1) were captured; SNPs of selected SNPs with $r^2 < 0.8$. All selected SNPs were, as noted, statistically different (p<0.05) from our previous GWAS (supplemental table 1). In blocks that contain competing SNPs, the most significant SNP was selected. Ultimately, three SNPs—rs11225138, rs11225161, and rs11225166—were selected to precede further replication study.

SNP genotyping
Whole-blood samples were obtained by peripheral venous puncture; EDTA was used as the anticoagulant. Genomic DNA was extracted with QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. All SNPs were analysed by TaqMan-MGB probe assay ([Invitrogen Trading, Shanghai, China](http://invitrogen.com)) using available primers and probes (supplemental table 2). Reactions were performed on 384-well plates, Roche Lightcycle 480, carried out by pre-incubation at 95° for 4 min followed by 45 cycles of denaturation at 95° for 15 s, annealing, extension, and detection for 40 s at 60°C. Direct sequencing of 5% randomly selected samples were applied to validate the genotyping assays.

Statistical analysis
Basic characteristics of patients and controls are expressed as means±SD. The case–control genetics power was calculated by Genetic Power Calculator ([http://pngu.mgh.harvard.edu/~purcell/gpc/](http://pngu.mgh.harvard.edu/~purcell/gpc/)). Linkage disequilibrium (LD) was assessed using the Haploview software (Broad Institute, Cambridge, Massachusetts, USA). PLINK ([v.1.05, http://pngu.mgh.harvard.edu/](http://pngu.mgh.harvard.edu/)).

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**Figure 1** Regional plots of *YAP1*.
Logistic regression, the Padjusted value of rs11225161 in replication reached 3.98e-09 (OR 1.393, table 1), implying a genome-wide index. Genetic models were divided into additive (+/+ vs +/− population as a strata. By the additive effects of risk allele dosage, the logistic regression for disease trait was conducted to exclude the potential confounding effects of age and body mass index. Genetic models were divided into additive (+/+ vs +/− vs −/−), dominant (+/+ vs +/− vs −/−), and recessive (+/+ vs +/− vs −/−). Dominant and recessive models were applied for the phenotype analysis which was compared by one way analysis of variance (ANOVA), SPSS, V16.0 (SPSS Inc). A value of p<0.05 was regarded as statistically significant.

RESULTS

Characteristics of PCOS and control subjects are summarised in supplemental table 3. The PCOS group was younger than the control group (p<0.001), and the PCOS group had a significantly higher body mass index (p<0.001) than the controls. There were also significantly statistical differences in hormone of LH and T (LH: 10.88±4.59 IU/l vs 4.68±2.64 IU/l, p<0.001; T: 53.38±18.59 ng/dl vs 27.91±12.79 ng/dl, p<0.001).

LD and haplotype of three SNPs were calculated by Haploview. Given little or moderate LD at rs11225158 (rs11225158, r² = 0.23), rs11225158 (vs rs11225166, r² = 0.33), and rs11225166 (vs rs11225161, r² = 0.17), haplotype associations did not reveal a more significant association than single marker analyses. Hardy–Weinberg equilibrium performed by PLINK was higher than 0.05, suggesting no deviation in the PCOS and the control groups in three SNPs.

The allele frequencies of rs11225161, rs11225138, and rs11225166 were also statistically different after combined (p<0.001), allele frequency differences, and genotype comparisons (p=0.041, supplemental table 7), but the difference was not found in control dominant model analysis (supplemental table 9). In addition, in rs11225166, glucose at 30 min also differed in the two groups (p=0.021, supplemental table 8).

DISCUSSION

Our recently published GWAS identified three susceptibility loci to PCOS having a p value around 10e-05, specifically variants in gene YAP1.8 In the current research we performed replication studies on SNPs of YAP1 and confirmed the susceptibility of YAP1 to PCOS.

One rs11225161 in the fifth intron of YAP1 has been identified. Meta-analysis of previous GWAS and current replication data showed a genome-wide association significance for allele frequency. The other two SNPs—rs11225138 in the third intron and rs11225166 in the seventh intron of YAP1—were also statistically significantly different in the adjusted combined study. These data showed variants in YAP1 are associated with PCOS. As reported, polymorphic change in the YAP1 promoter region results in a decrease of YAP1 expression,11 which implies SNPs in our study remain to be elucidated in functional significance.

YAP1 can bind to a number of transcription factors and serve as a modulator by phosphorylation. As one of the major manifestations of PCOS, ovary enlargement is regulated by some pathways that YAP1 may be involved in. Phosphorylated YAP1 can inhibit co-activation of the TEAD (TEA domain) transcription factor to upregulate pro-growth genes.12 Moreover, by participation of YAP1/TAZ (transcriptional co-activator with PDZ binding motif), Hippo signalling pathway can act on stem cell compartments and regulate organ size in mammals.8 YAP1 can also bind to p73 and selectively promote pro-apoptotic genes such as BAX (Bcl-2–associated X protein) and TPS313 (tumour protein p53 inducible protein 5).8 13 This binding could be attenuated by phosphorylation of YAP1 by Akt,14 which also participates in the endometrial hyperplasia in women with PCOS.15 Our association study has proved YAP1 to be a novel

| Table 1 Allele frequencies comparison of PCOS and controls and meta-analysis of the replication study and GWAS |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **SNPs** | **MAF** | **Allele frequency comparison** | **Meta-analysis** |
| | | **PCOS** | **CTRL** | **χ²** | **p Value** | **OR** | **p Value** | **P(R)** | **OR** | **Q** |
| rs11225161 | A | 0.24 | 0.19 | 14.51 | 1.4E-4 | 1.32 (1.144 to 1.523) | 3.98e-09 | 3.98e-09 | 1.383 | 0.385 |
| rs11225138 | G | 0.27 | 0.23 | 7.479 | 6.2E-3 | 1.212 (1.056 to 1.391) | 1.16e-04 | 1.16e-04 | 1.225 | 0.439 |
| rs11225166 | G | 0.27 | 0.25 | 1.738 | 0.19 | 1.097 (0.956 to 1.258) | 1.73e-03 | 9.27e-02 | 1.177 | 0.0469 |

In meta-analysis, the p values were calculated by fixed effect model and P(R) calculated by random effect model. Bold indicates statistical significance.

CTRL, control; GWAS, genome-wide association study; MAF, minor allele frequency; SNPs, single nucleotide polymorphisms.

For more detailed information, please refer to the original document.
candidate gene for PCOS, but the underlying function of YAP1 on PCOS still needs further study.

By genotype analysis, the risk alleles of these three SNPs in YAP1 seem to have a dominant effect on PCOS patients. Genotype—phenotype correlation analysis also showed that risk allele carriers have severe glucose metabolic disorder. In risk allele carriers of rs11225161, 50 min and 60 min glucose were statistically higher, meanwhile in rs11225166, 50 min glucose was also increased in the risk G group. It has been shown in several studies that patients with PCOS have greater insulin resistance than controls, independent of body mass index. In the compensatory phase, there is sufficient insulin secretion for the insulin action impairment. When the β cell function is impaired and no longer able to compensate for the defect mentioned above, glucose intolerance develops, presenting as elevated glucose levels after glucose load. Considering that PCOS and several metabolic disorders (eg, diabetes mellitus, metabolic syndrome, and cardiovascular disease) share insulin resistance as a crucial pathogenetic mechanism, impeded glucose tolerance as shown in the current study suggests that women carrying the risk allele of rs11225161 and rs11225166 are at a high risk of developing these complications.

The LH concentration in the rs11225138 risk allele G group was higher than in the non-G PCOS subjects. Increased secretion of LH in PCOS is evidence of anovulation due to the aberrant expression of the receptor, reverse LH/FSH value, and the absence of LH peak. High concentrations of LH will persistently stimulate the ovarian theca cell and result in an aberrant expression of the receptor, reverse LH/FSH value, and the absence of LH peak. High concentrations of LH will persistently stimulate the ovarian theca cell and result in anovulation. In addition, excess androstenedione will convert into oestrone, which will exert inhibitory feedback on the hypothalamus and result in anovulation.

In conclusion, we have identified a new susceptibility gene YAP1 on chromosome 11q13 for PCOS.

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Contributors Wrote the manuscript: LT, ZH, ZX; edited the manuscript: ZH, CL, WP; conceived the study: CZ, ZH; performed experiments: LT, ZB, LG; provided clinical data: SY, ZH.

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Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was provided by Institutional Review Board for Reproductive Medicine of Shandong University (Shandong, China).

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Data sharing statement The additional unpublished data from the study are available to nobody else but the authors.

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