An Inactivating Mutation of the Luteinizing Hormone Receptor Causes Amenorrhea in a 46,XX Female*

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ABSTRACT

Hypergonadotropic hypogonadism is characterized by decreased gonadal function due to the inability of the gonads to respond to pituitary gonadotropins. Hypergonadotropic hypogonadism in females has many causes, among which are ovarian dysgenesis and abnormalities of the ovarian receptors for the pituitary gonadotropins. We evaluated a woman who presented with amenorrhea due to hypergonadotropic hypogonadism, but who had structurally normal ovaries. She is a sister of two previously identified 46,XY male pseudohermaphrodites in a pedigree (Fig. 1) that was previously analyzed by us (4). The two male pseudohermaphrodites have Leydig cell hypoplasia. Injection of hCG did not cause any change in plasma levels of estradiol or progesterone, suggesting complete ovarian resistance to LH. Analysis of the DNA sequence of the LH receptor gene revealed that the patient is homozygous for the same single base change as her two brothers. This mutation causes substitution of an alanine residue by a proline at position 593. In vitro analysis of the mutant LH receptor in cultured human embryonic kidney 293 cells documented that the receptor is unable to stimulate adenyl cyclase in response to hCG. Plasma levels of estradiol and progesterone were low, whereas LH and FSH levels were increased. On histological analysis of the ovary, follicles were seen at all developmental stages. Nonetheless, primary amenorrhea had been present for 5 yr, and repeated measurements of plasma estradiol and progesterone indicate that ovulation does not occur. These results document the existence of inherited LH resistance as a cause of primary amenorrhea in women. The combined clinical and molecular observations are consistent with previous experimental data suggesting that in humans, LH is necessary for ovulation but follicular maturation can occur in the presence of FSH alone. (J Clin Endocrinol Metab 81: 3850–3854, 1996)

THE PITUITARY hormones FSH and LH have an essential role in the regulation of gonadal function (1, 2). In the cycling ovary, the growth of small follicles is dependent on FSH. Some small antral follicles develop into preovulatory (or Graafian) follicles. Ovulation is subsequently induced by a peak in the level of circulating LH. The pituitary secretion of LH and FSH is negatively controlled by the gonadal hormones estradiol and progesterone. In hypergonadotropic hypogonadal patients, the ovary does not respond to LH or FSH with the secretion of steroid hormones, and the absence of the negative feedback results in elevated levels of these gonadotropins (3). In this report we describe a patient with amenorrhea and infertility due to hypergonadotropic hypogonadism. She is a sister of two 46,XY male pseudohermaphrodites in a pedigree (Fig. 1) that was previously analyzed by us (4). The two male pseudohermaphrodites have Leydig cell hypoplasia due to a homozygous inactivating mutation in the LH receptor gene. This suggested that the primary amenorrhea and infertility in the present patient might also be caused by this mutation. Indeed, plasma levels of estradiol and progesterone were low, suggesting the absence or hypofunction of ovarian follicles with regard to LH-dependent hormone production. Histological examination of ovarian tissues revealed complete follicular development, suggesting uncompromised response to FSH. The subject was, therefore, evaluated for an inherited defect of the ovarian receptor for LH. DNA analysis revealed a homozygous nucleotide change in the LH receptor gene, rendering the LH receptor molecule unable to stimulate cAMP production upon stimulation with hCG. We conclude that inherited complete ovarian resistance to LH causes anovulation in the presence of normal follicular maturation.

Subjects and Methods

The patient

A 21-yr-old woman was referred for evaluation of amenorrhea and infertility. She is 1 of 25 siblings born to consanguineous parents (Fig. 1). Normal menses never occurred. From the age of 16 yr, irregular spotting occurred on the average every 6 months. Gynecological examination revealed a normal-sized vagina with thin walls and no secretory function. Bone mass measured by bone densitometry was significantly reduced. The patient was otherwise healthy and had normal development, with full maturation of primary and secondary characteristics. Pelvic ultrasonography revealed an enlarged right ovary (22 cm³) with a large cyst. The left ovary was of normal size (5 cm³). The uterus was small, but of normal shape. Uterine volume was determined by ultrasonography to be 20 cm³ (normal uterine volume at menarche, 25–90 cm³).
Hormonal evaluation

Progesterone, estradiol, and cortisol were measured by an immunofluorometric assay. 17-Hydroxyprogesterone, androstenedione, dehydroepiandrosterone (DHEA), DHEA sulfate, and testosterone were determined using RIAs. LH and FSH were determined using an immunochemiluminescence assay (Delphia, Wallac, Finland). Normal hormone levels are indicated in the table. GnRH-induced increases in plasma LH and FSH were determined 1 h after an iv injection of 100 μg GnRH (Ayerst Laboratories, Rouse Point, IL). For the ACTH challenge test, plasma hormone levels were measured 1 h after the injection of 250 μg Cosyntropin (Organon, Oss, The Netherlands). The hCG sensitivity of steroid hormone production was evaluated 48 h after a single injection of 6000 IU Pregnyl (Organon).

Ovarian histology

An ovarian biopsy was obtained during video laparoscopy. Tissue was fixed in buffered 4% p-formaldehyde, paraffin-embedded, sectioned, and stained with hematoxylin-eosin. All procedures were carried out in the course of normal patient care after appropriate informed consent had been obtained.

DNA analysis and receptor expression

DNA fragments were amplified with PCR from genomic DNA isolated from peripheral blood (5) using primers previously described (4).

Fig. 1. Pedigree of family. The pedigree of this family reveals consanguinity of the parents of the patient. The patient's two 46,XY siblings with Leydig cell hypoplasia are also indicated (square with a dot).

Table 1. Hormone levels

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>hCG</th>
<th>ACTH</th>
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</thead>
<tbody>
<tr>
<td>LH (IU/L)</td>
<td>10 (0.95–8.4)</td>
<td>10 (0.95–8.4)</td>
<td>35 (7.6–31.7)</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>8.7 (1.7–8.9)</td>
<td>8.7 (1.7–8.9)</td>
<td>11 (4.6–11.7)</td>
</tr>
<tr>
<td>Progesterone (nmol/L)</td>
<td>2.0 (1.2–7.0)</td>
<td>2.0 (1.2–7.0)</td>
<td>6.35 (4.3 ± 1.8)</td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td>270 (130–500)</td>
<td>270 (130–500)</td>
<td>34.7 (27.4 ± 16)</td>
</tr>
<tr>
<td>17-hydroxyprogesterone (nmol/L)</td>
<td>2.4 (0.7–3.3)</td>
<td>2.4 (0.7–3.3)</td>
<td>3031</td>
</tr>
<tr>
<td>DHEA (nmol/L)</td>
<td>22.9 (6.2–20.1)</td>
<td>22.9 (6.2–20.1)</td>
<td>12.13</td>
</tr>
<tr>
<td>DHEA sulfate (nmol/L)</td>
<td>1587 (1104–8956)</td>
<td>1587 (1104–8956)</td>
<td>34.7 (27.4 ± 16)</td>
</tr>
<tr>
<td>Androstenedione (nmol/L)</td>
<td>3.49 (2.0–6.6)</td>
<td>3.49 (2.0–6.6)</td>
<td>3.84</td>
</tr>
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Amplified DNA fragments were isolated after agarose gel electrophoresis and sequenced using the cycle sequencing kit from BRL (Life Technologies, Grand Island, NY). Human embryonic kidney 293 cells were transfected with LH receptor expression constructs (6) and, after 48 h, treated for 2 h with hCG. Binding of [125I]hCG and cAMP production were measured as described previously (4).

Results

Evaluation of the pituitary-gonadal axis indicates LH resistance

Determination of plasma hormones revealed low normal to normal levels of estradiol, progesterone, 17-hydroxyprogesterone, DHEA, androstenedione, and testosterone (Table 1). Estradiol and progesterone levels were 240 pmol/L (range, 84–447; n = 6) and 2.0 nmol/L (range, 0.6–3.8; n = 3), respectively. To exclude deficiencies in the steroidogenic enzymes, an ACTH challenge test was performed. Cortisol and the adrenal androgens showed a normal response (Table 1). In contrast, hCG injection did not induce any response in the plasma levels of the steroid hormones measured (Table 1), leading to the diagnosis of LH resistance. Plasma levels of LH and FSH were elevated and responded normally to a GnRH challenge (Table 1). Serum inhibin and PRL levels were within the normal range.

Ovarian histology

Histological analysis of an ovarian biopsy showed all stages of follicular development, including primordial follicles, preantral follicles with oocytes and a few granulosa cell layers, and follicles with a large antrum and well developed thecal cell layer. In these antral follicles, the thecal cells had a luteinized appearance, whereas the granulosa cells in the same follicle did not show any signs of luteinization (Fig. 2A). Other areas of the ovarian biopsy contained multiple primordial follicles (Fig. 2B). No preovulatory follicles, corpora lutea, or corpora albicands were present.

Mutation in the LH receptor gene

DNA sequence analysis of the sixth transmembrane segment and flanking regions of the LH receptor gene was performed, which indicated a homozygous single nucleotide change of guanine to cytidine at position 1787 of the gene (Fig. 3). This mutation is identical to that previously identified in the patient's two 46,XY brothers with Leydig cell hypoplasia (4) and causes substitution of a proline residue for alanine 593 at the border of the sixth transmembrane segment and the third extracellular loop of the receptor mol-
Fig. 2. Histology of ovarian biopsy. A, A partially collapsed antral follicle with granulosa cells (short arrow) and thecal cells (long arrow). The thecal cells have a luteinized appearance. The adjacent granulosa cells do not show luteal differentiation. B, A group of primordial follicles. Areas containing primordial follicles with a normal appearance were found near the ovarian cortex (hematoxylin-eosin staining; magnification, ×400).

cule. The father of these patients carried both the mutated and wild-type alleles of the LH receptor gene (Fig. 3).

In *in vitro* transfection experiments, the effect of this mutation on LH receptor function was determined. 293 cells transfected with the wild-type LH receptor expression construct responded to hCG treatment with a more than 300-fold increase in cAMP production (Fig. 4). In contrast, cells transfected with the mutant LH receptor expression plasmid did not respond (Fig. 4). Ligand binding to cells transfected with mutant receptors was reduced. To investigate whether this low binding is the cause of the inability of these receptors to couple to the intracellular Gs protein, 293 cells were transfected with decreasing amounts of wild-type LH receptor expression plasmid (Fig. 4). At 0.1 µg DNA, a robust hCG-induced cAMP response was found, although the cells showed hCG binding similar to that of cells transfected with 10 µg mutant LH receptor expression plasmid (Fig. 4).

**Discussion**

Hypergonadotropic hypogonadism is caused by the absence of the normal gonadal response to the pituitary hormones LH and FSH (1). This syndrome has many causes, among which chromosomal abnormalities such as Turner's syndrome are frequent (1, 3). However, in subjects with a normal karyotype, gonadotropin receptor defects could explain the reduced gonadal activity. Recently, an inactivating FSH receptor gene mutation was described in families with a hereditary form of ovarian dysgenesis (7, 8). In these women, the inability of the ovaries to respond to FSH resulted in the absence of follicle growth, low gonadal hormone production, and variably developed secondary sex characteristics in the presence of high levels of FSH and LH.

The patient described in this report presented with primary amenorrhea and infertility. The presence of two 46,XY
siblings with hypoplasia of Leydig cells in her pedigree prompted us to screen for LH receptor mutations. Indeed, homozygosity for the same mutation was found in both the patient with amenorrhea and infertility and her two siblings.

![DNA sequence](image)

**Fig. 3.** LH receptor mutation in a woman with hypergonadotropic hypogonadism. The DNA sequence surrounding nucleotide 1787 of the LH receptor gene is shown for the patient, her father, and a normal control. Note the homozygous conversion of G\textsubscript{1787} to C in the patient. The father is heterozygous for this mutation and carries both the G\textsubscript{1787} and C\textsubscript{1787} alleles.

with Leydig cell hypoplasia. Expression of the mutant LH receptor revealed that the receptor was unable to couple the hCG signal to cAMP production. Furthermore, this receptor is poorly expressed at the plasma membrane, as indicated by the low hCG binding, but this low expression is not the cause of the absence of coupling. The Ala\textsuperscript{593} to Pro change in the sixth transmembrane segment probably confers aberrant folding of the receptor molecule during its synthesis and modification in the endoplasmic reticulum and Golgi apparatus. This may result in faulty trafficking and subsequent low expression at the plasma membrane.

The ovarian LH resistance in this case is associated with normal development of female internal and external genitalia and infertility. Follicular development also occurs normally, at least up to the antral stage. However, ovulation does not occur, as borne out by the history of amenorrhea and the absence of signs of ovulation in the biopsy sample. The small size of the uterus as well as the presence of a thin walled hyposecreatory vagina in a subject with reduced bone mass suggest that this patient has had a long standing hypoestro-
genization. Neither estradiol nor progesterone, measured at six different time points, reached levels found during the ovulatory or luteal phase of the cycle in normal women. Because the FSH signal is unimpaired, conversion of androgens by FSH-induced aromatase activity in the growing follicles may explain the residual estradiol in plasma, which was in the borderline to low normal range for the follicular stage of the cycle. Whether these androgens originate from the adrenal or represent basal ovarian production cannot be determined, and a dexamethasone suppression test was not performed.

The presence of estradiol explains the relatively minor increase in plasma gonadotropin hormone concentrations compared to the very high LH and FSH levels found in ovarian dysgenesis caused by the FSH receptor mutation (8). The absence of follicular stimulation in these FSH receptor-deficient patients results in much lower estradiol production. The lack of FSH-induced inhibin removes the negative feedback effect on gonadotropin levels (3, 8).

Amenorrhea has previously been noted in female siblings of 46,XY male hermaphrodites with Leydig cell hypoplasia (9). The present results indicate that such patients have inherited unresponsiveness to LH as the basis for their amenorrhea. After submission of this manuscript, a woman was described with an inactivating mutation of the LH receptor gene that leads to a truncation of the LH receptor protein (10). The data in this report confirm the hormonal and LH receptor expression experiments described here (10). The fact that male pseudohermaphroditism and female amenorrhea are caused by the same genetic defect allows a tentative estimate of the frequency of inherited resistance to LH in females. At least 20 cases of Leydig cell hypoplasia have been reported in the literature, and many more have probably been erroneously diagnosed as androgen sensitivity (testicular feminization). Therefore, inherited resistance to LH should be considered in all amenorrheic females who fail to respond to hCG injection with an increase in steroid hormone levels.

Hypogonadism also occurs in cases with disrupted LH or FSH β-subunit genes. An inherited form of LH β-subunit inactivity was found in a man with hypogonadism (11). As none of the female siblings was homozygous for this mutation, the effect of genetic absence of LH on female function is unknown. Other genetic variant forms of LH have been reported, but these were without any obvious clinical effect (12). Primary amenorrhea and infertility associated with very low plasma estrogen levels and poorly developed secondary sex characteristics were observed in a patient with absence of FSH due to a mutation in the FSH β-subunit gene (13).

The endocrine observations in this case are comparable to the findings in ovarian dysgenesis (8), underscoring the idea that defects in the FSH pathway of ovarian stimulation have more severe clinical consequences than do changes in the LH receptor signal transduction pathway.

In conclusion, we show that complete ovarian resistance to LH due to a homozygous inactivating mutation in the LH receptor gene, results in amenorrhea and anovulation in the presence of normal female sex differentiation and follicular development. In humans, LH is necessary for ovulation, but normal follicular maturation is possible in the presence of FSH alone.

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References