

BRIEF REPORT: CONGENITAL HYPERTHYROIDISM CAUSED BY A MUTATION IN THE THYROTROPIN- RECEPTOR GENE

PETER KOPP, M.D., JACQUELINE VAN SANDE, M.D.,
JASMINE PARMA, M.D., LAURENCE DUPREZ, M.D.,
HANS GERBER, M.D., ETIENNE JOSS, M.D.,
J. LARRY JAMESON, M.D., PH.D.,
JACQUES E. DUMONT, M.D., PH.D.,
AND GILBERT VASSART, M.D., PH.D.

CONGENITAL hyperthyroidism is rare. Most cases occur in infants born of mothers with a history of Graves' disease.¹ The disorder is usually transient in such infants, because it is caused by transplacental passage of maternal thyrotropin-receptor-stimulating autoantibodies that are subsequently cleared.^{2,3} However, a few neonates with persistent nonautoimmune hyperthyroidism of unknown cause have been described.⁴⁻¹¹ The family history suggested an autosomal dominant disorder in some of these infants.^{5,12}

A molecular basis for autonomous thyroid function has been found in some patients with hyperfunctioning thyroid adenomas. Some of these tumors have somatic mutations in stimulatory G (guanine nucleotide-binding) protein subunits ($G_s\alpha$)^{13,14} or in the thyrotropin receptor that cause constitutive activation of the abnormal thyroid tissue.¹⁵ Activating mutations of the thyrotropin receptor have also been found in the germ line in two families with nonautoimmune hereditary hyperthyroidism.¹⁶ The thyrotropin receptor, a member of the superfamily of G-protein-coupled transmembrane receptors, controls both the function and the growth of thyroid cells through stimulation of adenylate cyclase and phospholipase C.¹⁷ In this report, we describe a boy with persistent congenital hyperthyroidism in whom the thyrotropin-receptor gene contained a germ-line mutation with a single amino acid substitution that resulted in constitutive activation of the receptor.

CASE REPORT

The patient was born prematurely at 32 weeks of gestation. His weight was 1660 g, his length 44 cm, and his head circumference 29 cm (normal range, 28 to 31 cm). Because of tachycardia (150 beats per minute), tachypnea, and a diffuse goiter (Fig. 1A), hyperthyroidism was suspected, and laboratory tests confirmed the diagnosis (Table 1).

From the Department of Internal Medicine and the Laboratory of Endocrinology (P.K., H.G.) and the Clinic of Pediatrics (E.J.), Inselspital, University of Bern, Bern, Switzerland; the Center for Endocrinology, Metabolism, and Molecular Medicine, Northwestern University, Chicago (P.K., J.L.J.); and the Institut de Recherche Interdisciplinaire and Department of Medical Genetics, Faculty of Medicine, University of Brussels, Brussels, Belgium (J.v.S., J.P., L.D., J.E.D., G.V.). Address reprint requests to Dr. Kopp at the Center for Endocrinology, Metabolism, and Molecular Medicine, Northwestern University, Tarry 15, 303 E. Chicago Ave., Chicago, IL 60611.

Supported by grants from the Belgian Program of Interuniversity Poles of Attraction of the Federal Service for Science, Technology, and Culture, the Fonds de la Recherche Scientifique Médicale and Biomed program, the Swiss National Foundation of Science, and the National Institutes of Health (DK42144). Dr. Kopp is the recipient of a fellowship from the Swiss National Foundation of Science.

The patient was treated with propylthiouracil in a dose sufficient to induce hypothyroidism, with an appropriate increase in the serum thyrotropin concentration. Discontinuation of propylthiouracil and administration of triiodothyronine resulted in a decrease in the serum thyrotropin concentration and an increase in the serum thyroxine concentration to a supranormal value, findings indicative of normal thyrotropin regulation and autonomous thyroid function. Tests for serum antithyroid peroxidase and antithyroglobulin autoantibodies were negative, as were tests for thyrotropin-binding inhibitory antibodies (TRAK-Assay, Henning, Berlin, Germany), thyroid cyclic-AMP-stimulating antibodies, and thyrotropin-blocking antibodies (performed by J. Orgiazzi, Lyon, France). The serum thyroxine-binding globulin concentration was normal, and the serum thyroglobulin concentration was elevated. There was no ophthalmopathy.

The patient's mother was euthyroid, with no history of any thyroid disease, and repeated tests for thyroid antibodies were negative. There was no history of thyroid disease in other family members except for a maternal aunt with hyperthyroidism of unknown cause. The patient's only sister was euthyroid.

From the age of 0.4 to 8.6 years, the patient was treated with carbimazole (7.5 to 25 mg daily) and thyroxine (25 to 37.5 μ g daily). Discontinuation of therapy on repeated occasions was followed by the prompt recurrence of hyperthyroidism. At the age of 8.6 years, hyperthyroidism recurred while the patient was receiving 15 mg of carbimazole and 37.5 μ g of thyroxine daily. The size of his goiter increased rapidly (Fig. 1B), and multiple nodules were detectable by palpation and ultrasonography. A subtotal thyroidectomy was performed at the age of 8.7 years. The thyroid gland weighed 70 g, and it contained multiple nodules between 0.5 and 3 cm in diameter (Fig. 1C). Histologic examination revealed hyperplasia of all the follicular cells. Within the sharply delimited nodules the nuclei were irregular in shape. There were no signs of a malignant transformation or lymphocytic infiltration (Fig. 1D).

Although all the thyroid tissue was removed except for a few grams, the patient remained hyperthyroid after surgery (Table 1). A thyroid radionuclide scan showed a small amount of pretracheal tissue and a small nodule in the left side of the neck. The nodule was excised; histologic examination revealed hyperplastic thyroid tissue. Because of persistent hyperthyroidism and tissue growth, radioiodine therapy was administered at the age of 9.2 years. Thereafter, the patient became euthyroid, and the serum thyrotropin concentration increased to a normal value.

The patient's weight from birth to the age of 12 years was persistently low (below the third percentile). His height was below the third percentile during the first 17 months of life but was subsequently normal. During the first five years of life, the bone age was advanced, but thereafter it was normal. The circumference of the head was below normal, but with the exception of frontal bossing, the development of the skull was normal. Neuropsychological testing at 1.5, 3.6, and 8.3 years of age revealed mental retardation and hyperactivity, requiring that the patient attend a special-education class; his IQ was between 75 and 85.¹⁸

METHODS

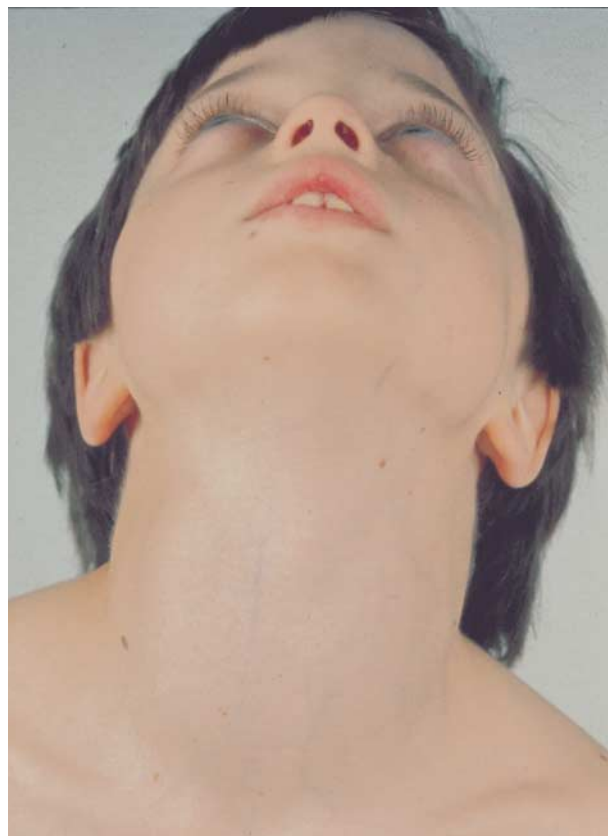
DNA Sequencing

DNA was extracted from leukocytes and nodular and nonnodular thyroid tissue obtained from the patient and from leukocytes obtained from his parents and sister. Paternity was verified by DNA fingerprinting. The major part of exon 10 of the thyrotropin-receptor gene was amplified with two sets of primers as described elsewhere.^{15,16} The products of the polymerase chain reaction were purified on streptavidin-coated magnetic beads (Dyna, Oslo, Norway) and sequenced with Sequenase 2.0 (U.S. Biochemical, Cleveland). DNA extracted from nodular and nonnodular tissue from two patients with hyperfunctioning thyroid adenomas was analyzed with the same methods.

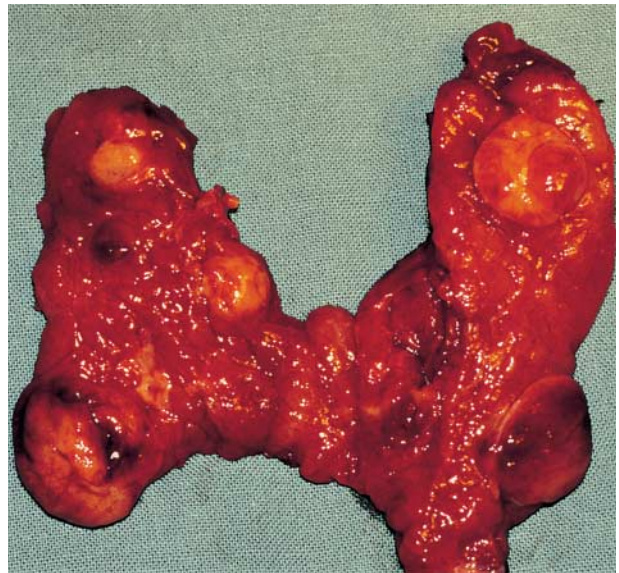
To exclude mutations in the regions known to contain activating mutations in $G_s\alpha$ (exons 8 and 9) or inhibitory G-protein subunits ($G_i\alpha$ [exons 5 and 6]), DNA from nodular and nonnodular thyroid tissue was amplified by the polymerase chain reaction.¹⁴ Both strands were sequenced with the Taq DyeDeoxy Terminator Cycle Sequenc-



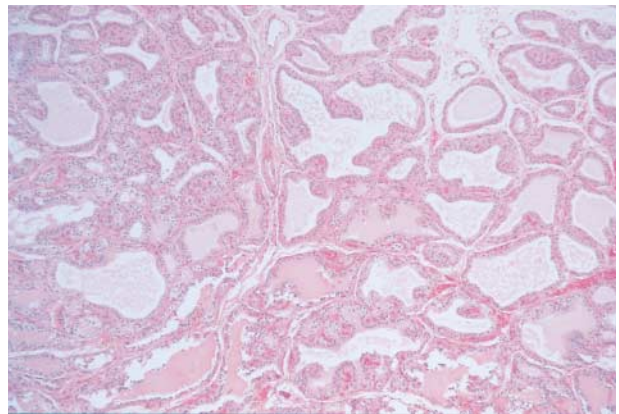
A



B



C



D

Figure 1. Goiter in a Patient with a Mutant Thyrotropin Receptor. Panel A shows the patient a few days after birth, and Panel B shows the patient at the age of 8.6 years, one month before he underwent a subtotal thyroidectomy. Panel C shows the multi-nodular goiter (70 g) removed when the patient was 8.7 years old. The largest nodule has a diameter of 3 cm. Panel D shows a photomicrograph of a representative section of the thyroid gland, with diffuse hyperplasia of follicular cells, nodular transformation, and slight nuclear polymorphism (hematoxylin and eosin, $\times 25$).

ing Kit and the 373A Sequencer (Applied Biosystems, Foster City, Calif.). Exons 1 and 2 of the *H-ras*, *K-ras*, and *N-ras* proto-oncogenes were sequenced by the same method.¹⁹

Expression and Function of the Mutated Receptor

The wild-type and mutated receptors were subcloned in pSVL-based constructs and verified by sequencing. COS-7 cells were transfected transiently with wild-type or mutant receptor constructs with the use of the diethylaminoethyl-dextran method and were analyzed 72 hours after transfection.¹⁵ All experiments were performed in triplicate and on at least three occasions.

For studies of cyclic AMP production, the cells were incubated for 60 minutes in Krebs-Ringer-HEPES buffer containing various quantities of thyrotropin and 25 μM of the phosphodiesterase inhibitor rolipram. Cyclic AMP was measured after the cells had been boiled in water.²⁰ For studies of inositol phosphate accumulation, trit-

Table 1. Thyroid Function and Treatment in a Patient with Congenital Hyperthyroidism Caused by an Activating Mutation in the Thyrotropin-Receptor Gene.*

CHRONOLOGIC AGE (YR)	SERUM THYROXINE	SERUM TRIIODOTHYRONINE	SERUM FREE THYROXINE	SERUM FREE TRIIODOTHYRONINE	SERUM THYROTROPIN	TREATMENT
	$\mu\text{g/dl}$	ng/dl	ng/dl	ng/dl	mU/liter	
Birth	22.0	300			<0.5	
0.3	1.0	50			25	Propylthiouracil
0.4	27.7	750			0.5	Triiodothyronine
8.6				0.9	<0.04	Carbimazole, thyroxine
8.7						Thyroidectomy
8.9	14.9		3.3	1.1	<0.04	
9.2						Radioiodine
11.6			0.8	0.4	0.6	
Normal range	4.3–10.8	80–200	0.8–2.1	0.2–0.6	0.3–4.0	

*The following assays were performed: radioimmunoassays for serum thyroxine and triiodothyronine, a luminoimmunoassay (Magic Lite, Ciba-Corning, Dietlikon, Switzerland) for serum free thyroxine, a radioimmunoassay (Amerlex-M, Amersham-Rahn, Zurich, Switzerland) for serum free triiodothyronine, and Irmaclone and Dynotest (Henning, Berlin, Germany) for serum thyrotropin. To convert values for thyroxine and triiodothyronine to nanomoles per liter, multiply by 12.87 and 0.015, respectively; to convert values for free thyroxine and free triiodothyronine to picomoles per liter, multiply by 12.87 and 15, respectively.

ium-labeled inositol (myo-[2-³H]inositol) (Dupont-NEN, Haren, Belgium) was added for the last 24 hours of culture, after which the medium was replaced by Krebs-Ringer-HEPES buffer containing 10 mM lithium chloride and various quantities of thyrotropin. After incubation for 30 minutes, ice-cold 3 percent perchloric acid was added, and tritium-labeled inositol phosphates were then isolated and assayed by chromatography.^{15,16} To determine the extent of thyrotropin binding to the receptor, the cells were washed twice with Hanks' medium (sodium chloride replaced by 280 mM sucrose) and 0.2 percent bovine serum albumin and then incubated with ¹²⁵I-labeled thyrotropin (Henning, Berlin, Germany) and various amounts of unlabeled thyrotropin for four hours at 4°C in the same medium. The cells were then rinsed twice with cold buffer and dissolved in 1 M sodium hydroxide, and the cell-bound radioactivity was measured in a gamma counter.

RESULTS

Identification of a Mutation in the Thyrotropin-Receptor Gene

A thymine-to-cytosine (T-to-C) transition in the gene segment encoding the sixth transmembrane region of the receptor was identified in the DNA from

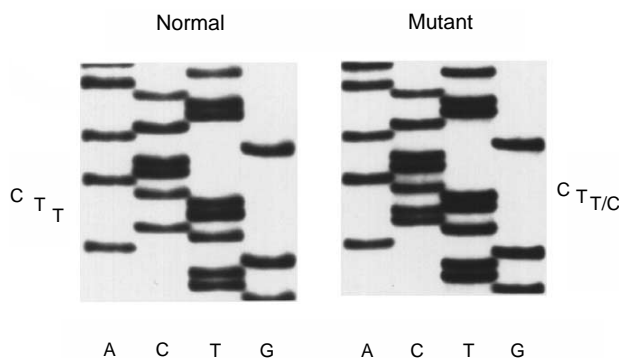


Figure 2. Nucleotide Sequence of the Gene Segment That Encodes Part of the Sixth Transmembrane Segment of the Thyrotropin Receptor, Showing the Heterozygous Mutation in the Patient with Congenital Hyperthyroidism.

The mutation, TTC→CTC, resulted in the substitution of leucine for phenylalanine at position 631 in one allele. The wild-type (normal) sequence is shown for comparison.

the patient's leukocytes and nodular and nonnodular thyroid tissue. The patient was heterozygous for the mutation, which resulted in the substitution of leucine (CTC) for phenylalanine (TTC) at position 631 in one allele (Fig. 2). In contrast, the patient's parents and sister had only the wild-type sequence, indicating that the patient had a new germline mutation. The location of this mutation, together with the other known mutations that constitutively activate the thyrotropin receptor, is shown in Figure 3. The same amino acid substitution has been found in DNA from hyperfunctioning thyroid adenomas in two patients but not in DNA from adjacent normal thyroid tissue. In these adenomas the wild-type codon TTC (phenyl-

alanine at position 631) had changed to TTA (also encoding leucine) (not shown).

Analysis of the patient's nodular thyroid tissue for activating mutations of *G_sα*, *G_iα*, and the *ras* oncogenes (*N-ras*, *H-ras*, and *K-ras*) did not reveal any mutations. Immunohistochemical expression of the *ras* p21 protein was markedly increased in nodular tissue (monoclonal antibody pan-Ras 11, Dupont, Regensdorf, Switzerland).

Functional Studies

The wild-type receptor had a low level of constitutive activity when expressed in COS-7 cells.^{15,16,21} Basal intracellular cyclic AMP concentrations were five to six times higher in COS-7 cells transfected with increasing amounts of the mutated receptor than in cells transfected with the wild-type thyrotropin receptor (Fig. 4). The binding of ¹²⁵I-labeled thyrotropin to cells transfected with the two types of receptors was similar (Fig. 4), indicating that the difference in the generation of basal cyclic AMP was not due to greater expression of the mutant receptors. The mutant receptors retained their ability to respond to thyrotropin, as indicated by the increased production of cyclic AMP and inositol phosphates in cells incubated with thyrotropin (data not shown). Like the mutant thyrotropin receptors found in patients with hyperfunctioning thyroid adenomas and hereditary hyperthyroidism,^{15,16} this mutant receptor caused no increase in basal inositol phosphate production, indicating that the constitutive activation was restricted to activation of the cyclic AMP regulatory cascade (data not shown).

DISCUSSION

Constitutive activation of G-protein-coupled receptors is involved in familial gonadotropin-independent precocious puberty in males (luteinizing hormone receptor),^{22,23} certain forms of retinitis pigmentosa²⁴ and night blindness (rhodopsin),²⁵ hyperfunctioning thyroid adenomas,¹⁵ and autosomal dominant hyperthy-

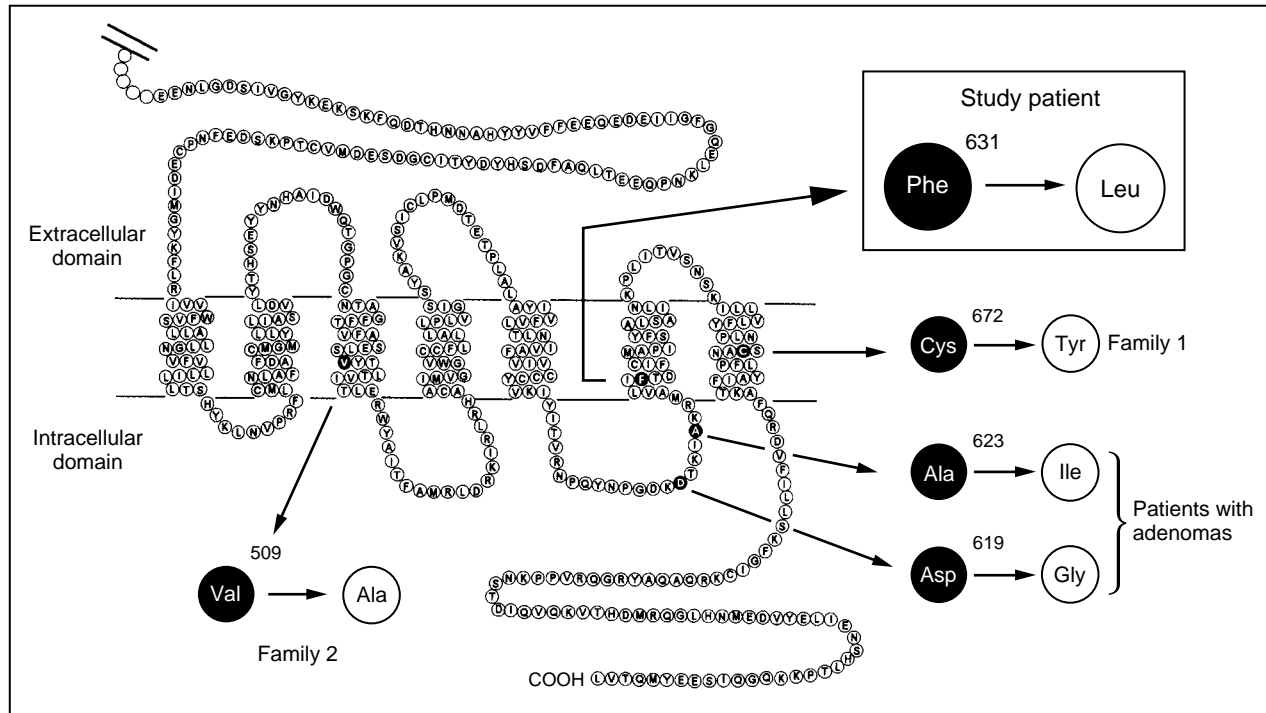


Figure 3. Amino Acid Structure of the Thyrotropin Receptor and Location of Gain-of-Function Mutations in the Study Patient, Patients with Hyperfunctioning Thyroid Adenomas, and Two Families with Germ-Line Mutations Causing Autosomal Dominant Nonautoimmune Hereditary Hyperthyroidism.

The long amino-terminal extension of the receptor is not shown. The numbering of residues starts at the initiator codon.

roidism.¹⁶ The new germ-line mutation of the thyrotropin-receptor gene reported here expands this notion.

In our patient, one of the alleles of the gene for the thyrotropin receptor had a mutation resulting in an amino acid substitution also found in the nodular tissue of two patients with hyperfunctioning thyroid adenomas, except that in the adenomas the mutations occurred somatically. The discovery of a common

pathogenic mechanism for the two conditions is not unexpected. For example, in the McCune–Albright syndrome, activating mutations in G_{α} occur early in development and affect multiple tissues.²⁶⁻²⁸ On the other hand, G_{α} mutations that occur specifically in the thyroid gland cause hyperfunctioning thyroid adenomas,^{13,14} whereas those occurring in the somatotroph cells of the pituitary gland can cause acromegaly.¹³

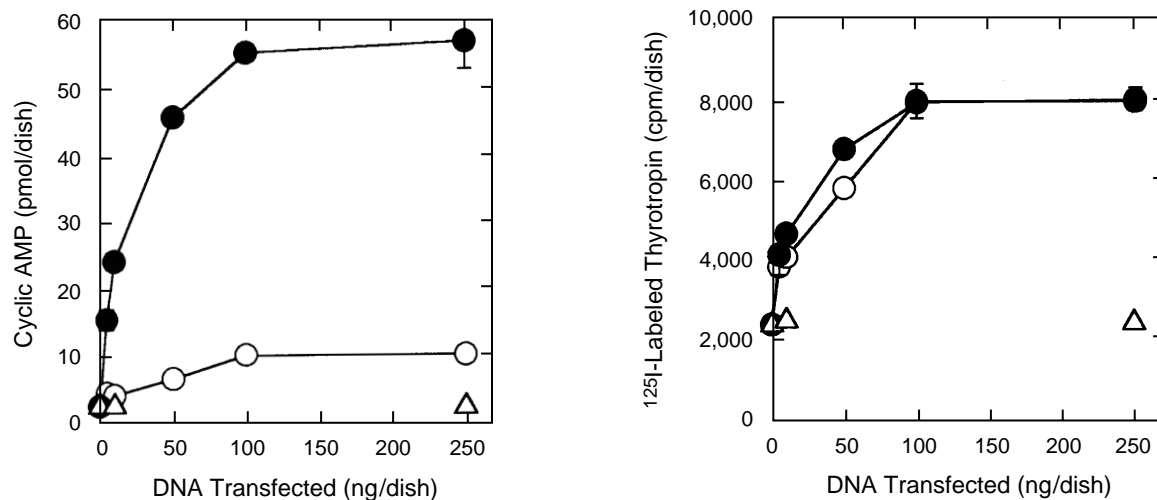


Figure 4. Functioning of the Mutant Thyrotropin Receptor in the Study Patient.

The left-hand panel shows basal intracellular accumulation of cyclic AMP in COS-7 cells transfected with increasing amounts of mutated DNA constructs (solid circles), wild-type DNA constructs (open circles), or pSVL plasmid alone (triangles). The right-hand panel shows the binding of ¹²⁵I-labeled thyrotropin to transfected COS-7 cells. Data are means (\pm SE) of triplicate transfections from at least three experiments.

Our data point to phenylalanine at position 631 as a residue that plays a key part in maintaining the thyrotropin receptor in the inactive state. At present, mutations in five residues have been found to cause constitutive activation of the thyrotropin receptor (Fig. 3). These mutations may perturb the structure of a domain that normally inhibits receptor coupling to G proteins, leading to thyrotropin-independent activation of the receptor. Although mutations at other locations in the transmembrane and intracytoplasmic domains of the thyrotropin receptor cause autonomous thyroid function,^{15,16} the independent occurrence of the same amino acid substitution in different patients suggests that certain receptor locations may be mutational "hot spots," either because the DNA sequence is susceptible to mutagenesis or, more likely, because mutations at this location lead to clonal expansion and cause a readily identifiable phenotype of hyperthyroidism.

The constitutive activation of cyclic AMP in our patient explains the ensuing hyperthyroidism,¹⁷ as well as the formation of goiter through the mitogenic effects of cyclic AMP on thyroid follicular cells.²⁹ In addition, the patient had thyroid nodules that were presumably caused by additional mutations in other genes.³⁰

Although congenital nonautoimmune hyperthyroidism is rare, it is important to distinguish this disorder from the more common congenital autoimmune hyperthyroidism, because nonautoimmune hyperthyroidism can be severe and does not remit. Furthermore, because of its presumably early onset during fetal development, the disorder could have irreversible consequences if untreated.

This work is dedicated to the memory of Professor K. Zuppinger. We are indebted to Mr. C. Christophe, Mrs. C. von Grünigen, Dr. J. Grüning, Mrs. V. Hänsele, Dr. J. Teuscher, Mr. C. Massart, Mr. Y. Mauquois, Dr. P. Mullis, and Professor J. Orgiazzi for their help and support.

REFERENCES

- Ramsay I, Kaur S, Krassas G. Thyrotoxicosis in pregnancy: results of treatment by antithyroid drugs combined with T4. *Clin Endocrinol (Oxf)* 1983; 18:73-85.
- Zakarija M, McKenzie JM. Pregnancy-associated changes in the thyroid-stimulating antibody of Graves' disease and the relationship to neonatal hyperthyroidism. *J Clin Endocrinol Metab* 1983;57:1036-40.
- Zakarija M, McKenzie JM, Hoffman WH. Prediction and therapy of intrauterine and late-onset neonatal hyperthyroidism. *J Clin Endocrinol Metab* 1986;62:368-71.
- Wilroy RS Jr, Etteldorf JN. Familial hyperthyroidism including two siblings with neonatal Graves' disease. *J Pediatr* 1971;78:625-32.
- Hollingsworth DR, Mabry CC, Eckerd JM. Hereditary aspects of Graves' disease in infancy and childhood. *J Pediatr* 1972;81:446-59.
- Hollingsworth DR, Mabry CC. Congenital Graves disease: four familial cases with long-term follow-up and perspective. *Am J Dis Child* 1976;130:148-55.
- Hollingsworth DR, Mabry CC, Reid MC. New observations in congenital Graves' disease. In: Stockigt JR, Nagataki S, eds. *Thyroid research VIII: proceedings of the Eighth International Thyroid Congress*, Sydney, Australia, 3-8 February, 1980. Oxford, England: Pergamon Press, 1980:587-90.
- Idem*. Congenital Graves' disease. In: La Cauza C, Root AW, eds. *Problems in pediatric endocrinology: proceedings of the Sernio Symposia*. Vol. 32. London: Academic Press, 1980:169-91.
- Hollingsworth DR. Neonatal hyperthyroidism. In: Delanges F, Fisher DA, Malvaux P, eds. *Pediatric thyroidology*. Vol. 14 of *Pediatric and adolescent endocrinology*. Basel, Switzerland: Karger, 1985:210-22.
- Horton GL. Hyperthyroidie héréditaire par hyperactivité diffuse non-autoimmune de la thyroïde avec autonomie de fonction et de croissance. (M.D. thesis. Switzerland: University of Lausanne, 1987.)
- Zannoli R, Breda L, Rosati E, Chiarelli F. Iperthyroidismo neonatale ad insorgenza precoce ed a decorso protratto. *Minerva Pediatr* 1989;41:33-40.
- Thomas JS, Leclère J, Hartemann P, et al. Familial hyperthyroidism without evidence of autoimmunity. *Acta Endocrinol* 1982;100:512-8.
- Lyons J, Landis CA, Harsh G, et al. Two G protein oncogenes in human endocrine tumors. *Science* 1990;249:655-9.
- O'Sullivan C, Barton CM, Staddon SL, Brown CL, Lemoine NR. Activating point mutations of the gsp oncogene in human thyroid adenomas. *Mol Carcinog* 1991;4:345-9.
- Parma J, Duprez L, Van Sande J, et al. Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature* 1993;365:649-51.
- Duprez L, Parma J, Van Sande J, et al. Germline mutations in the thyrotropin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. *Nat Genet* 1994;7:396-401.
- Vassart G, Dumont JE. The thyrotropin receptor and the regulation of thyrocyte function and growth. *Endocr Rev* 1992;13:596-611.
- Diagnostic and statistical manual of mental disorders, 3rd ed. rev.: DSM-III-R. Washington, D.C.: American Psychiatric Association, 1987.
- Karga H, Lee J-K, Vickery AL Jr, Thor A, Gaz RD, Jameson JL. Ras oncogene mutations in benign and malignant thyroid neoplasms. *J Clin Endocrinol Metab* 1991;73:832-6.
- Brooker G, Harper JF, Terasaki WL, Moylan RD. Radioimmunoassay of cyclic AMP and cyclic GMP. *Adv Cyclic Nucl Res* 1979;10:1-33.
- Kosugi S, Okajima F, Ban T, Hidaka A, Shenker A, Kohn LD. Substitutions of different regions of the third cytoplasmic loop of the thyrotropin (TSH) receptor have selective effects on constitutive, TSH-, and TSH receptor auto-antibody-stimulated phosphoinositide and 3',5'-cyclic adenosine monophosphate signal generation. *Mol Endocrinol* 1993;7:1009-20.
- Kremer H, Mariman E, Otten BJ, et al. Cosegregation of missense mutations of the luteinizing hormone receptor gene with familial male-limited precocious puberty. *Hum Mol Genet* 1993;2:1779-83.
- Shenker A, Laue L, Kosugi S, Merendino JJ Jr, Minegishi T, Cutler GB Jr. A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. *Nature* 1993;365:652-4.
- Robinson PR, Cohen GB, Zhukovsky EA, Oprian DD. Constitutively active mutants of rhodopsin. *Neuron* 1992;9:719-25.
- Rao VR, Cohen GB, Oprian DD. Rhodopsin mutation G90D and a molecular mechanism for congenital night blindness. *Nature* 1994;367:639-42.
- Schwindinger WF, Francomano CA, Levine MA. Identification of a mutation in the gene encoding the α subunit of the stimulatory G protein of adenylyl cyclase in McCune-Albright syndrome. *Proc Natl Acad Sci U S A* 1992;89:5152-6.
- Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E, Spiegel AM. Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *N Engl J Med* 1991;325:1688-95.
- Yoshimoto M, Nakayama M, Baba T, et al. A case of neonatal McCune-Albright syndrome with Cushing syndrome and hyperthyroidism. *Acta Paediatr Scand* 1991;80:984-7.
- Dumont JE, Jauniaux JC, Roger PP. The cyclic AMP-mediated stimulation of cell proliferation. *Trends Biochem Sci* 1989;14:67-71.
- Kopp P, Kimura ET, Aeschmann S, et al. Polyclonal and monoclonal thyroid nodules coexist within human multinodular goiters. *J Clin Endocrinol Metab* 1994;79:134-9.