

## BRIEF REPORT

## A Chorionic Gonadotropin–Sensitive Mutation in the Follicle-Stimulating Hormone Receptor as a Cause of Familial Gestational Spontaneous Ovarian Hyperstimulation Syndrome

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**S**PONTANEOUS OVARIAN HYPERSTIMULATION SYNDROME IS A RARE EVENT, in contrast to iatrogenic ovarian hyperstimulation syndrome, which is induced with the use of gonadotropins in assisted reproductive medicine. The pathological features of the syndrome, whether spontaneous or iatrogenic, include the presence of multiple serous and hemorrhagic follicular cysts lined by luteinized cells, a condition called hyperreactio luteinalis.<sup>1</sup> The pathogenesis of the syndrome and its systemic manifestations are poorly understood.<sup>2–6</sup> We describe a familial case of recurrent spontaneous ovarian hyperstimulation syndrome. The affected women were heterozygous for a mutation in the transmembrane domain of the gene encoding the follicle-stimulating hormone receptor. In vitro characterization of the mutated receptor revealed that its specificity had decreased, allowing human chorionic gonadotropin as well as follicle-stimulating hormone to stimulate it.

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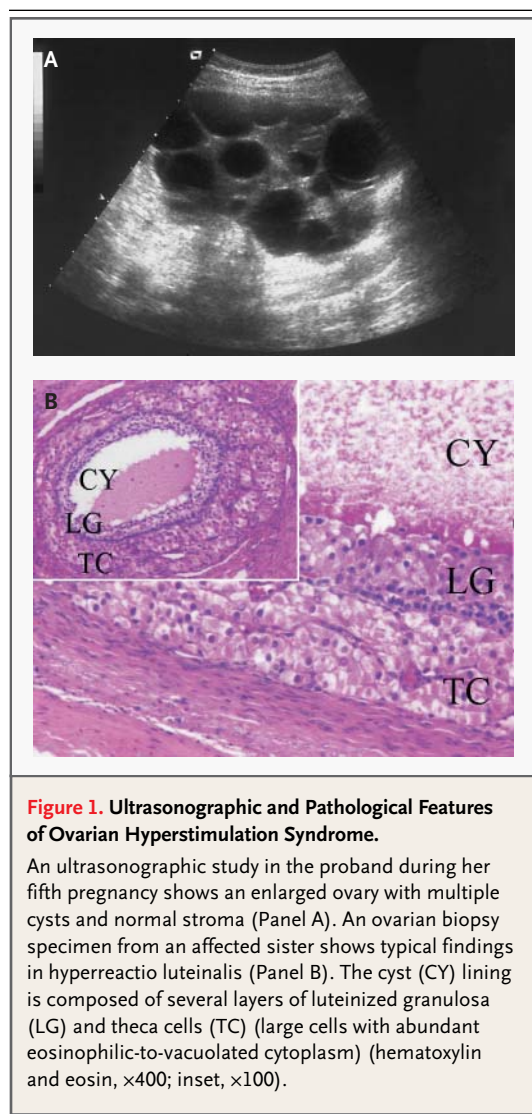
## CASE REPORT

The proband, a 25-year-old woman of Moroccan origin, was admitted to the hospital because of ovarian hyperstimulation syndrome during her fifth spontaneous pregnancy. All but one of her pregnancies had been complicated by this syndrome, which was first suspected because of abdominal pain and nausea after a therapeutic abortion carried out at eight weeks' gestation to terminate her first pregnancy. An ultrasonographic examination at that time revealed bilateral multilocular ovarian masses and ascites. Laparoscopy, performed 21 days after the abortion, confirmed the presence of a moderate amount of ascitic fluid (approximately 1 liter) and huge polycystic ovaries. Her second pregnancy ended in a miscarriage at six weeks, without evidence of ovarian hyperstimulation syndrome. During her third pregnancy, the syndrome was detected at eight weeks' gestation and lasted throughout the pregnancy. At 38 weeks, she delivered a baby girl, who was small (2450 g) for her gestational age. The fourth pregnancy, complicated by ovarian hyperstimulation syndrome, was terminated by therapeutic abortion at nine weeks.

The patient was referred to us during the eighth week of her fifth pregnancy with acute lower abdominal pain and nausea. Ultrasonographic examination revealed enlarged, multilocular ovaries measuring 14 cm in length (Fig. 1A). Hematologic laboratory values and the results of routine liver-, kidney-, and thyroid-function tests were nor-

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**Figure 1. Ultrasonographic and Pathological Features of Ovarian Hyperstimulation Syndrome.**

An ultrasonographic study in the proband during her fifth pregnancy shows an enlarged ovary with multiple cysts and normal stroma (Panel A). An ovarian biopsy specimen from an affected sister shows typical findings in hyperreactio luteinalis (Panel B). The cyst (CY) lining is composed of several layers of luteinized granulosa (LG) and theca cells (TC) (large cells with abundant eosinophilic-to-vacuolated cytoplasm) (hematoxylin and eosin,  $\times 400$ ; inset,  $\times 100$ ).

mal, as was the serum level of the  $\beta$  subunit of chorionic gonadotropin. The follicle-stimulating hormone was suppressed, as expected, during pregnancy. The level of inhibin B, which was markedly and inappropriately elevated, later declined in parallel with the chorionic gonadotropin level. The level of estradiol exceeded the upper limit of the normal range during the first trimester, stabilized within the normal range during the second trimester, and increased further, as is usual, during the third trimester. There was a moderate increase in the total testosterone level due to an increase in the level of sex hormone-binding globulin; the level of free testosterone was normal (Table 1). The ovaries remained enlarged throughout the pregnancy. The

patient delivered a normal baby girl. The patient, who reported having had regular menses between pregnancies, refused any gynecologic examination after the delivery.

#### FAMILY HISTORY

Two of the patient's four sisters reported similar histories, although the occurrence of ovarian hyperstimulation syndrome was not consistent among them. In one, the syndrome had occurred only during the four most recent of seven pregnancies, and it always began during the first trimester. A laparotomy, performed during the fourth pregnancy, showed enlarged ovaries and ascites. The other affected sister had had moderate ovarian hyperstimulation syndrome during the two most recent of four pregnancies, prompting a laparotomy that revealed enlarged ovaries and ascites on each occasion. Despite resection of a large ovarian wedge (500 g) bilaterally during the first laparotomy, enlarged ovaries (15 cm in length) were found on laparotomy during the subsequent pregnancy (Fig. 1B). A third sister had had two unremarkable pregnancies. The other members of the family declined to be interviewed.

The study was approved by the review boards of the participating institutions, and written informed consent was obtained from the proband and participating family members.

#### METHODS

##### HORMONE ASSAYS

Estradiol and total and free testosterone were measured by radioimmunoassays (DiaSorin, Immuno-*tech*–Beckman Coulter, and Dade Behring, respectively); chorionic gonadotropin was measured by electrochemiluminescence immunoassay (Elecsys 2010, Roche Diagnostics); inhibin B was measured by enzyme-linked immunosorbent assay (Argene Biosoft) as previously described,<sup>7</sup> with cross-reactivity with inhibin A below 1 percent; sex hormone-binding globulin was measured by immunoradiometric assay (CIS bio international–Schering); and follicle-stimulating hormone was measured by a time-resolved fluorescence assay (Perkin Elmer).

##### DNA SEQUENCING

DNA was extracted from peripheral-blood leukocytes. The 10 exons of the gene encoding the human follicle-stimulating hormone receptor were sequenced on both strands with the use of a Taq di-

**Table 1. Laboratory Values during the Proband's Fifth Pregnancy.\***

Variable	First Trimester			Second Trimester			Third Trimester	
	8 wk	12 wk	14 wk	16 wk	18 wk	26 wk	30 wk	40 wk
Human chorionic gonadotropin, $\beta$ subunit (U/liter) <sup>†</sup>	60,429	50,888	42,269	26,037	17,308	4850	5,841	15,486
Inhibin B (ng/liter) <sup>‡</sup>	401	230	225	—	96	8	33	80
Follicle-stimulating hormone (U/liter) <sup>§</sup>	0.025	0.027	0.003	—	0.004	0.036	0.024	0.080
Estradiol (ng/liter) <sup>¶</sup>	3,510	6,050	8,390	—	8,010	8990	10,520	13,870
Testosterone								
Total ( $\mu$ g/liter) <sup>  </sup>	2	3.9	4	3.9	5	2.6	1.5	1.6
Free ( $\mu$ g/liter) <sup>**</sup>	—	0.0036	—	0.0033	0.0035	0.0011	0.0007	0.0026
Sex hormone-binding globulin (nmol/liter) <sup>††</sup>	—	411	—	486	—	500	520	600

\* To convert the values for estradiol to picomoles per liter, multiply by 3.671. To convert the values for testosterone to nanomoles per liter, multiply by 3.467. The respective intraassay and interassay coefficients of variation were 2.8 percent and 3.2 percent for chorionic gonadotropin; 4.2 percent and 9.4 percent, 5.7 percent and 10.9 percent, and 7.4 percent and 12.3 percent for inhibin B at 225, 112, and 44 ng per liter, respectively; 2.0 percent and 1.8 percent for follicle-stimulating hormone; 2.7 percent and 8.3 percent, 2.9 percent and 6.8 percent, and 3.6 percent and 10.9 percent for estradiol at 193, 68, and 12 ng per liter, respectively; 7.0 percent and 8.2 percent for total testosterone; 4.3 percent and 7.4 percent for free testosterone; and 5.2 percent and 5.3 percent for sex hormone-binding globulin. The lower limit of detection of the assay for follicle-stimulating hormone was 0.01 U per liter.

<sup>†</sup> The normal range (according to the manufacturer) is 31,000 to 184,000, 14,300 to 75,800, and 3900 to 49,400 U per liter at 8, 14, and 18 weeks, respectively.

<sup>‡</sup> The normal value (in a cohort of 467 pregnant women, followed until normal delivery [Lahlou N: personal communication]) is less than 6 ng per liter during the first and second trimesters, less than 25 ng per liter at 30 weeks, and less than 40 ng per liter at 40 weeks.

<sup>§</sup> The normal value (in a cohort of 467 pregnant women, followed until normal delivery [Lahlou N: personal communication]) is less than 0.03 U per liter.

<sup>¶</sup> The normal range (in a cohort of 467 pregnant women, followed until normal delivery [Lahlou N: personal communication]) is 320 to 2300, 710 to 5000, 1000 to 6700, 2000 to 11,500, 3600 to 20,000, 4400 to 23,000, and 8500 to 31,000 ng per liter at 8, 12, 14, 18, 26, 30, and 40 weeks, respectively.

<sup>||</sup> The normal range (according to the manufacturer) is 0.1 to 0.6  $\mu$ g per liter.

<sup>\*\*</sup> The normal range (according to the manufacturer) is 0.00004 to 0.0039  $\mu$ g per liter.

<sup>††</sup> The normal range (according to the manufacturer) is 68 to 680 nmol per liter.

deoxy terminator cycle-sequencing kit and an automated sequencer (373A, Applied Biosystems), as previously described.<sup>8</sup>

#### CONSTRUCTION OF AN EXPRESSION VECTOR ENCODING THE MUTATED FOLLICLE-STIMULATING HORMONE RECEPTOR

Cloning of complementary DNA corresponding to the human follicle-stimulating hormone receptor gene into the pSG5 expression vector (pSG5-hFSHR) has been described elsewhere.<sup>8</sup> A mutation was introduced into pSG5-hFSHR by site-directed mutagenesis with the use of the polymerase chain reaction (PCR). A restriction fragment encompassing the mutated site was ligated to pSG5-hFSHR. The construct was verified by double-strand sequencing.

#### FUNCTIONAL STUDIES OF THE MUTATED RECEPTOR

COS-7 cells (a cell line derived from fibroblasts of the African-green-monkey kidney) were transfected with plasmid encoding mutated or wild-type follicle-stimulating hormone receptor and luteinizing hormone receptor, as previously described.<sup>8,9</sup> The efficiency of transfection was assessed by immunocytochemistry, and cell-surface expression of the follicle-stimulating hormone receptor was quantified with the use of a biotinylated monoclonal antibody, FSHR323, and iodine-125-labeled streptavidin.<sup>8</sup> The accumulation of cyclic AMP (cAMP) was measured as previously described<sup>8</sup> after incubation of the transfected COS-7 cells with recombinant human follicle-stimulating hormone (Gonal-f, Serono), purified human chorionic gonadotropin (Or-

ganon), and purified bovine thyroid-stimulating hormone (Sigma-Aldrich). Hormone binding assays were performed as described.<sup>8</sup> The dissociation constant and maximal binding capacity were calculated from Scatchard plots. The results of expression assays were analyzed by Student's *t*-test and the Mann-Whitney test; the results of hormone-binding and stimulation experiments were analyzed by analysis of variance and Mann-Whitney tests.

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## RESULTS

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### IDENTIFICATION OF THE MUTATION

Direct sequencing of the PCR products amplified from genomic DNA from the proband revealed a heterozygous substitution of a thymidine for a cytosine in exon 10, resulting in the substitution of isoleucine for threonine at position 449 of the follicle-stimulating receptor protein (Fig. 2A). This residue, located in the upper part of the third transmembrane domain of the receptor, is highly conserved among the receptors for follicle-stimulating hormone, thyroid-stimulating hormone, and luteinizing hormone in different species. The substitution was not detected in the DNA of 500 patients from France or North Africa who had been referred to us for unexplained hypergonadotropic infertility (unpublished data). The mutation was also found in the DNA of the two affected sisters but not in that of the third unaffected sister.

### FUNCTIONAL STUDIES OF THE MUTANT RECEPTOR

The transfection efficiency and cell-surface expression of the wild-type follicle-stimulating hormone receptor were similar to those of the mutant receptor in five independent experiments (mean [ $\pm$ SE] binding of FSHR323, 4530 $\pm$ 421 and 4640 $\pm$ 557 counts per minute, respectively;  $P=0.87$ ). There was no significant difference between the basal level of cAMP produced by the wild-type receptor and that produced by the mutant receptor. The responses of the two receptors to recombinant follicle-stimulating hormone were not statistically different in five experiments ( $P>0.13$ ). The doses of follicle-stimulating hormone producing a response halfway between base line and maximum (the EC<sub>50</sub>) were 22.4 $\pm$ 6.3 and 18 $\pm$ 6.8 U per liter for the wild-type and mutated receptors, respectively (Fig. 2B). In four experiments, the binding of follicle-stimulating hormone to the wild-type receptor was similar to its binding to the mutant receptor (dissociation constant, 5.5 $\pm$ 0.77 and 2.4 $\pm$ 0.14 nmol per liter, respec-

tively [ $P=0.25$ ]; maximal binding capacity, 55.6 $\pm$ 12.8 and 77.9 $\pm$ 17.9 pmol per liter, respectively [ $P>0.35$ ]). A dose-dependent increase in cAMP in response to chorionic gonadotropin was observed with the mutant receptor, whereas the wild-type receptor was insensitive to chorionic gonadotropin, except at a high concentration (1000 U per milliliter). In four experiments, the EC<sub>50</sub> of chorionic gonadotropin was 36.3 $\pm$ 1 U per milliliter for the mutant follicle-stimulating hormone receptor and 0.26 $\pm$ 0.07 U per milliliter for the wild-type luteinizing hormone receptor (Fig. 2C). Stimulation with bovine thyroid-stimulating hormone (up to 10 mU per milliliter) had no effect.

Thus, the mutated follicle-stimulating hormone receptor had lost specificity only for gonadotropins. No direct binding of iodine-125-labeled chorionic gonadotropin to the mutated receptor could be detected, in agreement with the low affinity detected in the stimulation experiments, and iodine-125-labeled follicle-stimulating hormone was not displaced by chorionic gonadotropin (data not shown).

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## DISCUSSION

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Little is known about the pathophysiology of the ovarian hyperstimulation syndrome.<sup>2-6</sup> Both the spontaneous and the iatrogenic forms of the syndrome involve a unique pathologic entity called hyperreactio luteinalis.<sup>1</sup> However, the onset of ovarian hyperstimulation syndrome is variable,<sup>4,10,11</sup> and its relation to the level of human chorionic gonadotropin is inconsistent.<sup>4,12</sup> Multiple putative risk factors have been proposed as an explanation.<sup>2-6,10</sup>

We found that a mutation in the follicle-stimulating hormone receptor leads to hypersensitivity to chorionic gonadotropin and that the mutation segregated with ovarian hyperstimulation syndrome in this family. The onset and evolution of symptoms, which coincided with the usual gestational time course of fluctuation in chorionic gonadotropin levels, and the parallel levels of inhibin B suggest that chorionic gonadotropin was a trigger for ovarian hyperstimulation syndrome in this family. Few mutations in the follicle-stimulating hormone receptor,<sup>13</sup> and only one resulting in a gain of function,<sup>14</sup> have been reported. This mutation broadens the specificity of the receptor so that it responds to another ligand, chorionic gonadotropin.

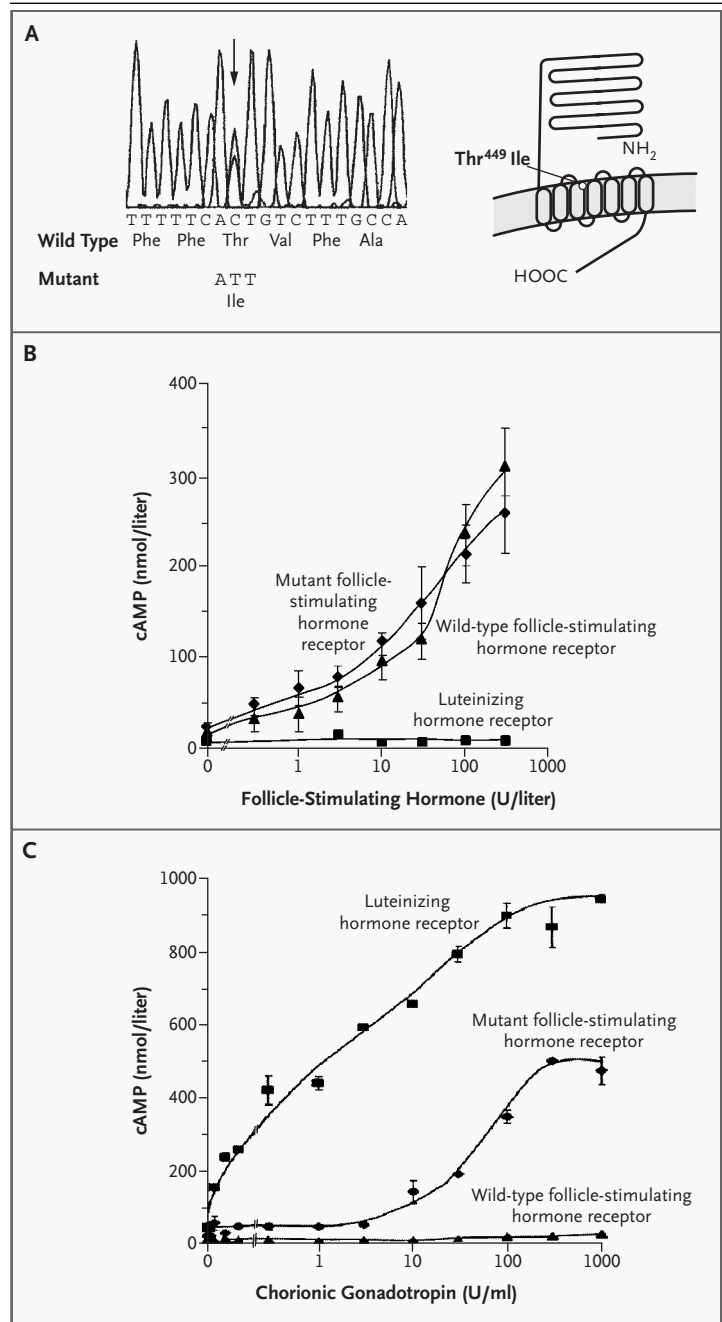
Alteration of the specificity of the follicle-stimulating hormone receptor by a mutation of a highly conserved residue located in the serpentine domain

**Figure 2. Sequencing and Functional Studies of the Wild-Type and Mutant Follicle-Stimulating Hormone Receptors.**

Panel A shows the sequence of exon 10 of the follicle-stimulating hormone receptor in the proband; the arrow indicates the heterozygous mutation at position 449. Also shown is a diagram of the receptor relative to the cell membrane; the mutated residue is located in the third transmembrane domain. Panel B shows the stimulation by follicle-stimulating hormone of cyclic AMP (cAMP) accumulation with the wild-type follicle-stimulating hormone receptor, the mutant follicle-stimulating hormone receptor, and the luteinizing hormone receptor. Each point represents the mean ( $\pm$ SE) of five independent experiments, except for the points on the curve for the luteinizing hormone receptor, which are the means ( $\pm$ ranges) of duplicate determinations in one experiment. Panel C shows the stimulation by chorionic gonadotropin of cAMP accumulation with the wild-type follicle-stimulating hormone receptor, the mutated follicle-stimulating hormone receptor, and the luteinizing hormone receptor. The data reflect one representative experiment of four and are presented as the means ( $\pm$ ranges) of duplicate determinations.

of the receptor, the threonine residue at position 449, is an unexpected finding. The ligand binds to the extracellular domain of the receptor, whereas the serpentine domain is usually regarded as the portion of the receptor responsible for the coupling to G proteins.<sup>13</sup> Discrimination among ligands by gonadotropin receptors depends on a combination of positive and negative determinants of the extracellular domain.<sup>15</sup> Accumulating data indicate that the extracellular and serpentine domains of this subclass of G-protein-coupled receptors interact<sup>16,17</sup> and that residues of the transmembrane domain and extracellular loops are involved in the modulation of hormone binding.<sup>18,19</sup> It is unlikely that conserved residue 449 specifically interacts with the ligand. It is more likely that the mutation induces a conformational change that affects the extracellular loops or the extracellular domain.

The mutant receptor has no apparent constitutive activity. This suggests that the mutation induces limited and precise conformational changes. The altered specificity, which results in increased responsiveness to chorionic gonadotropin but not to thyroid-stimulating hormone, is in keeping with such a moderate conformational change. A parallel change has been described in familial gestational hyperthyroidism, in which mutation of a conserved residue in the extracellular domain of the thyroid-stimulating hormone receptor renders it hypersensitive to chorionic gonadotropin but not to follicle-stimulating



lating hormone.<sup>20</sup> Both of these mutations support a model of different negative determinants that exclude one hormone. Whether these molecular determinants are exposed or covered may depend on conformational changes due to mutations of conserved residues distant from binding sites.

The molecular findings in this case help to elucidate the pathogenesis of both the spontaneous and iatrogenic forms of ovarian hyperstimulation

syndrome. It has been proposed that follicle-stimulating hormone and chorionic gonadotropin have synergistic roles in the development of ovarian hyperstimulation syndrome.<sup>21</sup> In our patient, the paradoxical activation of the mutant follicle-stimulating hormone receptor during gestation because of its hypersensitivity to chorionic gonadotropin, as indicated by the unusual inhibin B time course,<sup>22</sup> supports the concept that the follicle-stimulating hormone pathway has a role in the pathophysiology of ovarian hyperstimulation syndrome. The fact that estradiol originates not only in the ovary but also, as pregnancy progresses, in the placenta explains the observed time course of changes in estradiol levels and explains why estradiol is a poor marker of ovarian status later in pregnancy. Excessive follicular recruitment in association with luteinization of granulosa cells secondary to activation of both the follicle-stimulating hormone receptor and the luteinizing hormone receptor by chorionic gonadotropin is an explanation for iatrogenic ovarian hyperstimulation syndrome, in which each receptor is overstimulated by its cognate hormone. It is not known whether altered sensitivity or specificity of either of these receptors may be the cause of some cases of iatrogenic ovarian hyperstimulation syndrome. However, we suggest that screening for mu-

tations in the follicle-stimulating hormone receptor may be useful in women who have prolonged or delayed episodes of ovarian hyperstimulation syndrome.

Our findings do not elucidate the pathogenesis of the systemic symptoms of ovarian hyperstimulation syndrome, whether spontaneous or iatrogenic. Whereas estradiol levels may be predictive of iatrogenic ovarian hyperstimulation syndrome, the respective contributions of factors such as vascular endothelial growth factor, cytokines, prostaglandins, and components of the renin-angiotensin system, as emphasized in several studies, remain to be clarified.<sup>2,6,23</sup>

In this case, fertility in the proband and her two sisters was unaffected by the mutation, and surprisingly, there were no multiple gestations. Since we do not know whether the repetitive, long-lasting ovarian hyperstimulation in the affected women increases the risk of ovarian carcinoma,<sup>24,25</sup> careful follow-up is indicated.

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## REFERENCES

1. Scully RE, Young RH, Clement PB. Tumors of the ovary, maldeveloped gonads, fallopian tube, and broad ligament. In: Atlas of tumor pathology. Vol. 23. Washington, D.C.: Armed Forces Institute of Pathology, 1998:429-50.
2. Elchalal U, Schenker JG. The pathophysiology of ovarian hyperstimulation syndrome — views and ideas. *Hum Reprod* 1997;12:1129-37.
3. Ludwig M, Gembruch U, Bauer O, Diedrich K. Ovarian hyperstimulation syndrome (OHSS) in a spontaneous pregnancy with fetal and placental triploidy: information about the general pathophysiology of OHSS. *Hum Reprod* 1998;13:2082-7.
4. Schnorr JA Jr, Miller H, Davis JR, Hatch K, Seeds J. Hyperreactio luteinalis associated with pregnancy: a case report and review of the literature. *Am J Perinatol* 1996;13:95-7.
5. Csapo Z, Szabo I, Toth M, Devenyi N, Papp Z. Hyperreactio luteinalis in a normal singleton pregnancy: a case report. *J Reprod Med* 1999;44:53-6.
6. Rizk B, Aboulghar M. Classification, pathophysiology and management of ovarian hyperstimulation syndrome. In: Brinsden PR, ed. A textbook of in vitro fertilization and assisted reproduction: the Bourn Hall guide to clinical and laboratory practice. New York: Parthenon, 1999:131-55.
7. Lahlou N, Chabbert-Buffet N, Christin-Maitre S, Le Nestour E, Roger M, Bouchard P. Main inhibitor of follicle stimulating hormone in the luteal-follicular transition: inhibin A, oestradiol, or inhibin B? *Hum Reprod* 1999;14:1190-3.
8. Beau I, Touraine P, Meduri G, et al. A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor. *J Clin Invest* 1998; 102:1352-9.
9. Misrahi M, Meduri G, Pissard S, et al. Comparison of immunocytochemical and molecular features with the phenotype in a case of incomplete male pseudohermaphroditism associated with a mutation of the luteinizing hormone receptor. *J Clin Endocrinol Metab* 1997;82:2159-65.
10. Wajda KJ, Lucas JG, Marsh WL Jr. Hyperreactio luteinalis: benign disorder masquerading as an ovarian neoplasm. *Arch Pathol Lab Med* 1989;113:921-5.
11. Quereda F, Acien P, Hernandez A. Hyperreactio luteinalis: intraoperative finding during a cesarean section in a twin pregnancy. *Eur J Obstet Gynecol Reprod Biol* 1996; 66:71-3.
12. Bidus MA, Ries A, Magann EF, Martin JN. Markedly elevated beta-hCG levels in a normal singleton gestation with hyperreactio luteinalis. *Obstet Gynecol* 2002;99: 958-61.
13. Themmen APN, Huhtaniemi IT. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. *Endocr Rev* 2000;21:551-83.
14. Gromoll J, Simoni M, Nieschlag E. An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. *J Clin Endocrinol Metab* 1996; 81:1367-70.
15. Moyle WR, Campbell RK, Myers RV, Bernard MP, Han Y, Wang X. Co-evolution of ligand-receptor pairs. *Nature* 1994;368:251-5.
16. Vlaeminck-Guillem V, Ho SC, Rodien P, Vassart G, Costagliola S. Activation of the cAMP pathway by the TSH receptor involves switching of the ectodomain from a tethered inverse agonist to an agonist. *Mol Endocrinol* 2002;16:736-46.
17. Nishi S, Nakabayashi K, Kobilka B, Hsueh AJ. The ectodomain of the luteinizing hormone receptor interacts with ex-

- olooop 2 to constrain the transmembrane region: studies using chimeric human and fly receptors. *J Biol Chem* 2002;277:3958-64.
18. Ryu K, Gilchrist RL, Tung CS, Ji I, Ji TH. High affinity hormone binding to the extracellular N-terminal exodomain of the follicle-stimulating hormone receptor is critically modulated by exolooop 3. *J Biol Chem* 1998;273:28953-8.
19. Quintana J, Wang H, Ascoli M. The regulation of the binding affinity of the luteinizing hormone/choriogonadotropin receptor by sodium ions is mediated by a highly conserved aspartate located in the second transmembrane domain of G protein-coupled receptors. *Mol Endocrinol* 1993;7:767-75.
20. Rodien P, Bremont C, Sanson ML, et al. Familial gestational hyperthyroidism caused by a mutant thyrotropin receptor hypersensitive to human chorionic gonadotropin. *N Engl J Med* 1998;339:1823-6.
21. White AW, Bradbury JT. Ovarian theca lutein cysts: experimental formation in women prior to repeat cesarean section. *Am J Obstet Gynecol* 1965;92:973-80.
22. Petraglia F, Luisi S, Benedetto C, et al. Changes of dimeric inhibin B levels in maternal serum throughout healthy gestation and in women with gestational diseases. *J Clin Endocrinol Metab* 1997;82:2991-5.
23. Wang TH, Horng SG, Chang CL, et al. Human chorionic gonadotropin-induced ovarian hyperstimulation syndrome is associated with up-regulation of vascular endothelial growth factor. *J Clin Endocrinol Metab* 2002;87:3300-8.
24. Edie-Osagie EC, Hopkins RE. Recurrent idiopathic ovarian hyperstimulation syndrome in pregnancy. *Br J Obstet Gynaecol* 1997;104:952-4.
25. Fauser BC, Hsueh AJ. Genetic basis of human reproductive endocrine disorders. *Hum Reprod* 1995;10:826-46.

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