Association of genetic variants of insulin degrading enzyme with metabolic features in women with polycystic ovary syndrome

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Objective: To evaluate the influence of the four single nucleotide polymorphisms of the insulin-degrading enzyme (IDE) gene on metabolic features in women with polycystic ovary syndrome (PCOS) in a Chinese population.
Design: Prospective, case-control study.
Setting: University-based hospital.
Patient(s): Three hundred fifteen patients with PCOS and 327 healthy controls.
Intervention(s): Peripheral venous puncture, ultrasonography, oral glucose tolerance test (OGTT).
Main Outcome Measure(s): Genotype analysis of four single nucleotide polymorphisms in the IDE gene, hormonal and metabolic phenotypes.
Result(s): No significant differences in genotypes of these polymorphisms were found between PCOS patients and healthy controls. But the frequency of the C allele of rs2209972 was significantly higher in the PCOS group than that in the control group. The single nucleotide polymorphisms rs4646953, rs1887922, and rs1544210 had no impact on clinical and biochemical characteristics of women with PCOS. There were significant differences in body mass index (BMI) and insulin level in the rs2209972 genotype of women with PCOS. The women with PCOS with the CC genotype of rs2209972 had statistically significantly higher fasting insulin level and homeostasis model assessment for insulin resistance than the women with PCOS with the TT genotype.
Conclusion(s): The single nucleotide polymorphism rs2209972 in the human IDE gene is associated with metabolic features of PCOS women in a Chinese population. (Fertil Steril® 2008;90:378–84. ©2008 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovary syndrome, insulin resistance, single nucleotide polymorphism, insulin-degrading enzyme, gene

Polycystic ovary syndrome (PCOS) is a common complex and heterogenous endocrine disorder that is characterized by oligomenorrhea or amenorrhea, hyperandrogenism, and multiple small subcapsular cystic follicles in the ovary on ultrasonography (1). It affects 5%–10% of women of reproductive age (2–4). More recently, it has been recognized that in addition to endocrine abnormalities, many patients with PCOS demonstrate other metabolic aberrations including insulin resistance (IR), hyperinsulinemia, and impaired glucose tolerance (5–9). These metabolic derangements are similar to those seen in groups at increased risk of future type 2 diabetes. The frequent co-occurrence of polycystic ovaries (PCO) and type 2 diabetes strongly suggests shared etiological determinants underlying the two conditions. Their cause are multifactorial, individual susceptibility being determined by the action of multiple genetic and environmental risk factors. Studies at the insulin gene are consistent with the hypothesis that shared genetic susceptibility effects contribute to the physiological and epidemiological overlap between type 2 diabetes and PCOS (10–13). On this basis, any gene known to influence susceptibility effects to type 2 diabetes merits evaluation as a candidate for involvement in PCOS.

Recent work has identified the insulin-degrading enzyme (IDE) gene as such a candidate. Also known as insulysin or insulinase, IDE is the major enzyme responsible for insulin proteolysis in vitro (14), which may function in the termination of the insulin response (15). The gene encoding IDE is located on chromosome 10q23-q25, within a region linked to type 2 diabetes and related quantitative traits (16–19). In mice, IDE hypofunction induced by IDE gene disruption leads to hyperinsulinemia (20). Furthermore, IDE activity in the diabetic Goto-Kakizaki rat is reduced by 30%, where polymorphism in IDE is likely to be the main contributing factor (21). More recently, a number of linkage and association studies for various single nucleotide polymorphisms (SNPs) in the IDE gene expected to be associated with IR, hyperinsulinemia, and type 2 diabetes have been carried out by several research groups (20, 22, 23). Congruence of

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positional and functional data indicates that sequence variation in IDE may play a role in modifying insulin metabolism in human populations.

On the basis of the role of IDE in IR and hyperinsulinemia, we hypothesized that the IDE gene polymorphism can be regarded as a "candidate gene" for PCOS or for IR in PCOS and is a marker for the clinical characteristics of affected women. We have analyzed the IDE gene for the presence of single nucleotide base changes using restriction fragment length polymorphism in a large case-control sample of Chinese women to examine the possibility that IR in subjects with PCOS is a consequence of point mutations in the region of the IDE gene.

MATERIALS AND METHODS

Patients with PCOS

Patients with PCOS were recruited between January 1, 2004 and August 30, 2006, from the reproductive center clinic at the Shandong Provincial Hospital in Jinan. None of the patients had used hormonal preparations, including oral contraceptives (OC), for at least 3 months before the start of the study. According to the revised diagnostic criteria, announced in the 2003 American Society for Reproductive Medicine/European Society for Human Reproduction and Embryology (ASRM/ESHRE) Rotterdam consensus, PCOS is diagnosed when the phenotypes of patients are satisfied with two of three criteria: oligomenorrhea or amenorrhea, clinical or biochemical hyperandrogenism, and ultrasonographic polycystic ovarian morphology; whereas other causes such as nonclassic congenital adrenal hyperplasia are excluded (24).We recruited 315 women with PCOS.

Controls

The control population consisted of 327 healthy age-matched women with normal cycles, also recruited during this period and evaluated consecutively. All of the controls were carefully evaluated to avoid any selection bias. Each of them had normal ovulatory menstrual cycles, absence of hirsutism and other manifestations of hyperandrogenism, and absence of sonographic signs of PCOS. None of them had sign of galactorrhea and thyroid dysfunction or personal or family history of diabetes. They had normal hormonal status, were not receiving OCs or any drug therapy for at least 3 month before starting the study. Women in the control group were recruited from the same area as the patients with PCOS. All the subjects in this study were Chinese women.

After undergoing a history and physical examination, including measurement of abdominal and hip circumferences, blood sampling in the fasting state of all subjects was performed on days 2–4 of the menstrual cycle or during amenorrhea, after excluding pregnancy by appropriate testing for the measurement of hormone.

In patients with PCOS, an oral glucose tolerance test (OGTT) was performed. After a 12-hour overnight fast, a 75-g dose of glucose was ingested, and blood samples were collected through a venous catheter from the antecubital vein after 0, 30, 60, and 120 minutes for measurement of serum glucose and insulin.

Blood samples for molecular genetic studies were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and stored at −20°C.

Institutional review board (IRB) approval was obtained for this study, and informed consent was obtained from all women before inclusion.

Genetic Analysis

Four SNPs, rs4646953, rs1887922, rs2209972, and rs1544210, of IDE gene, which is associated with the phenotypes of type 2 diabetes, were studied. Details of reported SNPs may be found at the dbSNP Web site (http://www.ncbi.nlm.nih.gov/SNP/) under their respective identification numbers.

DNA was extracted from human leukocyte nuclei isolated from whole blood. Restriction fragment length polymorphism assays were designed for polymorphisms identified. Primers for SNP rs4646953 were forward 5′-GTGCA GAAGCCACGCTAGCC-3′ and reverse 5′-CGCTAGT CAGTGGGGGTGCC-3′ and the enzyme used was Ava II. For analysis of SNP rs1887922, the forward primer was 5′-CTGCTTTCTAGGTAGTGAGG-3′ and the reverse primer was 5′-GAGAGTTGCATTCTGAATTA-3′ and the enzyme used was Nla III. The SNP rs2209972 primers were 5′-TAAATTCGCCTTTCGCAACA-3′ and 5′-GTTTTC ACTCTCCGGGGACA-3′ with BsaH I digestion. Analysis of SNP rs1544210 involved the use of a forward primer 5′-CAAACAGCCAGGGAAACTCT-3′and a reverse primer 5′-CTGCTGGGTCTCATCCTTAC-3′ with digestion by Alu I. All enzyme digests were carried out at 37°C for 4 hours. Analysis of products was by agarose gel electrophoresis in 2% 89 mM tris-HCl, 89 mM borate, 2 mM EDTA (TBE) gels.

Cycling parameters were denaturation at 95°C for 12 minutes, 10 cycles at 94°C for 30 seconds, from 65°C for 30 seconds to 54°C for 30 seconds, descending 1°C per cycle, 72°C for 30 seconds, and 25 cycles at 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 30 seconds, and then 72°C for 7 minutes.

Of the 642 samples, 611 samples for SNP rs4646953, 597 samples for SNP rs1887922, 593 samples for SNP rs2209972, and 605 samples for rs1544210 were successfully genotyped the first time. Therefore the success rate of the first genotyping was 93.7%. The test continued until all the samples were successful genotyped, and all the samples were double genotyped in a blinded fashion with concordant results.

Statistical Analysis

Calculated values included body mass index (BMI) and the waist-to-hip ratio. The measurements derived from the OGTT were: [1] homeostasis model assessment for IR