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# Relationship between leptin receptor and polycystic ovary syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, which is involved in the multi-system disease, and its etiology is still not clearly understood. It is currently considered that not only the genetic factors but also the environment factors play a crucial role in the pathogenesis of PCOS. Obesity plays an important role through the insulin, leptin and endocannabinoid system in the pathological process of PCOS, leading to more severe clinical manifestations. The aim of our present study is to investigate whether there is association between single nucleotide polymorphisms (SNPs) of Gln223Arg and Pro1019Pro in the *leptin receptor* gene (*LEPR*) and PCOS in a Korean population. Interestingly, a significant association was found between the Pro1019Pro in *LEPR* gene and PCOS, and a highly significant association was found between the Gln223Arg in *LEPR* gene and PCOS ($P = 0.033$, OR = 1.523, 95% confidence interval and $P < 0.0001$, OR = 0.446, 95% confidence interval). Moreover, genotype combination and haplotype analyses indicate that Gln223Arg and Pro1019Pro polymorphisms of *LEPR* are significantly associated with the risk of PCOS.

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## 1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting approximately 6–8% of women of reproductive age (Shi et al., 2012). It is characterized by hyperandrogenism, chronic oligoanovulation and polycystic ovarian morphology (Shi et al., 2012). It is also known as a complex multi-system syndrome, with important features of hyperandrogenism, hyperinsulinemia and insulin resistance (IR). As a complex disease, PCOS is largely affected by multiple genetic and environment factors through their joint together. A considerable percentage of PCOS patients have both obesity and reproductive dysfunction disease (Zhai et al., 2012). So far, most of the genetic studies of PCOS have focused on the variants of interested genes which are examined for relation to PCOS or several other traits. It has been reported that PCOS is closely related to several kinds of metabolisms, such as glucose, lipid, leptin, resistin, and iron metabolism, to name a few (Bu et al., 2012; Escobar-Morreale, 2012; Glintborg and Andersen, 2010; Mahde et al., 2009). The adipose tissue generates many other factors, which play important roles in the complicate processes of metabolisms, including food intake, insulin regulation, energy expenditure and lipid metabolisms. In addition, they also take part in the development of relevant diseases such as obesity and IR (Bjorndal et al., 2011; Greenberg and Obin, 2006; Mukherjee and Maitra, 2010; Ouellet et al., 2012). Obesity has been identified as a risk factor for PCOS in reproductive age women. Leptin, one of important factors secreted by lipocytes, can affect reproduction, angiogenesis, hematopoiesis, and immune processes (Snoussi et al., 2006).

Women with PCOS typically have dramatic IR and leptin levels seem to have relationship with IR in young PCOS women (Yildizhan et al., 2011). Leptin levels were increased not only in obese women with PCOS but also in the non-obese subjects compared with controls (Yildizhan et al., 2011). Furthermore, correlation data have shown that leptin is not likely to play major pathogenetic roles in overweight/obese patients with PCOS (Spanos et al., 2012). However, the studies on the relationship between leptin level and PCOS have shown contradictory results (Glintborg and Andersen, 2010; Mendonca et al., 2004).

Leptin is a protein of 167 amino acids and produced in the adipocyte of white adipose tissue. Different animal model studies demonstrated that leptin is closely correlated with IR, and adiposity rather than IR might be the major determinant factor for the leptin levels (Sepilian et al., 2006). Leptin treatment to obese leptin-deficient animals has reversed their hyperthermia, neuroendocrine and immunological

*Abbreviations:* PCOS, polycystic ovary syndrome; SNP, single nucleotide polymorphism; LEPR, leptin receptor; IR, insulin resistance; FSH, follicle-stimulating hormone; E2, estradiol; LH, luteinizing hormone; PRL, prolactin; TSH, thyroid-stimulating hormone; DHEA-S, dehydroepiandrosterone sulfate; PCR-RFLP analysis, polymerase chain reaction-restriction fragment length polymorphism analysis; HWE, Hardy–Weinberg equilibrium; TNF-α, tumor necrosis factor α; PAI-1, plasminogen activator inhibitor-1; ADPOQ, adiponectin; NSCLC, non-small cell lung cancer; ADRB2, ß-2 adrenergic receptor.

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abnormalities (Mantzoros et al., 2011; Skibicka and Grill, 2009). It is worth noting that the role of leptin is supported by the universal distribution of leptin receptor (LEPR), and LEPR is selectively expressed in the central and peripheral tissues. LEPR localizes to chromosome 1p31 and the long form has 18 exons (Sun et al., 2010). LEPR, a single-transmembrane-domain receptor, shows structural similarity to the class I cytokine receptor family (Fruhbeck, 2006). Genetic association studies have shown that LEPR gene polymorphisms are closely related to the obesity, IR and dyslipidemia (Fruhbeck, 2006). Additionally, several genetic variants of the *LEPR* gene have been demonstrated that they are associated with metabolic diseases among different specific ethnic groups, including Lys109Arg, Gln223Arg, Ser343Ser, Ser492Thr, Lys656Asn, Ala976Asp, and Pro1019Pro (Matsuoka et al., 1997). Here, the aim of present investigation was to assess the association of the *LEPR* gene polymorphisms of Gln223Arg and Pro1019Pro in the case of Korean women with PCOS.

## 2. Materials and methods

### 2.1. Subjects

A total of 379 Korean women were recruited from the Fertility Center of the CHA General Hospital in Korea in the present study. Among them, 229 women had PCOS while the other 150 were healthy control subjects. Informed consent was obtained from all patients. The diagnosis of PCOS was based on the criteria proposed by the 2003 ASRM/ESHRE Rotterdam consensus. We used 100 ng/μl genomic DNA for PCR, and blood samples were collected in tubes containing EDTA as an anti-clotting factor and stored at −20 °C until use. Genomic DNA was extracted from the blood of PCOS patients and control women. We manually extract genomic DNA from blood, and amplified products were purified using Bioneer's AccuPrep PCR purification kit (http://us.bioneer.com/Protocol/AccuPrep%20PCR%20Purification%20Kit.pdf).

The study was approved by the Institutional Review Board. Blood samples were collected from normal controls and PCOS patients to analyze plasma follicle-stimulating hormone (FSH), estradiol ($E_2$), luteinizing hormone (LH), prolactin (PRL), thyroid-stimulating hormone (TSH), and dehydroepiandrosterone sulfate (DHEA-S).

### 2.2. Genetic analysis

Two SNPs of Gln223Arg and Pro1019Pro were genotyped by the restriction fragment length polymorphism (RFLP) analysis for all samples. The variant of Gln223Arg for LEPR was amplified using a forward primer 5′-ACC CTT TAA GCT GGG TGT CC-3′ and a reverse primer 5′-GAA GCC ACT CTT AAT ACC CCC-3′ by polymerase chain reaction (PCR). The cycling parameters for Gln223Arg were as follows: denaturation at 94 °C for 5 min, 30 cycles at 94 °C for 30 s, 57.8 °C for 30 s, 72 °C for 30 s, and finally at 72 °C for 5 min. The other variant Pro1019Pro of LEPR was amplified using a forward primer 5′-TGA GGC TGA GGG TAC TGA GGT A-3′ and a reverse primer 5′-GAA AGA ATC CGT CAA CGG AG-3′ by PCR. The cycling conditions for Pro1019Pro were as follows: denaturation at 94 °C for 5 min, 30 cycles at 94 °C for 30 s, 61.8 °C for 10 s, 72 °C for 15 s, and finally at 72 °C for 5 min. Amplification products were purified using Bioneer's AccuPrep PCR purification kit (Bioneer, Daejeon, Korea). The PCR fragment of Gln223Arg was 481 bp in length, and was digested with the enzyme *Msp* I (New England Biolabs, Beverly, MA, USA) for 6 h at 37 °C. Digestion of the allele G produced two fragments with a length of 291 bp and 190 bp. The resulting fragment of Pro1019Pro was 178 bp in length. The polymorphism was typed using the enzyme *Hinc* II at 37 °C for 6 h. Digestion of allele C produced two fragments with a length of 165 bp and 13 bp (Fig. 1). In the genotyping experiments for both SNPs, the digested DNA fragments were electrophoresed on a 2% agarose gels containing ethidium bromide and visualized by an ultraviolet transilluminator.

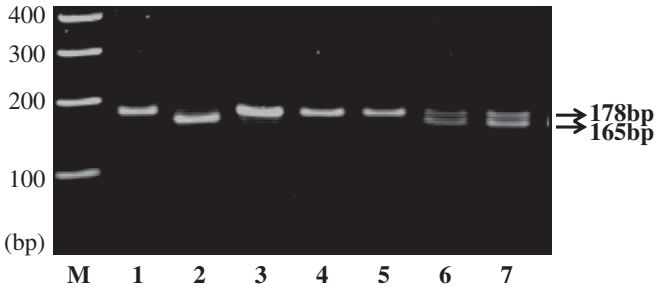


**Fig. 1.** RFLP analysis of the G/A polymorphism in exon 20 of the *LEPR* gene.

**Table 1**
Comparison of disorders/symptoms between normal controls (n = 150) and PCOS (n = 229).

| Characteristics | Controls (n = 150) | PCOS (n = 229) |
|---|---|---|
| Hyperandrogenism and oligo- or amenorrhea | n = 0 | n = 25 (10.9%) |
| Hyperandrogenism and polycystic ovaries | n = 0 | n = 23 (10.1%) |
| Oligo- or amenorrhea and polycystic ovaries | n = 0 | n = 153 (66.8%) |
| Hyperandrogenism, oligo- or amenorrhea and polycystic ovaries | n = 0 | n = 28 (12.2%) |

### 2.3. Statistical analysis

Statistical analysis was carried out using Hap Analysis (NGRI, Seoul, Korea; www.hap.ngri.re.kr) and GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA, US) and $\chi^2$ tests were used to analyze the association between PCOS and healthy controls. $P < 0.05$ was considered statistically significant. The HAPSTAT program (v.3.0, www.bios.unc.edu/~lin/hapstat/) was used for case–control haplotype analysis.

## 3. Result

Regarding the diagnostic criteria of PCOS, we followed the 2003 ASRM/ESHRE Rotterdam consensus. In accordance with the criteria, a total of 229 patients were diagnosed with PCOS when they exhibit two of the following three features: oligo- or amenorrhea, clinical or biochemical hyperandrogenism, and ultrasonographic polycystic ovarian morphology. Among 229 PCOS patients, 153 patients (68.2%) had

**Table 2**
Clinical and biochemical characteristics of normal controls (n = 150) and PCOS (n = 229).

| Characteristics | Controls (n = 150) | PCOS (n = 229) |
|---|---|---|
| Age (y) | 32.06 ± 3.20 (28–36) | 32.56 ± 3.57 (28–37) |
| Body mass index ($kg/m^2$) | 22.63 ± 3.15 (16.99–36.01) | 26.12 ± 5.72 (18.9–40.22) |
| Waist/hip ratio (WHR) | 0.75 ± 0.05 (0.65–1.2) | 1.26 ± 0.06 (1.11–2.27) |
| FSH levels (mIU/ml) | 8.78 ± 2.87 (1.62–22.87) | 5.96 ± 1.76 (2.22–12.25) |
| LH levels (mIU/ml) | 3.06 ± 1.56 (0.94–6.92) | 8.19 ± 6.63 (1.62–24.82) |
| Prolactin (ng/ml) | 11.98 ± 6.22 (3.84–46.14) | 13.16 ± 9.38 (2.04–71.94) |
| E2 (pg/ml) | 32.04 ± 14.7 (3.82–63.67) | 42.5 ± 34.15 (4.57–88.07) |
| TSH (μIU/ml) | 1.79 ± 0.89 (0.625–3.87) | 2.82 ± 1.79 (6.58–13.10) |
| DHEA-S (μg/dl) | 150.41 ± 52.84 (64.03–245.26) | 248.28 ± 95.94 (56.78–472.81) |
| Free testosterone (ng/ml) | 0.24 ± 0.14 (0.01–0.51) | 0.56 ± 0.26 (0.08–0.84) |

Numerical data were presented as means ± standard deviation (SD). Abbreviations: BMI, Body mass index: WHR, Waist–hip ratio: FSH, Follicle-stimulating hormone: LH, Luteinizing hormone: E2, Estradiol: TSH, Thyroid-stimulating hormone; DHEA-S, Dehydroepiandrosterone-sulfate.

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