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Family-based analysis of GGT1 and HNF1A gene polymorphisms in patients with polycystic ovary syndrome

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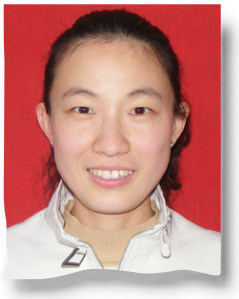

Xinghua Xu works in the Liaocheng People's Hospital, China. She received her PhD and carries out postdoctoral research in the Center for Reproductive Medicine of Shandong University. Dr Xu has been involved in reproductive medicine for 10 years and she is especially interested in the study of PCOS.

KEY MESSAGE

A total of 310 family trios were studied and the transmission disequilibrium test (TDT) was used to assess the linkage between PCOS and rs4820599 of GGT1, rs7305618 and rs2393791 of HNF1A. No significant evidence supported a relationship between genes GGT1 and HNF1A and PCOS in the current family trios.

A B S T R A C T

Polycystic ovary syndrome (PCOS) is a complex endocrine-metabolic disease. Previous studies indicate that genes GGT1 and HNF1A may contribute to the abnormal glucose metabolism and altered lipid profile that are important clinical features of PCOS. In the current study, the correlation between polymorphisms in the GGT1 and HNF1A genes and PCOS was explored. A total of 310 family trios were studied and the transmission disequilibrium

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test (TDT) was used to assess the linkage between PCOS and three single-nucleotide polymorphisms (SNP) (rs4820599 of GGT1, rs7305618 and rs2393791 of HNF1A). No deviations from HWE were detected. None of the three SNP markers showed significant transmission disequilibrium in PCOS family trios (rs4820599: GGT1 gene, $\chi^2 = 1.067$; rs7305618: HNF1A gene, $\chi^2 = 0.013$; rs2393791: HNF1A gene, $\chi^2 = 0.031$). In conclusion, no significant evidence supported a relationship between genes GGT1 and HNF1A and PCOS in the current family trios.

Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine-metabolic disease affecting 6–8% of women of child-bearing age (Azziz et al., 2004). It is associated with a high risk of complications including obesity, hyperinsulinemia, insulin resistance, type 2 diabetes mellitus (T2DM), dyslipidaemia and cardiovascular diseases (Azziz, 2016; de Groot et al., 2011; Kandaraki et al., 2009; Wild et al., 2000; Xu et al., 2014). Complex interactions between environmental factors and predisposing polygenic backgrounds contribute to this complicated and heterogeneous disorder (Vink et al., 2006). The strong familial clustering of PCOS allows the possibility of genetic investigation (Li et al., 2012). Over recent decades, many genes have been interrogated as potential susceptibility genes for PCOS, such as LHCGR, FSHR, THADA, DENND1A, YAP1, RAB5B and SUOX (Azziz, 2016). Based on the genome-wide association study (GWAS), several loci, such as 8p23.1, 9q22.32 and 11p14.1, were identified to be associated with PCOS (Chen et al., 2011; Hayes et al, 2015; Shi et al., 2012). However, the molecular mechanisms underlying PCOS are still not fully clarified and the exact aetiology remains unknown.

The serum level of γ-glutamyl transferase (GGT), which is used clinically as a biomarker for liver dysfunction and/or excessive alcohol consumption, is correlated with an elevated risk of metabolic syndrome, diabetes and cardiovascular diseases (Morita et al., 2015; Targher, 2010). As an active enzyme, serum GGT activity is increased in subjects with metabolic syndrome and/or obesity (Middelberg et al., 2012). Its activity is associated with SNP in and near GGT1 (Middelberg et al., 2012). Variant rs4820599 is predicted to be located within a transcription factor binding site in the GGT1 promoter (Morita et al., 2015). The risk G allele of rs4820599 is associated with the levels of GGT, fasting plasma glucose, high-density lipoprotein and low-density lipoprotein (Jinnouchi et al., 2015). Another gene which may influence GGT levels is the HNF1A gene on chromosome 12 (Yuan et al., 2008). HNF1A encodes a transcription factor (TF) that binds to promoters of a variety of genes expressed predominantly in liver and also in pancreatic islet cells (Wakil et al., 2014). An association between polymorphism rs7305618 of HNF1A and the risk of T2DM has been identified in a Chinese population (Li et al., 2014). Besides, mutations found in HNF1A could cause maturity-onset diabetes in youth type 3, primarily through impaired insulin secretion (Middelberg et al., 2012). In addition, rs2393791 of HNF1A was found to be linked to hypertension and hypertriglyceridemia (Wakil et al., 2014). Our previous GWAS, including 744 PCOS cases and 895 controls (Chen et al., 2011), also showed that rs2393791, rs7305618 of HNF1A and rs4820599 of GGT1 might be associated with PCOS. Although the *P*-values of these three SNPs have not reached genome significance (5×10^{-8}), they were around 10^{-4} to 10^{-3} (**Supplementary Table S1**), implying their potential risks to PCOS.

To further validate our GWAS research results and to avoid potentially complicating factors such as population stratification, genetic heterogeneity or environmental factors, a family-based analysis was performed using transmission disequilibrium test (TDT) (Spielman et al., 1993) to assess the linkage between PCOS and the three polymorphisms.

Materials and methods

PCOS families

A total of 310 Han Chinese family trios, each containing a patient diagnosed with PCOS and the parents, were evaluated in this study. The 930 participants were recruited from the Centre for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University, during the period from May 2009 to March 2011. All the PCOS subject probands were of Han Chinese origin. Written informed consent was obtained from all participants. This study was approved by the Institutional Review Board of Reproductive Medicine of Shandong University on 23 October 2015 (reference number 47). All of the PCOS probands were diagnosed according to the revised 2003 Rotterdam criteria, which is satisfied by at least two of the three features (Rotterdam, 2004). Patients with other related diseases (including adrenal congenital hyperplasia, Cushing syndrome and androgen-secreting tumours) were excluded.

To qualify as a PCOS index case, women with an interval between menstrual bleedings of more than 35 days were recruited as oligomenorrheic/amenorrhoeic. Hyperandrogenism was defined either by total testosterone (T) ≥ 60 ng/dl and/or m-FG scores ≥6 (Ferriman and Gallwey, 1961; Xu et al., 2012). Polycystic ovaries (PCO) were diagnosed by ultrasound (transvaginal ultrasound or ultrasound examination was performed rectally if subjects were virginal) if at least one ovary was >10 cm^3 or if an ovary contained at least 12 follicles 2–9 mm in diameter.

Genotyping

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. High-resolution melting (HRM) analysis was used for genotyping analysis. All three SNPs (rs4820599, rs7305618 and rs2393791) were amplified using PCR. The following sets of primers were used to amplify target genes: rs4820599 forward primer 5'-GGACGCTCACATCTCCTGCGT-3' and reverse primer 5'-AACTGCAGATCAGTGCCTCAA-3'; rs7305618 forward primer 5'-TCTGGCCTCTTCTGCGATCGCT-3' and reverse primer 5'-ACACACAGAGCAGGGTGGAGAC-3'; rs2393791 forward primer 5'-AGAGTCCCCAGTTCCTTGC-3' and reverse primer 5'-CTACATATTTGTGCACGTGTAA-3'. The PCR reaction was performed using a Gene Amp PCR System 9700 (Applied Biosystems by Life Technologies, USA). The reaction was carried out under the following

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