Low-density lipoprotein receptor–related protein-5 C/T polymorphism in exon 18 is associated with C peptide and proinsulin levels in control women and patients with polycystic ovary syndrome

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Objective: To assess the previously unstudied potential role of C/T (A1330V) polymorphism of the low-density lipoprotein receptor–related protein-5 gene in insulin sensitivity and secretion in polycystic ovary syndrome. The low-density lipoprotein receptor–related protein-5 gene has been found to play a role in determining insulin secretion in animal models.
Design: Case–control study.
Setting: Tertiary outpatient clinic.
Patient(s): Women with polycystic ovary syndrome (n = 299; age, 27.5 ± 7.1 y [mean ± SD]), according to the European Society of Human Reproduction and Embryology criteria, as well as healthy control women (n = 187, age, 28.9 ± 9.8 y).
Intervention(s): Oral glucose tolerance test, blood sampling.
Main Outcome Measure(s): Glucose, insulin, C peptide, proinsulin during oral glucose tolerance tests, and lipids. Genotyping of C/T (A1330V) polymorphism by polymerase chain reaction–restriction fragment length polymorphism.
Result(s): There was no difference in the frequency of genotypes between women with polycystic ovary syndrome (CC/CT/TT: 80.3%, 18.4%, 1.3%) and the control women (79.1%, 19.8%, and 1.1%). Carriers of the T allele had statistically significantly higher basal and stimulated C peptide and proinsulin levels than CC homozygotes, both basally and at the 180th minute. Regarding insulin sensitivity, there was no difference between T carriers and CC homozygotes.
Conclusion(s): Polymorphism of C/T in the low-density lipoprotein receptor–related protein-5 gene is associated with C-peptide and proinsulin secretion but does not influence insulin sensitivity in either healthy women or women with polycystic ovary syndrome. (Fertil Steril® 2008;90:699–708. ©2008 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovary syndrome, C peptide, proinsulin, insulin resistance, LRP5 gene, genetic polymorphism

Polycystic ovary syndrome is the most common cause of ovarian dysfunction in women and is associated with infertility as well as with metabolic and cardiovascular aberrations that are seen in the so-called insulin resistance syndrome. Polycystic ovary syndrome is a heterogeneous condition, the pathophysiology of which appears to be both multifactorial and polygenic.

Polycystic ovary syndrome (PCOS) is often, but not consistently, associated with insulin resistance (1). The issue of β-cell function in PCOS remains even more controversial, with findings ranging from increased to defective insulin secretion (2–8). Genetic factors determine not only insulin resistance but also, and probably more strongly, insulin secretion (9). Which particular genes are most heavily involved, and whether these genetic variants contribute to the pathophysiology of PCOS, remains to be clarified.

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Low-density lipoprotein receptor–related protein 5 (LRP5), a novel member of the LDL receptor superfamily, originally was cloned on the basis of its association with type 1 diabetes mellitus in human beings. Low-density lipoprotein receptor–related protein 5 is coded by the gene located at the insulin-dependent diabetes mellitus-4 locus, chromosome 11q13 (10). The LRP5 is expressed in four functionally important cell types: the distributed mononuclear phagocyte system, retinoid-storing and metabolizing cells, central nervous system neurons, and the pancreatic islets of Langerhans (11). Recent data have provided evidence that LRP5 (as a coreceptor of Wnt) maintains the normal function of β-cells (12). The LRP5-knockout mouse model exhibits abnormalities of carbohydrate and lipid metabolism, as well as defective insulin secretion and higher blood glucose after oral glucose challenge. However, insulin tolerance tests revealed that the LRP5$^{-/-}$ mice were not insulin resistant. The targeted ablation of the LRP5 gene led, therefore, to impaired insulin secretion without having any effect on peripheral insulin sensitivity (13). The experiments suggested that Wnt–LRP5 signaling contributes to glucose-induced insulin secretion in the β-cells. Wnt signaling is an important regulator of adipogenesis or insulin secretion and may be involved in the pathogenesis of type 2 diabetes (T2DM) (14). Wnt is involved in the transcriptional regulation of several genes (HNF-4α, Tcf11, and Tcf12), mutations of which impair insulin secretion and lead to diabetes mellitus (15). Besides this, the altered Wnt signal transduction in PCOS theca cells was found by using gene expression profiling (16). The C>T polymorphism (c.4037, A1330V) in exon 18 of the LRP5 gene is a coding polymorphism that results in a substitution of alanine with valine at codon 1330. This domain of the LRP5 receptor is involved in ligand binding. It is conceivable that mutations of these regions could alter protein conformation and ligand affinity or specificity and, thus, the function and signaling of this pathway.

In human beings, mutations in the LRP5 gene cause monogenic disorders such as pseudoglioma-osteoporosis and high–bone mass syndromes (17, 18). The A1330V polymorphism recently has been associated with bone mass variations in the general population (19). The relationship between LRP5 genetic variants and metabolic phenotype, however, hitherto has not been studied.

The aim of this study was to evaluate the potential effect of the LRP5 coding polymorphism (c.4037, A1330V) on insulin sensitivity, secretion, and lipid spectrum in women with PCOS, in comparison to healthy women.

MATERIALS AND METHODS

The study group comprised 299 women with PCOS who were 27.5 $\pm$ 7.1 years of age (mean $\pm$ SD) and who matched the European Society of Human Reproduction and Embryology criteria. In 182 women with PCOS, polycystic ovaries were detected by using vaginal ultrasound, according to the criteria published by Balen et al. (20). A normal ultrasonographic picture of the ovaries was found in 62 women. They had a combination of chronic anovulation and clinical signs of hyperandrogenism, together with an elevation of total testosterone (T), free T index, or androstenedione above the upper limit of the normal range (normal ranges were as follows: for T, 0.40–2.65 nmol/L; for free T index, value of $\leq$6, and for androstenedione, 1.6–5.4 nmol/L). Fifty-five women did not undergo ultrasound examination, mostly for noncompliance or personal reasons; in all of them, a combination of chronic anovulation and the clinical signs of hyperandrogenism, together with an elevation of total T, of free T index, or of androstenedione above the upper limit of the normal range was seen. The control group comprised 187 healthy eumenorrheic women, recruited via advertisement, who were 28.9 $\pm$ 9.8 years of age, did not have symptoms of hyperandrogenism, and had a regular menstrual cycle (21–35 d). Pelvic ultrasonography was not performed in the control women. All probands were examined consecutively at the outpatient department of the Institute of Endocrinology (Prague, Czech Republic) between the years 1999 and 2006. All of the subjects were healthy, without the presence of any active endocrinopathy or severe internal disease. The only medication being taken was combined oral contraception (COC), which 87 of the healthy control women were taking.

After signing informed consent as approved by the local ethics committee, two blood pressure readings were obtained from seated patients after a 10-minute rest; the mean was determined from the two values and was used for further analysis. Weight (to the nearest 0.1 kg) and height (to the nearest cm) were measured. Waist circumference was measured in a standing position, halfway between the lower ribs and the crest of the pelvis. Hip circumference was measured as the widest gluteal circumference. Basal blood samples for lipid spectrum, glucose, C peptide, insulin, and proinsulin were taken from each patient's cubital vein.

Oral glucose tolerance tests (oGTTs) with sampling for blood glucose, insulin, and C peptide in the 0th, 30th, 60th, 120th, and 180th minutes were performed in 182 of the women with PCOS and in all control women. The oGTT with sampling in the 0th, 60th, and 120th minutes only was performed in another 52 patients with PCOS, and the basal values of insulin, C peptide, and blood glucose were determined in the remaining 65 patients with PCOS. The samples were processed by centrifuge, and serum was frozen at -20°C until analysis. In addition, 69 women with PCOS also underwent a euglycemic hyperinsulinemic clamp, as described elsewhere (21).

We estimated C peptide by using an immunoradiometric assay (Immunotech, CR) with an interassay coefficient of variation of 5.1%. Insulin also was estimated by using an immunoradiometric assay (Immunotech, CR), with an interassay coefficient of variation of 5.3%. Proinsulin was determined in the 0th and 180th minutes of an oGTT in all control women and in a subgroup of women with PCOS