

## Leptin receptor variant in women with polycystic ovary syndrome

The polycystic ovary syndrome (PCOS) is one of the most common abnormalities in women of reproductive age. It is characterized by menstrual irregularities, chronic anovulation, hirsutism, obesity, increased LH levels and androgen secretion, and insulin resistance.

Leptin is a protein hormone that is secreted almost exclusively by the adipocytes and acts by binding to their specific receptors. The main action of leptin is to regulate the eating behavior and energy metabolism (1). Recently, associations between leptin receptor gene mutations and obesity and diabetes have been shown in humans (2, 3).

Because PCOS is often associated with obesity and insulin resistance, we aimed to investigate the frequency of the leptin receptor gene variant, which is caused by the amino acid replacement Gln<sup>223</sup>Arg at exon 4, in women with PCOS.

Fifty-six women with PCOS and 58 healthy women (controls) were screened for the Gln<sup>223</sup>Arg mutation. Blood samples from these 114 women had been stored in our genetic bank. The institutional review board approved the study. Each woman underwent a screening history and physical examination. No woman had taken hormonal preparations for at least 3 months before study entry.

The polycystic ovary syndrome was diagnosed according to the following criteria: [1] bilateral polycystic ovaries on ultrasonography; [2] oligomenorrhea or amenorrhea, hirsutism, obesity, or chronic anovulation, or any combination of these conditions; [3] hyperandrogenemia (elevated serum levels of testosterone, free testosterone, or androstenedione); [4] elevated serum LH levels; and [5] exclusion of late-onset congenital adrenal hyperplasia and ovarian and adrenal tumors. All women with PCOS were euthyroid and had normal serum prolactin levels.

Controls were not hirsute or infertile, had normal-appearing ovaries on ultrasonography, and had normal serum androgen and LH levels. Women with PCOS had significantly higher baseline serum levels of LH, androstenedione, total testosterone, free testosterone, and insulin compared with controls.

Nuclear DNA was isolated from peripheral blood lymphocytes as described elsewhere (4). The 80–base pair (bp) fragment of the leptin receptor gene that encompasses the region of the Gln<sup>223</sup>Arg mutation was amplified by polymerase chain reaction (PCR) using sense and antisense oligonucleotide primers, 5′-AAA CTC AAC GAC ACT CTC CTT-3′ and 5′-TGA ACT GAC ATT AGA GGT GAC-3′, respectively (Intek Inc., Bursa, Turkey) (5).

After an initial denaturation cycle (94°C for 3 minutes), total genomic DNA was subjected to 35 cycles of denaturation (94°C for 1 minute), annealing (57°C for 1 minute), and extension (72°C for 1 minute) by using a thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT). The 50-μL reaction mixture contained 500 ng of genomic DNA, 10 pmol of each primer, 0.2 mM of deoxynucleoside triphosphate, 1.5 mM of MgCl<sub>2</sub>, 0.5 U of Taq DNA polymerase (MBI Fermentas Inc., New York, NY), 5.0 μL of PCR buffer (500 mmol/L of KCl, 100 mmol of Tris [hydroxymethyl]aminomethane chloride, and 0.8% Nonidet P40 [MBI Fermentas Inc.]). The PCR products were electrophoresed in 4% NuSieve agarose gel and visualized by ethidium bromide staining to confirm the presence of the specific 80-bp product. A negative control was used to show that no contamination occurred during DNA isolation and PCR analysis.

The mutation was screened by restriction enzyme analysis by using *MspI* (Promega Corp., Madison, WI) in all PCR products. Because the Gln<sup>223</sup>Arg mutation in the leptin receptor gene creates the recognition site of *MspI*, PCR products were digested with this restriction enzyme (5). One unit of the enzyme was added to the PCR reaction mixture, and the mixture was incubated overnight at 37°C for *MspI* digestion. An aliquot of the mixture then was examined by 4% NuSieve agarose gel electrophoresis and visualized by ethidium bromide staining.

*MspI* digestion in persons who are homozygous for the normal leptin receptor results in one band of 80 bp. In the presence of the mutation, since the recognition site of the enzyme is created, two bands of 58 and 22 bp are observed in homozygotes and three bands of 80, 58, and 22 bp are observed in heterozygotes (Fig. 1).

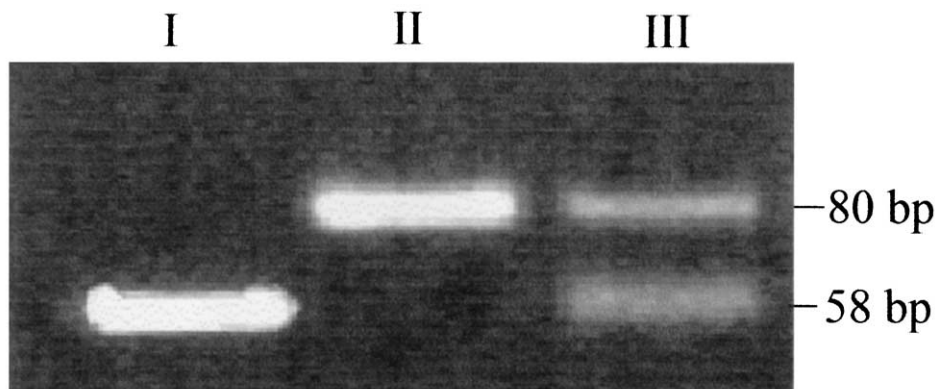
Thirteen (23%) of 56 women with PCOS were homozygous for the mutation, 24 (43%) were heterozygous, and 19 (34%) were homozygous for the wild-type leptin receptor gene. Among the controls, 7 (12%) were

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**FIGURE 1**

Genotyping of the human leptin receptor gene. Lane I: patients who were homozygous for the mutation. Lane II: patients who were homozygous for the normal genotype. Lane III: patients who were heterozygous for the mutation. Sizes of DNA fragments are indicated on the right side of the figure.



Erel. *Leptin receptor variant in women with PCOS. Fertil Steril* 2002.

homozygous for the Gln<sup>223</sup>Arg mutation, 30 (52%) were heterozygous, and 21 (36%) were homozygous for the wild-type leptin receptor gene. The frequency of this mutation did not significantly differ between women with PCOS and controls ( $\chi^2=2.53$ ;  $P=.28$ ).

We performed a power analysis for the probability of detecting a true difference. The power of the study was 0.56 when we were willing to accept a type I error of 0.05 and an increase in the rate of mutation from 12% in the control group to 23% in the PCOS group. The power is low because the samples in each group were smaller to detect the presence of a reasonable difference, at which the sample sizes were calculated to be at least 249 cases in each group for a type II error of 0.2 or less. However, our main objective was to document the frequency rate of mutation of this type in women with PCOS and thus provide basic knowledge for future studies. Therefore, statistical comparison of the frequency rate of mutation in PCOS in controls should not carry further importance.

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