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Family association study between melatonin receptor gene polymorphisms and polycystic ovary syndrome in Han Chinese

X. Song [a,b], X. Sun [c], G. Ma [a], Y. Sun [d], Y. Shi [a], Y. Du [d,e], Zi-Jiang Chen [a,e,f,g,h,i,*]

[a] Centre for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, China
[b] Department of Gynaecology and Obstetrics, Dongying People's Hospital, China
[c] Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, China
[d] Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[e] Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai, China
[f] Key Laboratory for Reproductive Endocrinology of Ministry of Education, Jinan, China
[g] Shandong Provincial Key Laboratory of Reproductive Medicine, Jinan, China
[h] National Research Centre for Assisted Reproductive Technology and Reproductive Genetics, Jinan, China
[i] Reproductive Medical Hospital Affiliated to Shandong University, Jinan, China

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ABSTRACT

Objective: The melatonin receptor (MTNR) gene, reported to be associated with insulin sensitivity, diabetes and metabolic syndrome, could be a plausible candidate gene for polycystic ovary syndrome (PCOS). This study was designed to investigate whether an association exists between two single nucleotide polymorphism (SNP) variants (rs2119882 and rs10830963) of the MTNR gene and PCOS in Han Chinese.

Study design: In total, 263 family trios (789 participants) were enrolled in this family-based transmission disequilibrium test (TDT). Genotypes were obtained by sequencing. In total, 135 trios of rs2119882 and 127 trios of rs10830963 were tested.

Results: An association was detected between rs2119882 ($p = 0.0209$) and PCOS, suggesting that the MTNR gene may indicate increased susceptibility to PCOS in Chinese. No significant association was found for rs10830963 (transmitted:non-transmitted = 76:51, $p = 0.1573$). The association between the MTNR gene variants and clinical characteristics of women with PCOS was investigated. CC genotype carriers had higher levels of clinical and metabolic features than the TC and TT genotypes. A significant difference in transmission of allele C of rs2119882 was found between obese and non-obese women with PCOS (Chi-squared = 5.5983, $p = 0.018$).

Conclusion: This study may provide a basis for further studies of the MTNR gene in the aetiology of PCOS.

Introduction

Polycystic ovary syndrome (PCOS), first described by Stein and Lenventhal in 1935, is the most common and complex endocrine disorder, affecting 6–10% of women of reproductive age [1,2]. It is characterized by oligo-ovulation and/or anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovarian morphology [3]. To date, the inheritance mode of PCOS and the molecular mechanisms underlying PCOS aetiology remain unknown. The disorder has been reported to be associated with metabolic syndromes, such as type 2 diabetes, dyslipidaemia and hypertension [4–7]. The strong clinical, phenotypic and pathophysiological overlap between type 2 diabetes and PCOS promotes an argument for aetiological determinants underlying the two conditions [8].

Melatonin, the principal hormone of the pineal gland, is most widely recognized for its linkage with circadian rhythms and metabolic conditions, including diabetes and obesity [9,10]. Its function is mainly mediated by the melatonin receptor 1A (MTNR1A) gene and the melatonin receptor 1B (MTNR1B) gene, both of which belong to the G-protein-coupled receptor superfamily. The MTNR1A gene is mainly expressed in alpha cells, while the MTNR1B gene is mainly expressed in beta cells. These findings suggest an important role of the MTNR1A and MTNR1B genes in

* Corresponding author at: Centre for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, Jingliu Road 157, Jinan 250001, China.
Tel.: +86 531 85181173; fax: +86 531 87068226.
E-mail address: chenzijiang@hotmail.com (Z.-J. Chen).

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the aetiology and pathophysiology of PCOS. Previous studies have found that the polymorphisms rs2119882 in the MTNR1A gene and rs10830963 in the MTNR1B gene may play a common causative role in the pathogenesis of PCOS [11,12].

Familial aggregation and heritable tendencies of PCOS have long been recognized for this complex and heterogeneous disorder. However, information about family-based analysis of MTNR gene polymorphisms and PCOS is still lacking. To address this issue, a family-based analysis using the transmission disequilibrium test (TDT) was performed in 263 PCOS family trios (789 participants in total) to investigate the linkage and association between PCOS and the two single nucleotide polymorphisms (SNPs) rs2119882 and rs10830963 for a better understanding of the contribution of the MTNR gene to PCOS.

Materials and methods

Subjects

Seven hundred and eighty-nine participants, consisting of 263 trios (mother, father and offspring with PCOS), were recruited from the Centre for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University from July 2007 to April 2014. They were all Han Chinese and came from Shandong province. All subjects gave their informed consent for molecular studies. The study was approved by the Ethics Committee of Shandong University. PCOS was diagnosed according to the 2003 Rotterdam criteria as at least two of the following three features: oligomenorrhoea or amenorrhoea, clinical or biochemical hyperandrogenism, and polycystic ovaries on ultrasound. Other related diseases, such as adrenal congenital hyperplasia, Cushing's syndrome, no classic 21-hydroxylase deficiency, hypothyroidism, significant elevation in serum prolactin, and androgen-secreting tumours were excluded [3,13].

Methods

Anthropometric variables, such as waist circumference (WC), hip circumference (HC), body height and weight, were measured in all subjects during the first visit to the outpatient department. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Fasting venous blood samples were collected from 8.00 a.m. to 10.00 a.m. after a 12-h overnight fast. All blood samples of patients with PCOS were obtained on days 3–5 of their menstrual cycle. Serum levels of follicle-stimulating hormone, luteinizing hormone, total testosterone and oestrogen were measured enzymatically on an automated biochemistry analyser (Olympus 600, Olympus Diagnostica GmbH, Co. Clare, Ireland). All patients with PCOS underwent a 75-g oral glucose tolerance test. Fasting blood glucose (FBG) and 2-h glucose level were detected using the oxidase method (AU640 Automatic Biochemistry Analyser, Olympus Company, Hamburg, Germany), and fasting insulin (FINS) and 2-h insulin levels were detected by chemiluminescence immunoassay (endocrine hormone determination kit, Haier Import and Export, Ningbo, China). The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated according to the following equation: fasting blood glucose (mmol/l) × fasting insulin (mU/l)/22.5. Serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations were measured by the precipitation and enzymatic method (Ft-7060, Beckman Coulter, Galway, Ireland).

Genotyping

For each participant, a 5-ml whole blood sample was collected in a tube containing ethilene diamine tetraacetic acid via peripheral venous puncture, and stored at −20 °C. Genomic DNA was extracted using a QIAamp DNA minikit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The two valid SNPs (rs2119882 and rs10830963) in the MTNR gene were amplified using polymerase chain reaction with pairs of primers as follows.

- rs2119882: 5′-GCGAGCCCGCTTGGATTCC-3′ (forward), 5′-GCGG-TGCAATACACAACGGAAGAC-3′ (reverse).
- rs10830963: 5′-GTTTAGTGAGTGCCGAATTGG-3′ (forward), 5′-GAGCCTTTGTTCAGAACCATGC-3′ (reverse).

The positional information for the two SNPs is shown in Table 2. Reactions were performed under the following conditions: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 60 °C, extension at 72 °C for 45 s, and finally 72 °C for 5 min. The polymerase chain reaction products were initially analyzed by melting curve. Outliers lacking high peaks during melting curve analysis were alternately analyzed by 1% agarose gel electrophoresis, and then sequenced on an automated sequencer (ABI PRISM 310; Applied Biosystems, Foster City, CA, USA). All samples were double genotyped with 100% concordance.

Statistical analyses

Descriptive statistics for individual SNPs, including minor allele frequency and Hardy–Weinberg equilibrium, were obtained using Haploview 4.2 (Boston, USA) [14]. Next, the association between the two SNPs and PCOS was tested by TDT analysis, which was also performed using Haploview 4.2. Statistical significance was considered at the two-tailed level of $p = 0.05$. TDT is a valid Chi-squared test statistic for the linkage hypothesis, regardless of population history. In the TDT test, by collecting unrelated PCOS family trios, the difference between the probability of parents-to-offspring transmission and the hypothesis of no association (probability of transmission 50%) is analyzed. If a discrepancy exists, the reason would be an association between MTNR polymorphisms and PCOS.

Clinical and biochemical data for different subgroups were analyzed by independent samples *t*-test using Statistical Package for the Social Sciences Version 17.0 (IBM Corp., Armonk, NY, USA).

Categorical data have been expressed as frequency and percentage, and descriptive characteristics have been reported as mean [standard deviation (SD)]. Genotypic and allelic distributions were compared using Pearson's Chi-squared test and one-way analysis of variance in the patients with PCOS. $p < 0.05$ was considered to indicate statistical significance.

Results

Clinical and metabolic characteristics

The mean age of the 263 patients with PCOS was 27.14 (SD 3.42) years and mean BMI was 25.3 (SD 4.64) kg/m^2. Mean FBG and 2-h glucose were 5.61 (SD 1.14) mmol/l and 6.85 (SD 2.78) mmol/l, respectively. Mean FIN and 2-h insulin were 14.60 (SD 9.01) mIU/l and 74.68 (SD 67.29) mIU/l, respectively. HOMA-IR was 2.8 (SD 2.68), and TG was 1.44 (1.09) mmol/l.

Minor allele frequencies and TDT analysis

In total, 263 families were analyzed. The minor allele frequencies of these SNPs were 0.271 (rs2119882) and 0.43 (rs10830963), and the two SNPs were in Hardy–Weinberg