0021-972X/97/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 82, No. 3
Printed in U.S.A.

CLINICAL CASE SEMINAR

Detection of an Activating Mutation of the Thyrotropin Receptor in a Case of an Autonomously Hyperfunctioning Thyroid Insular Carcinoma*

DIEGO RUSSO, SALVATORE TUMINO, FRANCO ARTURI, PAOLO VIGNERI†, GIUSEPPE GRASSO, ALFREDO PONTECORVI, SEBASTIANO FILETTI, AND ANTONINO BELFIORE

Dipartimento di Medicina Sperimentale e Clinica, Cattedra di Endocrinologia, and Facoltà di Farmacia, Cattedra di Farmacologia (D.R.), University of Reggio Calabria, Catanzaro; Istituto di Clinica Medica I (S.T.), Istituto di Medicina Interna e di Malattie Endocrine e del Metabolismo, Cattedra di Endocrinologia (A.B., P.V.), University of Catania, Catania; Servizio di Anatomia Patologica, Ospedale S. Pietro e Gravina (G.G.), Caltagirone; and Cattedra di Endocrinologia, Università Cattolica, Laboratorio di Oncogenesi Molecolare, Istituto Tumori Regina Elena (A.P.), Rome, Italy

ABSTRACT

Thyroid carcinomas, even when well differentiated, usually appear as hypofunctioning at scintigraphy. We report a case of an aggressive insular thyroid carcinoma presenting as an autonomously functioning thyroid nodule and causing severe thyrotoxicosis. The tumor was metastatic to a cervical lymph node and both lungs.

An activating mutation of the TSH receptor gene in both the primary tumor and the lymph node metastasis was found, due to a base substitution at codon 633 (normal guanine at position 1896 replaced by cytosine CAC for GAC causing aspartic acid substitution by histidine). Other known oncogenes (*gsp*, *ras*, PTC/*ret*, *trk*, *met*, and p53) were not involved.

This is the first description of an activating TSH receptor mutation in a thyroid hyperfunctioning carcinoma in which an aggressive malignant phenotype coexisted with activation of the cAMP cascade and differentiated thyroid functions. (*J Clin Endocrinol Metab* **82:** 735–738, 1997)

THYROID CARCINOMAS, even when well differentiated, usually appear as cold or hypofunctioning at scintigraphy because their iodine uptake, like other differentiated functions, is retained at a much lower level in tumoral tissue than in the adjacent normal tissue (1–3). Rare reports exist of malignant nodules that are hot at scintiscan, as autonomously functioning thyroid nodules (AFTN) are almost invariably benign thyroid adenomas. Definitive proof of malignancy is lacking in some of these reports (4). The molecular alterations underlying malignant AFTN are unknown.

TSH receptor (TSHR) genetic alterations, responsible for constitutive activation of the cAMP cascade, have been detected in several thyroid diseases, including thyroid hyperfunctioning adenomas and differentiated thyroid carcinomas (5).

We now report a case of a malignant AFTN that had spread to a cervical lymph node and to both lungs and caused thyrotoxicosis. Both the primary tumor and the lymph node metastasis were carrying an activating mutation of the TSHR, suggesting that this type of mutation may play a role in cancer hyperfunction and possibly in the carcinogenic process.

Case Report and Methods

A 60-yr-old woman was referred to us in September 1992 because of a thyroid nodule and hyperthyroidism. She weighed 73 kg and was 146 cm tall. The patient complained of weight loss (10 kg in the last 7 months), palpitations, nervousness, tremor, and fatigue. Her resting pulse rate was 90 beats/min, and her blood pressure was 130/75 mm Hg. Upon neck examination, a 5-cm nodule was evident at the right thyroid lobe. A second nodule was present, approximately 3 cm in size and cranial to the other nodule.

Scintigraphy with 25 μCi ^{131}I showed the presence of two areas of increased iodine uptake corresponding to the two nodules felt at physical examination (Fig. 1). The left lobe was suppressed, but it was detectable and of normal size at ultrasound evaluation. Radioiodine uptake of the neck region was 35% after 6 h and 21% after 24 h. Serum T_4 was 12 μg/dL (normal, 5.7–12.1), T_3 was 2.8 ng/mL (normal, 0.8–1.8), and TSH was less than 0.06 mU/L (normal, 0.4–5.0). On the basis of these findings, a diagnosis of multiple toxic AFTN was made, and surgical removal was advised. In February 1993, a right total lobectomy with removal of the isthmus was carried out. The upper nodule was also removed. At histology, the diagnosis of an insular thyroid carcinoma with a lymph node metastasis was made.

Received October 8, 1996. Revision received November 8, 1996. Accepted November 12, 1996.

Address all correspondence and requests for reprints to: Sebastiano Filetti, M.D., Cattedra di Endocrinologia, Dipartimento di Medicina Sperimentale e Clinica, Via T. Campanella, 88100 Catanzaro, Italy. E-mail: filetti@mbox.vol.it.

* This work was supported by a grant from the Associazione Italiana per la Ricerca sul Cancro (to S.F.).

† Present address: Divisione di Oncologia Sperimentale, Istituto Tumori Milano, Milan, Italy.

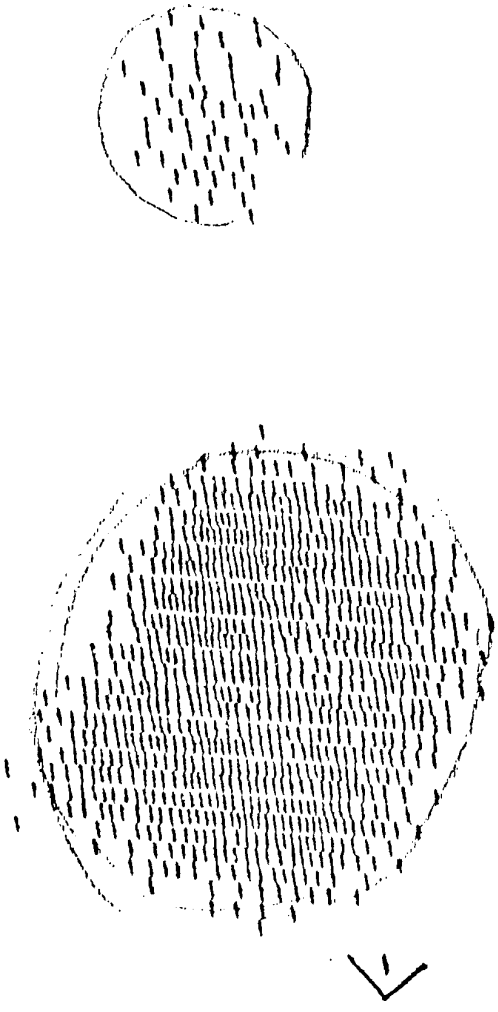

FIG. 1. ^{131}I scintigraphy showing two hot areas at the level of palpable nodules on the right side of the thyroid, with no uptake in the remaining gland.

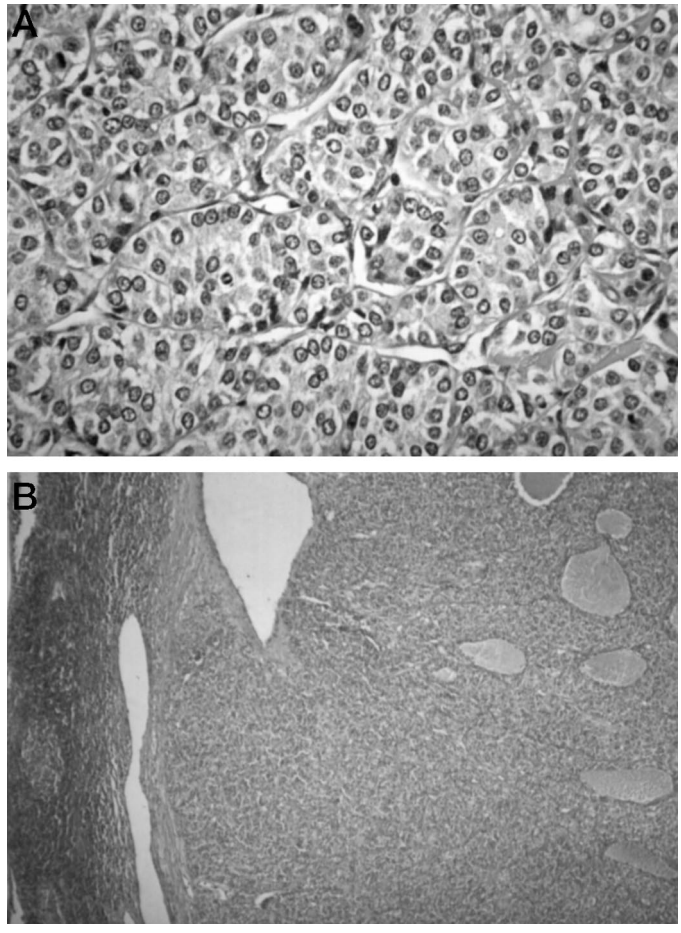

FIG. 2. Histopathological aspects of the primary (A) and the metastatic (B) thyroid tumor (hematoxylin and eosin). A, Neoplastic cells arranged in well defined insulae surrounded by thin fibrovascular septa with artifactual clefts (magnification, ×125); B, thin subcapsular lymphocytic rim in a massively metastatic lymph node. Neoplastic cells are arranged in a solid/trabecular pattern with occasional follicle formation (magnification, ×60).

Histological examination

Macroscopic examination revealed a 6-cm nodule with small necrotic areas at the right thyroid lobe. The second nodule was 3.5 cm. Microscopically, both nodules were constituted by neoplastic cells arranged in solid nests or insulae, surrounded by hyalin stroma (Fig. 2). Trabecular patterns and occasional microfollicles were also present. No papillary patterns were observed. Nuclei showed finely granular chromatin and prominent nucleoli. Grooved nuclei and nuclear inclusions were absent. Mitoses were rare. Focal infiltration of the nodule capsule and vascular infiltration were present. The lymph node excised was totally occupied by the neoplastic tissue, with the exception of a thin subcapsular rim. The diagnosis was insular thyroid carcinoma.

Clinical course

After thyroidectomy the patient appeared clinically euthyroid. Serum free T_4 (FT_4) was 1.0 ng/dL (normal, 0.7–1.7), FT_3 was 4.4 pg/mL (normal, 2.1–5.3), and TSH was less than 0.06 mU/L. The thyroglobulin (Tg) serum level was 513.0 ng/mL. Anti-Tg, antithyroperoxidase, and thyroid-stimulating antibodies were undetectable. Surprisingly, the radioiodine uptake in the neck was very low (5% after 6 h and 3% after 24 h). Therefore, the residual thyroid tissue could not be evaluated by scintigraphy. Urinary iodine excretion was 200 μg/mL. A total body scan with 5 mCi ^{131}I was then carried out and evidenced the left thyroid nodule and diffuse uptake at both lungs. The diagnosis of autonomously functioning thyroid cancer lung metastases suppressing the residual thyroid tissue function was made, and the patient was advised to undergo completion thyroidectomy followed by radioiodine treatment of the lung metastases. The patient refused further treatment until August 1993, when she agreed to undergo completion thyroidectomy. The left thyroid lobe was surgically removed and was found to be unaffected at histology. After completion thyroidectomy, in the absence of L-T_4 therapy, the patient was slightly hyperthyroid. Serum FT_3 was 6.9 pg/mL, and serum TSH was still suppressed (<0.06 mU/L). Serum Tg was 1170 ng/mL. Radioiodine treatment (150 mCi) was then given to treat lung metastases. Six months later, a second radioiodine dose (150 mCi) was given. The posttreatment total body scan showed a small area of uptake in the neck and no uptake in the lung. The patient was hypothyroid (FT_3, 1.9 pg/mL; FT_4, 0.3 ng/dL; TSH, 254.0 mU/L). Serum Tg was 11.2 ng/mL. The patient underwent two more 100-mCi radioiodine treatments at 8-month intervals. At the last control, during suppressive L-T_4 therapy, serum Tg (16.5 ng/mL) was still elevated.

Immunohistochemistry

Formalin-fixed, paraffin-embedded sections (5 mm thick) from neoplastic nodules were cut for staining with a panel of monoclonal antibodies. Immunoperoxidase staining was performed as previously described; counterstaining was with Meyer's hematoxylin. Tg was expressed in approximately 80% of the neoplastic cells, although at a low medium level. The titer of proliferating cells, as evidenced by staining

with an anti-proliferating cell nuclear antigen (PCNA) antibody, was approximately 1:150.

TSHR gene analysis

Genomic DNA was obtained from primary and metastatic tumor tissues and from the normal contralateral lobe using paraffin-embedded tissue, as previously described (6). PCR-amplified fragments encompassing all exon 10 of the TSHR gene were obtained using 500 ng genomic DNA, 250 nmol/L of each primer, 200 mmol/L deoxy-NTPs, *Taq* polymerase buffer, and 1 U *Taq* DNA polymerase (Pelkin-Elmer, Milan, Italy). Forty cycles of denaturation (94 C for 1 min), annealing (58 C for 1 min), and extension (72 C for 1 min) were carried out in a programmable heat block (Pelkin-Elmer, Norwalk, CT). Two couples of primers (Genosys, Cambridge, UK), according to the published sequence of the human TSHR (7), were used to amplify exon 10 of the TSHR gene. The primer oligonucleotides were 24 bases in length: 1) 5′-sense primer, 5′-TTTGACAGCCATTATGACTACACC-3′; 3′-antisense primer, 5′-TTGGAGTTGCTAACAGTGATGAGA-3′; and 2) 5′-sense primer, 5′-GCCTCTGTAGACCTCTACACTCAC-3′; 3′-antisense primer, 5′-GTTTTCAATCAGTTCATAGACATC-3′. To confirm the presence of a mutation, we also used primers closer to the mutated TSHR region and carried out sequence analysis in both sense and antisense directions. DNA sequencing was carried out by the dideoxynucleotide method (8), using the double strand DNA cycle sequencing system kit (Promega, Florence, Italy).

Results

The sequence of the PCR-amplified products encompassing the entirety of exon 10 of the TSHR gene revealed the presence of a base substitution at codon 633 (Fig. 3) of the TSHR gene in both the primary tumor and the metastatic lymph node, but not in normal thyroid tissue of the contralateral lobe. The normal guanine at position 1896 was replaced by cytosine (CAC for GAC), causing aspartic acid substitution by histidine. The mutation was located in the sixth transmembrane domain of the receptor in an area important for signal transduction.

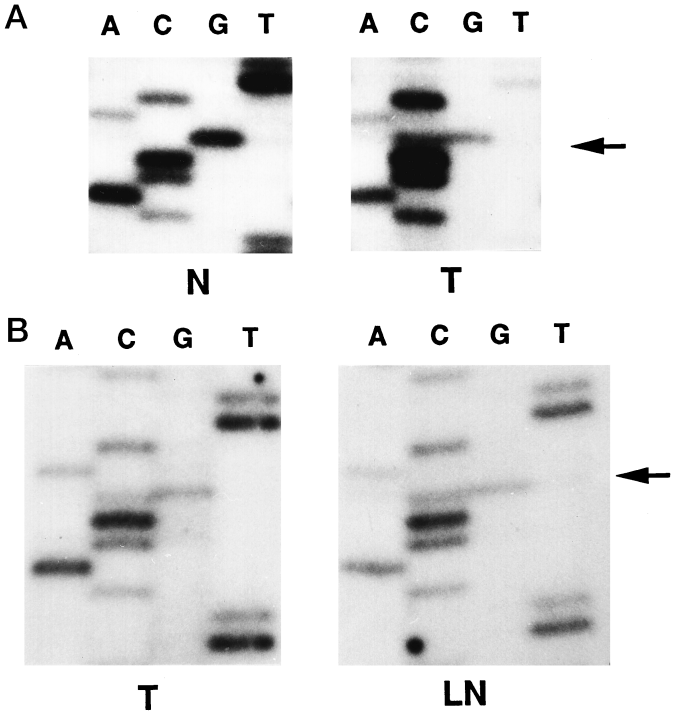

FIG. 3. Presence of a point mutation in codon 633 of the TSHR gene. A, *Right*, Tumoral tissue (T; coexistence of GAG and CAC); *left*, normal thyroid tissue from the same individual (N). B, *Left*, Tumoral tissue (T); *right*, lymph node metastasis from the same individual (LN).

Other oncogenes were also examined, but no alterations were detected at the gene (*gsp*, *ras*, PTC/*ret*, and *trk*) or protein (*met* and p53) levels (data not shown).

Discussion

Differentiated thyroid cancer usually has a reduced iodine uptake and a reduced function (1, 3). Therefore, it rarely causes hyperthyroidism. This may occur when either a well differentiated cancer is concomitant to Graves' disease and tumor metastases are stimulated by thyroid-stimulating antibodies (9) or differentiated metastases are bulky, especially when a large amount of iodine is available for the (hypo)-functioning large tumoral mass (10, 11). A third, more rare occurrence is a cancer presenting as hyperfunctioning AFTN (hot nodule), where the malignant phenotype coexists with hyperactivated differentiated thyroid functions (4).

The case described here provides evidence that an activating mutation of the TSHR gene may be responsible for autonomous hyperfunction of a thyroid carcinoma. A base substitution at codon 633 of the TSHR gene was present in both the primary tumor and the lymph node metastasis, but not in the normal contralateral tissue. Mutations at this codon were previously reported to constitutively activate the cAMP cascade (12). This mutation was probably also present in the lung metastases, because after surgery, lung metastases produced enough thyroid hormone to cause hyperthyroidism and suppress iodine uptake in the residual lobe.

The constitutive activation of the cAMP cascade, caused by mutations at the level of either the *gsp* or the TSHR gene, has been detected in AFTN (13–19). It is still unknown whether such mutations are responsible for both the autonomous growth and the hyperfunction of the adenomas (20, 21). Activating mutations of either *gsp* or TSHR have also been reported in a small proportion of thyroid carcinomas (22, 23). These carcinomas, however, were cold at scintigraphy, possibly because of concomitant activation of other oncogenes (*e.g. ras*) and the consequent suppression of the differentiated functions (23).

The present case represents the first description of an activating TSHR gene mutation in a hyperfunctioning carcinoma. Such a mutation is believed to provide constitutive thyroid function to an insular carcinoma. The insular phenotype is considered a variant of poorly differentiated carcinomas and has been reported to have a recurrence/metastasis rate of 60% and a 10-yr mortality rate of 13% (24–26). In the present case, however, the tumor retained the biochemical ad structural machinery necessary for hormone synthesis and secretion. It is likely that other undetected oncogene abnormalities were involved in determining the malignant phenotype of the tumor; however, they did not impair the expression of differentiated functions. We investigated a variety of other oncogenes (*gsp*, *ras*, PTC/*ret*, *trk*, *met*, and p53), but none was found to be abnormal.

In conclusion, we found that a mutation of the TSHR gene may explain the activation of differentiated thyroid functions

in an aggressive and metastatic insular carcinoma that presented as an AFTN causing symptomatic hyperthyroidism. The activation of the cAMP cascade and of differentiated functions may, therefore, coexist with an aggressive malignant behavior, as previously described in thyroid cancer associated with Graves' disease (9, 27). The role of activating mutations of the TSHR gene in thyroid carcinogenesis is still unclear (5) and requires further study.

References

1. **Pinsky SM.** 1986 Current status of thyroid imaging. In: Freeman LM, ed. Current concepts in diagnostic nuclear medicine, vol 3, no. 1. Chicago: MacMillan Professional Journals; 4–12.
2. **Belfiore A, La Rosa GL, Giuffrida D, et al.** 1995 The management of thyroid nodules. J Endocrinol Invest. 18:155–158.
3. **Fabbro D, Di Loreto C, Beltrami CA, Belfiore A, Di Lauro R, Damante G.** 1994 Expression of thyroid specific transcription factors TTF-1 and PAX-8 in human thyroid neoplasm. Cancer Res. 54:4744–4749.
4. **Rubenfeld S, Wheeler TM.** 1988 Thyroid cancer presenting as a hot thyroid nodule: report of a case and review of the literature. Thyroidology. 1:63–68.
5. **Russo D, Arturi F, Filetti S.** 1996 Thyroid-stimulating hormone receptor gene mutations and polymorphism in thyroid diseases. Curr Opin Endocrinol Diabetes. 3:428–432.
6. **Russo D, Arturi F, Wicker R, et al.** 1995 Genetic alterations in thyroid hyperfunctioning adenomas. J Clin Endocrinol Metab. 80:1347–1351.
7. **Nagayama Y, Kaufman KD, Seto P, Rapoport B.** 1989 Molecular cloning, sequence and functional expression of the cDNA for the human thyrotropin receptor. Biochem Biophys Res Commun. 165:1184–1190.
8. **Sanger F, Nicklen S, Coulson AR.** 1977 DNA sequencing with chain terminating inhibition. Proc Natl Acad Sci USA. 82:488–492.
9. **Filetti S, Belfiore A, Amir S, et al.** 1988 Thyrotoxicosis due to thyroid cancer: role of thyroid stimulating antibodies. N Engl J Med. 318:753–759.
10. **Gross JL, Vasques Moraes I.** 1996 Thyroid hormone-producing metastases in differentiated thyroid cancer. J Endocrinol Invest. 19:21–24.
11. **Ehrenheim Ch. Heintz P, Schober O, Schicha H, Hundeshagen H.** 1986 Jodinduzierte T_3-Hyprthyreose beim metastasierenden follikularen Schilddrusenkarzinom. Nuklearmedizin. 25:201–204.
12. **Kosugi S, Shenker A, Mori T.** 1994 Constitutive activation of cyclic AMP but not phosphatidylinositol signaling caused by four mutations in the 6th transmembrane helix of the human thyrotropin receptor. FEBS Lett. 356:291–294.
13. **Lyons J, Landis CA, Harsh G, et al.** 1990 Two G protein oncogenes in human endocrine tumors. Science. 249:655–659.
14. **O' Sullivan C, Barton CM, Staddon SL, Brown CL, Lemoine NR.** 1991 Activating point mutations of the *gsp* oncogene in human thyroid adenomas. Mol Carcinogen. 4:345–349.
15. **Said S, Schlumberger M, Suarez HG.** 1994 Oncogenes and anti-oncogenes in human epithelial thyroid tumors. J Endocrinol Invest. 17:371–379.
16. **Parma J, Duprez L, Van Sande J, et al.** 1993 Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature. 365:649–651.
17. **Porcellini A, Ciullo I, Laviola L, Amabile G, Fenzi G, Avvedimento VE.** 1994 Novel mutations of thyrotropin receptor in thyroid hyperfunctioning adenomas. Rapid identification by fine needle aspiration biopsy. J Clin Endocrinol Metab. 79:657–661.
18. **Paschke R, Tonacchera M, Van Sande J, Parma J, Vassart G.** 1994 Identification and functional characterization of two new somatic mutations causing constitutive activation of the thyrotropin receptor in hyperfunctioning autonomous adenomas of the thyroid. J Clin Endocrinol Metab. 79:1785–1789.
19. **Russo D, Arturi F, Suarez HG, et al.** 1996 Thyrotropin receptor gene alterations in thyroid hyperfunctioning adenomas. J Clin Endocrinol Metab. 81:1548–1551.
20. **Van Sande J, Parma J, Tonacchera M, Swillens S, Dumont J, Vassart G.** 1995 Somatic and germline mutations of the TSH receptor gene in thyroid diseases. J Clin Endocrinol Metab. 80:2577–2585.
21. **Derwahl M, Hamacher C, Russo D, et al.** 1996 Constitutive activation of the $G_s\alpha$ protein-adenylate cyclase pathway may not be sufficient to generate toxic adenomas. J Clin Endocrinol Metab. 81:1898–1904.
22. **Suarez HG, duVillard JA, Caillou B, Schlumberger M, Parmentier C, Monier R.** 1991 *gsp* mutations in human thyroid tumours. Oncogene. 6:677–679.
23. **Russo D, Arturi F, Schlumberger M, et al.** 1995 Activating mutations of the TSH receptor in differentiated thyroid carcinomas. Oncogene. 11:1907–1911.
24. **Carcangiu ML, Zampi G, Rosai J.** 1984 Poorly differentiated ("insular") thyroid carcinoma. Am J Surg Pathol. 8:655–667.
25. **Flynn SD, Forman BH, Stewart AF, Kinder BK.** 1986 Poorly differentiated ("insular") carcinoma of the thyroid gland: an aggressive subset of differentiated thyroid neoplasms. Surgery. 104:963–970.
26. **Papotti M, Botto Mica F, Favero A, Palestini N, Bussolati G.** 1993 Poorly differentiated thyroid carcinomas with primordial cell component. Am J Surg Pathol. 17:291–301.
27. **Belfiore A, Garofalo MR, Giuffrida D, et al.** 1990 Increased aggressiveness of thyroid cancer in patients with Graves' disease. J Clin Endocrinol Metab. 70:830–835.