

Brief Report

**FAMILIAL GESTATIONAL
HYPERTHYROIDISM CAUSED BY A
MUTANT THYROTROPIN RECEPTOR
HYPERSENSITIVE TO HUMAN
CHORIONIC GONADOTROPIN**

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SOME degree of stimulation of the thyroid gland by human chorionic gonadotropin is common during early pregnancy.¹⁻³ When serum chorionic gonadotropin concentrations are abnormally high — for example, in women with molar pregnancies — overt hyperthyroidism may ensue. The pathophysiologic mechanism is believed to be promiscuous stimulation of the thyrotropin receptor by the excess chorionic gonadotropin.^{4,5} The explanation for this stimulation is the close structural relations between chorionic gonadotropin and thyrotropin and between their receptors.⁶

Hyperemesis gravidarum is characterized by excessive vomiting in early pregnancy, leading to the loss of 5 percent or more of body weight.⁴ It is usually self-limited and therefore of little clinical consequence.^{5,7} Some women with the disorder have high serum thyroid hormone concentrations, and a few have sufficient clinical manifestations of hyperthyroidism to warrant short-term treatment with anti-thyroid drugs.^{8,9} Many but not all women with hyperemesis gravidarum and hyperthyroidism have high serum chorionic gonadotropin concentrations, raising the possibility that other factors contribute to the hyperthyroidism.^{3,8,9}

We describe a woman and her mother who had

recurrent gestational hyperthyroidism and normal serum chorionic gonadotropin concentrations. Both women were heterozygous for a missense mutation in the extracellular domain of the thyrotropin receptor. The mutant receptor was more sensitive than the wild-type receptor to chorionic gonadotropin, thus accounting for the occurrence of hyperthyroidism despite the presence of normal chorionic gonadotropin concentrations.

CASE REPORT

The proband was a 27-year-old woman who was 10 weeks' pregnant when referred for the evaluation and treatment of hyperthyroidism. This was her third pregnancy, the first and second having resulted in early miscarriage accompanied by severe nausea and vomiting. She again reported severe nausea and vomiting and had recently lost 5 kg in weight. Physical examination revealed persistent tachycardia (heart rate, 120 beats per minute), excessive sweating, and tremor of the hands. There was a small, diffuse goiter and no ophthalmopathy. Laboratory studies revealed the following values: serum thyrotropin concentration, $<0.07 \mu\text{U}$ per milliliter (normal, 0.2 to 6); serum free thyroxine concentration, 4.7 ng per deciliter (60 pmol per liter; normal, 0.8 to 1.9 ng per deciliter [11 to 24 pmol per liter]); and serum triiodothyronine concentration, 605 ng per deciliter (9.77 nmol per liter; normal, 65 to 170 ng per deciliter [1.05 to 2.75 nmol per liter]). No antibodies to thyroid peroxidase or the thyrotropin receptor were detected in serum.

The patient was treated with 450 mg of propylthiouracil per day for eight weeks, and the dose was then tapered to 150 mg per day. Her condition improved rapidly, and she remained clinically and biochemically euthyroid for the rest of her pregnancy. She delivered a normal girl at 38 weeks of gestation, at which time the propylthiouracil was discontinued. The patient did not return for a follow-up examination post partum.

Eighteen months later, after seven weeks of amenorrhea, she was referred for a recurrence of hyperthyroidism associated with hyper-

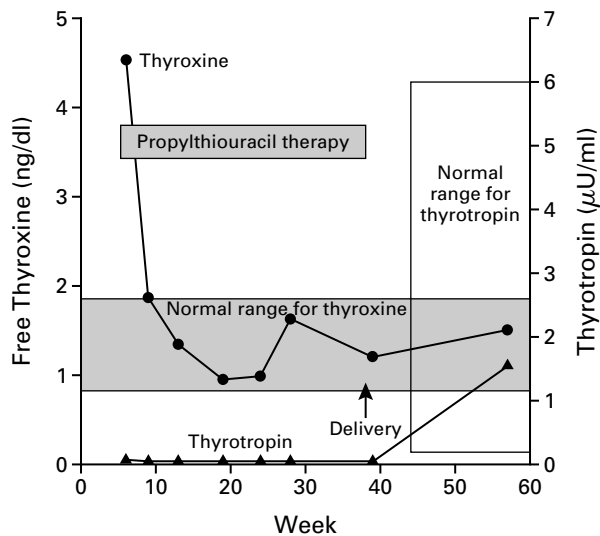


Figure 1. Serum Free Thyroxine and Thyrotropin Concentrations during the Index Patient's Fourth Pregnancy.

To convert values for serum free thyroxine to picomoles per liter, multiply by 12.9.

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emesis gravidarum. The findings on examination were similar to those during the previous pregnancy. The patient denied having any symptoms during the intervening period. The results of laboratory studies were as follows: serum thyrotropin concentration, $<0.07 \mu\text{U}$ per milliliter; serum free thyroxine concentration, 4.5 ng per deciliter (58 pmol per liter); and serum chorionic gonadotropin, 70 U per milliliter (normal range for the first trimester of pregnancy, 38 to 173). Serum thyroid-stimulating activity was undetectable.

The patient was treated with 250 mg of propylthiouracil per day for four weeks, and because her serum thyrotropin concentrations remained low, the dose was tapered to between 50 and 150 mg per day for the rest of the pregnancy (Fig. 1). She delivered a normal boy at 38 weeks of gestation, at which time the propylthiouracil was again discontinued. She was clinically and biochemically euthyroid when last examined four months after delivery.

The patient's mother reported a similar history. She had given birth to the patient at the age of 27, two years after having a miscarriage. The pregnancy was complicated by nausea, vomiting, and weight loss of 7 kg during the first trimester. Delivery was without complications and occurred at term. The same symptoms recurred during a subsequent pregnancy, and the woman was treated with carbimazole for what was believed to be hyperthyroidism due to Graves' disease, despite the absence of goiter and ophthalmopathy. She delivered a normal boy at 40 weeks of gestation. Carbimazole was discontinued two months after delivery. Since that time she has remained euthyroid and has had no more pregnancies.

The study was approved by the local ethics committee, and both women gave informed consent.

METHODS

Hormone Assays

Serum free thyroxine was measured by radioimmunoassay (Sorin Biomedica, Anthony, France), and serum thyrotropin and chorionic gonadotropin were measured by chemiluminescence assays (ACS180, Chiron, Cergy Pontoise, France). Thyroid-stimulating activity in the serum was assayed in cultures of Chinese-hamster-ovary cells, stably transfected with the human thyrotropin receptor.¹⁰

Sequencing of the Thyrotropin-Receptor Gene

To determine the sequence of the thyrotropin-receptor gene, DNA was extracted from peripheral-blood leukocytes, and the sequences of all exons of the thyrotropin-receptor gene and the intron-exon junctions were determined as described previously.¹¹

Functional Characterization of the Mutant

The mutation identified in the thyrotropin-receptor gene of the patient and her mother was introduced in wild-type thyrotropin-receptor complementary DNA (cDNA) by site-directed mutagenesis based on the polymerase chain reaction (PCR). In brief, an *XhoI*-*AflIII* restriction fragment encompassing the mutated site was ligated to wild-type thyrotropin-receptor cDNA that had been inserted in the expression vector pSVL (Pharmacia, Roosendaal, the Netherlands). The sequence of the segment containing the mutation was verified on both strands.

Plasmids encoding wild-type or mutant thyrotropin receptors and wild-type luteinizing hormone or follicle-stimulating hormone receptors were transfected into COS-7 cells by the DEAE-dextran method.¹¹ Cell-surface expression of the wild-type and mutant receptors was assessed by a FACScan flow cytometer (Becton Dickinson, San Diego, Calif.) with a mouse monoclonal antibody (BA-8) that recognizes a conformational epitope of the extracellular domain of the human thyrotropin receptor.¹²

The production of cyclic AMP (cAMP) was measured in the cells at base line and after incubation for 60 minutes¹¹ with bovine thyrotropin (Sigma, Bornem, Belgium), chorionic gonadotropin from the urine of pregnant women (Intervet, Chorulon, Turnhout, Belgium), and recombinant follicle-stimulating hormone (Organon, Brussels, Belgium).

RESULTS

Sequence Determination

Direct sequencing of PCR products amplified from genomic DNA from the patient identified the substitution of guanine for adenine at codon 183 in exon 7 in one allele of the thyrotropin-receptor gene. This substitution results in the replacement of a lysine residue with an arginine (K183R) at position 183 of the receptor, which is in the middle of its extracellular N-terminal domain. The patient's mother was heterozygous for the same mutation. The mutation nullifies an *AluI* restriction site, a characteristic that allows easy screening of the general population. None of 100 unrelated normal subjects carried the mutation, ruling out the possibility that it is a common polymorphism.

Functional Characterization of the Mutant Receptor

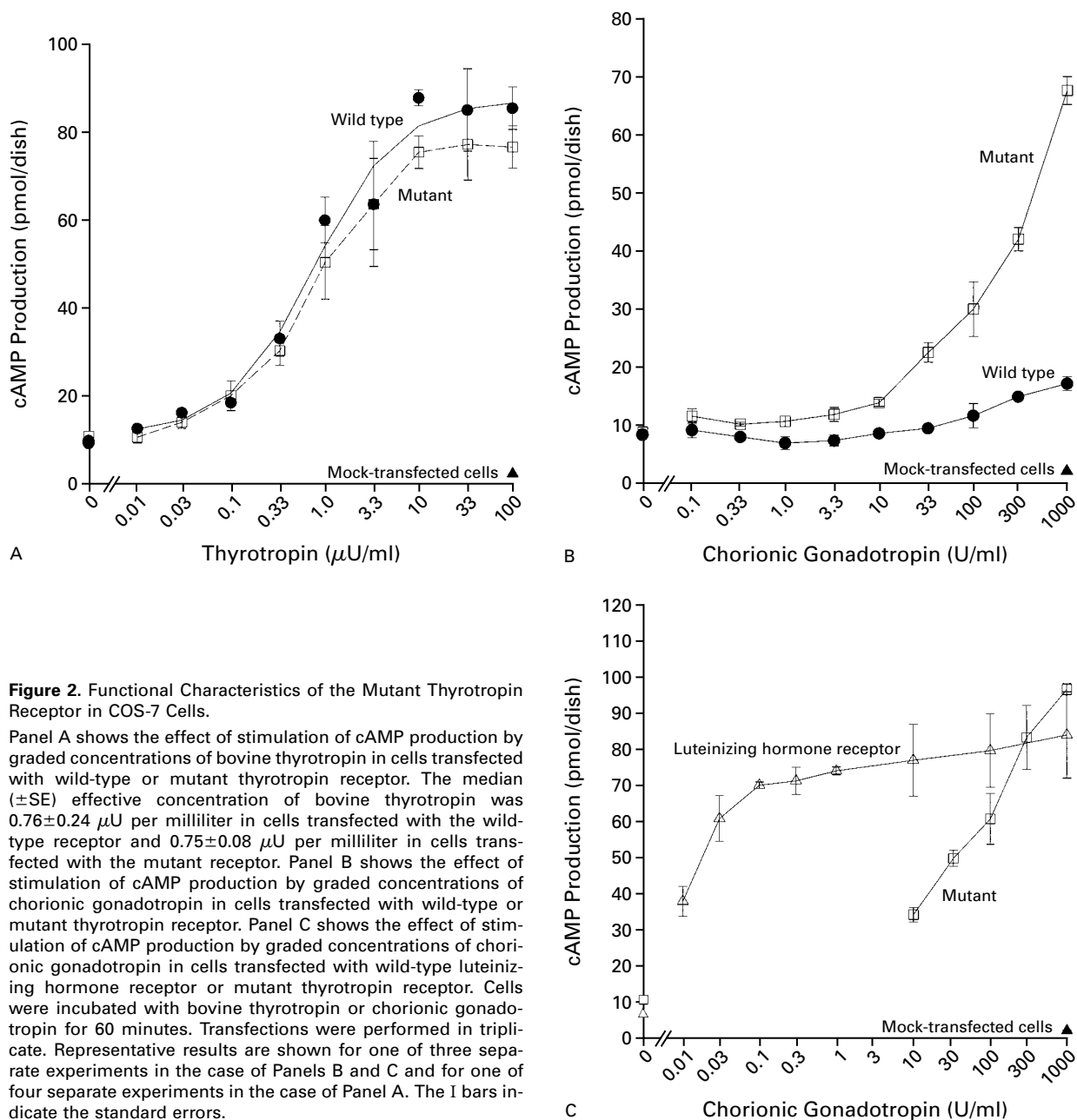
When transfected into COS-7 cells, the mutant cDNA was expressed at the cell surface at a concentration similar to that of the wild-type receptor (data not shown). Basal production of cAMP in cells expressing the mutant receptor was similar to that in cells expressing the wild-type receptor, as were the sensitivity and maximal responsiveness of the cells to bovine thyrotropin (Fig. 2A). In contrast, chorionic gonadotropin in concentrations of up to 1000 U per milliliter caused a dose-dependent increase in cAMP production by cells containing the mutant receptor, but only a minimal increase in cells containing the wild-type receptor (Fig. 2B). Comparison of the stimulation of cAMP production by chorionic gonadotropin in cells containing the wild-type luteinizing hormone receptor or the mutant thyrotropin receptor indicated that the mutant was about 1000 times less responsive (Fig. 2C).

Serum from a pregnant woman with a high serum chorionic gonadotropin concentration (280 U per milliliter) was diluted to achieve a concentration (65 U per milliliter) that was similar to the values recorded during the first trimester of pregnancy. This concentration of chorionic gonadotropin caused cAMP production to increase by a factor of 3.5 in COS-7 cells expressing the mutant thyrotropin receptor, but it had no effect on cells expressing the wild-type receptor. Follicle-stimulating hormone (200 mU per milliliter) had no effect on cells expressing either the mutant or the wild-type receptor.

The results of assays of direct binding of radiolabeled chorionic gonadotropin or the displacement of radiolabeled thyrotropin by chorionic gonadotropin from the mutant receptors were negative (data not shown).

DISCUSSION

The phenotype and genotype of our patient and her mother, together with the functional characteristics of the mutant thyrotropin receptor, led to a



syndrome of hereditary gestational hyperthyroidism caused by hypersensitivity of the thyrotropin receptor to chorionic gonadotropin. The mutation is remarkable in that it broadens the ligand specificity of a G-protein-coupled receptor without altering sensitivity to the native ligand, thyrotropin.

The mechanism responsible for gestational hyperthyroidism in these women differs from that of the hyperthyroidism associated with molar pregnancies and, at least in some women, hyperemesis gravidarum. In women with the latter two conditions, hyperthyroidism results from the activation of the

thyrotropin receptor by excessive quantities of chorionic gonadotropin or by chorionic gonadotropin molecules with increased thyrotropin-like activity.¹³⁻¹⁶ These two situations may be viewed as exaggerated forms of the thyroid stimulation that occurs at the time of maximal chorionic gonadotropin production in many normal pregnant women.¹ In contrast, in our patient and her mother, both of whom were heterozygous for the K183R mutation, normal serum chorionic gonadotropin concentrations that would not stimulate the wild-type thyrotropin receptor excessively caused hyperthyroidism that was severe

enough to necessitate antithyroid-drug therapy during pregnancy.

The relation between hyperemesis gravidarum and gestational hyperthyroidism is not clear. In some studies, high serum free thyroid hormone concentrations were found in 30 to 70 percent of women with hyperemesis gravidarum,^{5,7} but in other studies, few women had high values.¹⁷ Whether hyperemesis results from hyperthyroidism, from the hyperestrogenic state associated with hyperstimulation by chorionic gonadotropin, or from other mechanisms is not known.^{9,18-20} The finding of severe recurrent hyperemesis in the presence of normal serum chorionic gonadotropin concentrations in our patient suggests that hyperemesis is related to hyperthyroidism.

Considering its functional consequences, the mutation identified — the substitution of arginine for lysine at position 183 — is surprisingly conservative. This position is in a region of the receptor that constitutes the putative surface of interaction with thyrotropin.^{21,22} An arginine at position 183 may increase the stability of the illegitimate complex between chorionic gonadotropin and the thyrotropin receptor enough to cause signal transduction by the increased serum chorionic gonadotropin concentrations present in pregnant women.²³ However, despite the fact that luteinizing hormone activates the wild-type thyrotropin receptor more easily than does chorionic gonadotropin,²⁴ the serum luteinizing hormone concentrations after menopause would remain too low to cause activation of the mutant receptor. Indeed, the mother of our patient remained euthyroid after menopause. This finding is compatible with a relatively small gain of function in the mutant thyrotropin receptor in response to stimulation by chorionic gonadotropin, in agreement with our inability to detect binding of chorionic gonadotropin.

Unlike other mammals, primates rely on chorionic gonadotropin for the maintenance of the corpus luteum in early pregnancy.²⁵ Our data, together with the fact that thyrotropin secretion is often partially suppressed during the period when chorionic gonadotropin concentrations are maximal, suggest that evolution has led to the selection of physiologic mechanisms that operate close to the border of hyperthyroidism during normal pregnancy.

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REFERENCES

- Glinoe D. The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. *Endocr Rev* 1997; 18:404-33.
- Burrow GN. Thyroid function and hyperfunction during gestation. *Endocr Rev* 1993;14:194-202.
- Harada A, Hershman JM, Reed AW, et al. Comparison of thyroid stimulators and thyroid hormone concentrations in the sera of pregnant women. *J Clin Endocrinol Metab* 1979;48:793-7.
- Goodwin TM, Montoro M, Mestman JH, Pekary AE, Hershman JM. The role of chorionic gonadotropin in transient hyperthyroidism of hyperemesis gravidarum. *J Clin Endocrinol Metab* 1992;75:1333-7.
- Swaminathan R, Chin RK, Lao TT, Mak YT, Panesar NS, Cockram CS. Thyroid function in hyperemesis gravidarum. *Acta Endocrinol (Copenh)* 1989;120:155-60.
- Grossmann M, Weintraub BD, Szkudlinski MW. Novel insights into the molecular mechanisms of human thyrotropin action: structural, physiological, and therapeutic implications for the glycoprotein hormone family. *Endocr Rev* 1997;18:476-501.
- Bouillon R, Naesens M, Van Assche FA, et al. Thyroid function in patients with hyperemesis gravidarum. *Am J Obstet Gynecol* 1982;143: 922-6.
- Jeffcoate WJ, Bain C. Recurrent pregnancy-induced thyrotoxicosis presenting as hyperemesis gravidarum: case report. *Br J Obstet Gynaecol* 1985;92:413-5.
- Krentz AJ, Redman H, Taylor KG. Hyperthyroidism associated with hyperemesis gravidarum. *Br J Clin Pract* 1994;48:75-6.
- Ludgate M, Perret J, Parmentier M, et al. Use of the recombinant human thyrotropin receptor (TSH-R) expressed in mammalian cell lines to assay TSH-R autoantibodies. *Mol Cell Endocrinol* 1990;73:R13-R18.
- Parma J, Duprez L, Van Sande J, et al. Diversity and prevalence of somatic mutations in the thyrotropin receptor and Gs alpha genes as a cause of toxic thyroid adenomas. *J Clin Endocrinol Metab* 1997;82:2695-701.
- Costagliola S, Rodien P, Many MC, Ludgate M, Vassart G. Genetic immunization against the human thyrotropin receptor causes thyroiditis and allows production of monoclonal antibodies recognizing the native receptor. *J Immunol* 1998;160:1458-65.
- Hoermann R, Keutmann HT, Amir SM. Carbohydrate modifications transform human chorionic gonadotropin into a potent stimulator of adenosine 3',5'-monophosphate and growth responses in FRTL-5 thyroid cells. *Endocrinology* 1991;128:1129-35.
- Tsuruta E, Tada H, Tamaki H, et al. Pathogenic role of asialo human chorionic gonadotropin in gestational thyrotoxicosis. *J Clin Endocrinol Metab* 1995;80:350-5.
- Kimura M, Amino N, Tamaki H, et al. Gestational thyrotoxicosis and hyperemesis gravidarum: possible role of hCG with higher stimulating activity. *Clin Endocrinol (Oxf)* 1993;38:345-50.
- Goodwin TM, Hershman JM. Hyperthyroidism due to inappropriate production of human chorionic gonadotropin. *Clin Obstet Gynecol* 1997; 40:32-44.
- Wilson R, McKillop JH, MacLean M, et al. Thyroid function tests are rarely abnormal in patients with severe hyperemesis gravidarum. *Clin Endocrinol (Oxf)* 1992;37:331-4.
- Evans AJ, Li TC, Selby C, Jeffcoate WJ. Morning sickness and thyroid function. *Br J Obstet Gynaecol* 1986;93:520-2.
- Rosenthal FD, Jones C, Lewis SI. Thyrotoxic vomiting. *BMJ* 1976;2: 209-11.
- Depue RH, Bernstein L, Ross RK, Judd HL, Henderson BE. Hyperemesis gravidarum in relation to estradiol levels, pregnancy outcome, and other maternal factors: a seroepidemiologic study. *Am J Obstet Gynecol* 1987;156:1137-41.
- Kajava AV, Vassart G, Wodak SJ. Modeling of the three-dimensional structure of proteins with the typical leucine-rich repeats. *Structure* 1995; 3:867-77.
- Nagayama Y, Russo D, Chazenbalk GD, Wadsworth HL, Rapoport B. Extracellular domain chimeras of the TSH and LH/CG receptors reveal the mid-region (amino acids 171-260) to play a vital role in high affinity TSH binding. *Biochem Biophys Res Commun* 1990;173:1150-6.
- Mrabet NT, Van den Broeck A, Van den Brande I, et al. Arginine residues as stabilizing elements in proteins. *Biochemistry* 1992;31:2239-53.
- Yoshimura M, Hershman JM, Pang XP, Berg L, Pekary AE. Activation of the thyrotropin (TSH) receptor by human chorionic gonadotropin and luteinizing hormone in Chinese hamster ovary cells expressing functional human TSH receptors. *J Clin Endocrinol Metab* 1993;77:1009-13.
- Stewart HJ, Jones DSC, Pascall JC, Popkin RM, Flint APE. The contribution of recombinant DNA techniques to reproductive biology. *J Reprod Fertil* 1988;83:1-57.