Case Report

Concurrent Lymph Node Metastases of Medullary and Papillary Thyroid Carcinoma in a Case with *RET* Oncogene Germline Mutation

G. Papi, MD,[1] S. Corrado, MD,[2] M. G. Pomponi, MD,[3] C. Carapezzi, MD,[1] A. Cesinaro, MD,[2] and V. A. LiVolsi, MD[4]

Abstract

We report the case of a 72 yr-old woman who underwent total thyroidectomy and resection of neck lymph nodes because of a firm nodule in the right lobe, which was consistent with medullary thyroid carcinoma (MTC) on cytological examination. Histology showed multifocal bilateral MTC; a 2 mm papillary thyroid carcinoma (PTC) was also detected in the right lobe, next to a focus of MTC; five cervical lymph nodes contained MTC. In one right perithyroidal lymph node, concurrent metastases of MTC and PTC were demonstrated. DNA analysis showed a point mutation in exon 14 at codon 804 of the *RET* proto-oncogene locus, as frequently found in cases of familial MTC (FMTC).

To our knowledge, this case represents the first documented case of concurrent lymph node metastases of MTC and PTC in a patient with *RET* proto-oncogene germline mutation. We report this unique case, discuss related thyroid malignancies, and suggest possible underlying pathogenetic mechanisms.

Key Words: Thyroid; medullary carcinoma; papillary carcinoma; lymph node.

Introduction

Medullary carcinoma of the thyroid (MTC) is an uncommon thyroid tumor, comprising 5–10% of all thyroid malignancies [1]. MTC evolves from the neural crest–derived parafollicular C-cells, and occurs sporadically in most patients (80%); the remaining 20% have a familial background [2]. It has been demonstrated that distinct germline mutations in the *RET* proto-oncogene—which encodes a transmembrane receptor with cytoplasmic tyrosine kinase activity—are associated with the dominantly inherited cancer syndromes multiple endocrine neoplasia types 2A and 2B (MEN 2A and MEN 2B) and familial medullary thyroid carcinoma (FMTC), all sharing MTC as part of the disease phenotype [3].

Papillary carcinoma of the thyroid (PTC) occurs more frequently than MTC, accounting for approx 80% of all thyroid malignancies [1]; it is a well-differentiated carcinoma of the follicular cells, is assumed to be of endodermal derivation [4], and has a better prognosis than MTC.

The simultaneous occurrence of two distinct neoplasms derived from different cells of origin is well known, but it rarely occurs in the thyroid. In particular, few papers exist in the literature that describe the coexistence of MTC and PTC in the same patient [5–12], some of these report concurrent lymph node metastases of both tumors [9–11].

To our knowledge, no case has ever been described of concurrent lymph node metastases of MTC and PTC in a patient

[1]Department of Internal Medicine, ASL Modena, [2]Department of Morphologic and Forensic Sciences, Section of Pathology, University of Modena, [3]Department of Genetics, Catholic University of Rome, Italy, and [4]Department of Pathology, University of Pennsylvania Medical Center, Philadelphia, PA, USA.

Address correspondence to Dr. Giampaolo Papi, Dipartimento di Medicina Interna-Reparto di Medicina 1, Ospedale Ramazzini, Via S. Giacomo, 2-41012 Carpi (Modena)-Italy. E-mail: papigiampaolo@hotmail.com

Endocrine Pathology, vol. 14, no. 3, 269–276, Fall 2003

1046–3976/03/14:269–276/ $25.00

with *RET* proto-oncogene germline mutation. In the present paper, we report this unique case, discuss related thyroid malignancies, and suggest possible underlying pathogenetic mechanisms.

Report of a Case

A 72-yr-old Caucasian woman was referred for a swelling in her anterior neck. She did not complain of pressure symptoms, and did not manifest recurrent laryngeal nerve injury or signs/symptoms of thyroid dysfunction. She was diagnosed with depression, but was not taking medication. Pertinent family history included a maternal aunt who died of a malignant tumor of the neck, but if was unclear if this arose from the thyroid. The patient had a 78-yr-old sister with Alzheimer's disease, and a 45-yr-old healthy son.

On physical examination, a hard mass measuring about 20 mm was palpated in the right thyroid lobe; no cervical adenopathy was detected. Ultrasonography showed a $2 \times 2 \times 1.6$ cm hypoechoic nodule in the right thyroid lobe; neck lymph node enlargement was also bilaterally evident, the largest node measuring $1.5 \times 1 \times 1$ cm. Fine needle aspiration biopsy (FNAB) of the thyroid nodule was performed; cytological examination was consistent with MTC.

Lab data included: Thyrotropin (TSH) 2.1 μUI/mL (n.v.: 0.27–4.2); free-triiodotironine (FT3) 3.1 pg/mL (n.v.: 1.5–4.2); free-thyroxine (FT4) 1.3 pg/mL (n.v.: 0.6–1.8); calcitonin 1500 pg/mL (n.v.: <10); carcinoembryonic antigen (CEA) 35 μg/L (n.v.: <5). Parathormone (PTH) plasma values and 24-h urinary metanephrine and catecholamines excretion were normal.

The patient underwent total thyroidectomy with resection of neck lymph nodes in the central and lateral compartments, without postsurgical complications.

The final pathological diagnosis was: "Bilateral MTC with cervical lymph node metastases (pT2N1Mx); concurrent PTC classic variant, with cervical lymph node metastasis (pT1N1Mx)."

At 3-mo postoperative follow-up, the patient is taking L-thyroxine 125 μg/day, and is asymptomatic; lab analysis shows: TSH 0.2 mIU/mL, FT4 1.6 pg/mL, calcitonin 360 pg/mL, CEA 5.5 mcg/L, thyroglobulin (TG) 1 ng/mL, anti-TG antibodies (TG-Ab) 15 IU/L. Ultrasound of the neck, computed tomography (CT) scans of head, thorax, and abdomen, and ^{111}I-pentetreotide whole body scan (WBS) did not demonstrate any evidence of local recurrence or distant metastases; ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) showed a radionuclide uptake within the thorax, consistent with mediastinal and hilar lymph node metastases.

The patient is going to have ^{131}I-WBS to eventually ablate residual thyroid, and has been offered external radiation therapy to the neck and upper mediastinum.

Materials and Methods

Ultrasound examination was performed using an Esaote Biomedica instrument with 7.5-MHz linear probe. FNAB was ultrasound-guided and was obtained with 25-gauge needle. Multiple direct smears were prepared; air-dried samples were stained by the May–Grumwald–Giemsa method, and the alcohol-fixed samples were stained by the Papanicolau method.

Serum TSH (0.005 μUI/mL representing the lowest detection limit), FT3, FT4, and CEA concentrations were determined using an electrochemiluminescence immunoassay. Calcitonin plasma levels were measured through a modified two-site

radioimmunometric assay. Serum TG concentrations (0.5 ng/mL representing the lowest detection limit) were determined by an immunoradiometric assay involving monoclonal antibodies; TG-Ab levels (n.v. <100 IU/L) were measured by an hemoagglutination assay.

During surgery, the entire thyroid as well as cervical lymph nodes of paratracheal and tracheoesophageal areas and of jugulocarotid chain were resected; frozen sections disclosed the tumor. All surgical specimens were fixed overnight in 10% neutral buffered formalin and paraplast-embedded. For histological examination, 5-μm-thick sections were cut, and stained with hematoxylin and eosin (HE) and Congo red.

Immunohistochemistry was performed using the streptavidin–biotin method and antibodies directed against the following antigens: chromogranin (CH) (clone LK2H10; NeoMarkers, Fremont, CA), CT (Euro Diagnostica, Milan, Italy), TG (Clone 2H11+6E1; Cell Marque, Austin, TX), and (after the demonstration of PTC) CK19 (Novocastra, Newcastle upon Tyne, U.K.). Blank sections of two previously diagnosed cases of MTC and PTC, found in our files, were stained with the same antibodies, and served as positive controls. Negative control specimens were thyroids from two patients with nodular goiter.

The patient, her sister, and her son were screened for germline mutations in exons 10, 11, 13, 14, and 16 of the *RET* proto-oncogene. DNA analyses were performed in our laboratory, as follows: mutations in *RET* were detected in leukocyte genomic DNA by direct sequencing of amplified individual exons and flanking intron sequences. Polymerase chain reactions (PCR) were carried out under standard conditions, containing 50 ng of genomic DNA, 1 m*M* of forward and reverse primers, and 10 μL of Promega Taq Master Mix, as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C (1 min), annealing at 55–65°C (1 min), and extension at 72°C (1 min), followed by final extension at 72°C for 5 min, using the Gene Amp PCR System 9600-Perkin Elmer. PCR products were purified with a PCR product presequencing kit, composed of exonuclease 1 and shrimp alkaline phosphatase, by USB Corporation, according to their instructions, and later using the Centri-Sep Spin Columns by Applied Biosystems. Sequencing reactions were carried out using the ABIPRISM dye terminator cycle sequencing kit, and the products were analyzed on an ABIPRISM 310 Genetic Analyzer DNA sequencer. Sequences were analyzed manually.

Results

Cytological examination of the FNAB revealed highly cellular smears, on a background of blood and scarce colloid. At low-power observation, the overall impression was that of a neoplasm with low cellular cohesion. The aspirates contained a population of several single cells, rarely joining in loose groups. The prevalent cell shape was plasmacytoid, and binucleate cells were often seen as well. The cytoplasm was abundant and pale, while the nuclei contained hyperchromatic chromatin. These features led to the cytological diagnosis of MTC.

During operation, debulking of the thyroid gland and nodal tissues was relatively easy: no adherence to the perithyroidal tissues was noted. Metastatic disease was found in 4 of 23 lymph nodes, the largest node measuring 1.5 × 1 × 1 cm, all in the central compartment. At gross examination, the left thyroid lobe measured 5 × 4 × 3 cm, and did not apparently show remarkable changes; the right lobe mea-

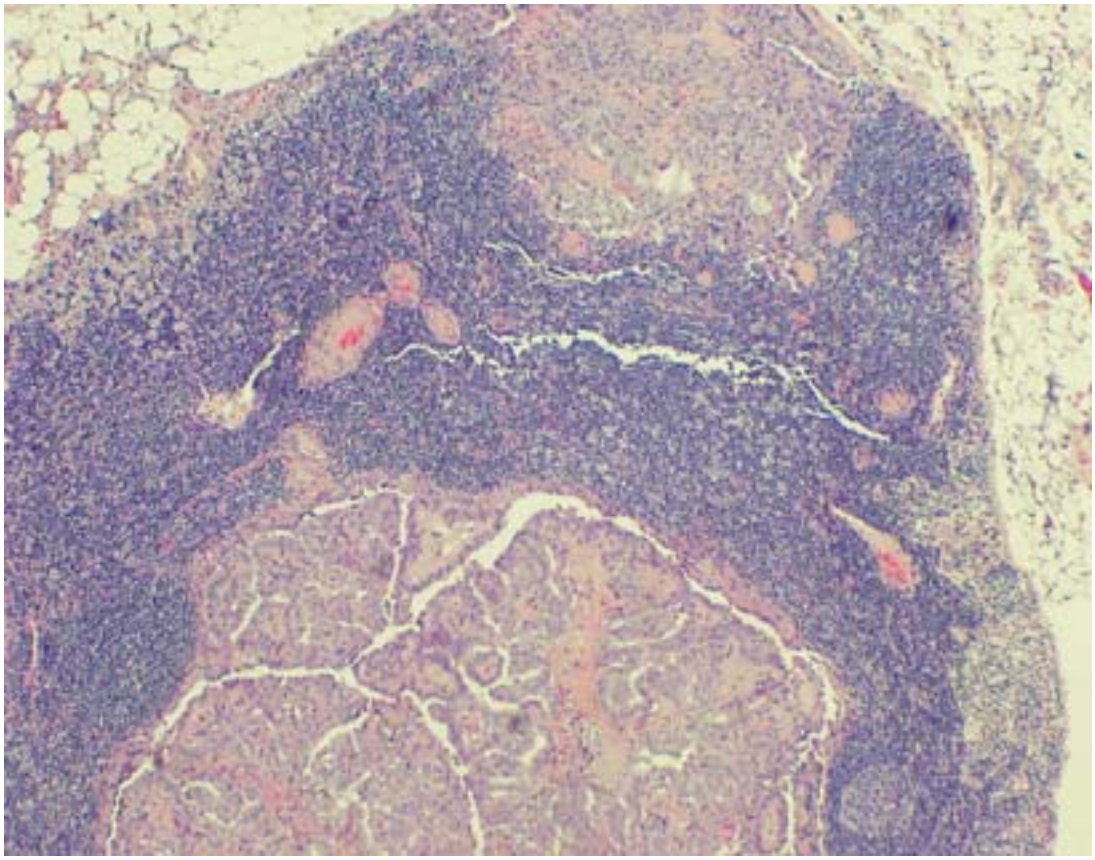

Fig. 1. Cervical lymph node. In the upper side of the figure, a metastasis of MTC is evident; in the lower side, a PTC focus can be seen. (HE stain, 400×, original magnification.)

sured 5.5 × 4.5 × 2.5 cm, and contained a 2 × 2.2 × 1.5 cm yellow, firm nodule. Although not encapsulated, it was well demarcated from the surrounding normal thyroid parenchyma.

Histologic examination of the right lobe showed a tumor composed of plasmacytoid cells, exhibiting well-defined cell borders, light eosinophilic cytoplasm, and round hyperchromatic nuclei with a fine chromatin pattern. Neoplastic cells were arranged in solid nests and sheets, closely approaching and in some areas invading the thyroid capsule. Within the stroma—which was densely fibrotic—and between tumor cells, abundant Congo-red-positive amyloid deposits were present. In the left lobe, an identical 2 mm neoplasm was observed. Furthermore, surrounding the two neoplasms, numerous areas of C-cell hyperplasia were detected. The diagnosis of MTC was made. Metastases that showed the same cytological and histological characteristics of primary MTC were demonstrated in a total of five cervical lymph nodes (four in the central compartment, and one in the jugulocarotid chain). In one right perithyroidal lymph node, beside the metastasis of MTC, another neoplastic area was observed; the tumor was well demarcated from the surrounding normal lymph node parenchyma, exhibited a papillary architecture, and was composed of cells containing ground-glass nuclei, intranuclear grooves, and inclusions (Fig. 1): this picture was consistent with metastasis of PTC. Further histologic evaluation demonstrated a 2 mm PTC at the right thy-

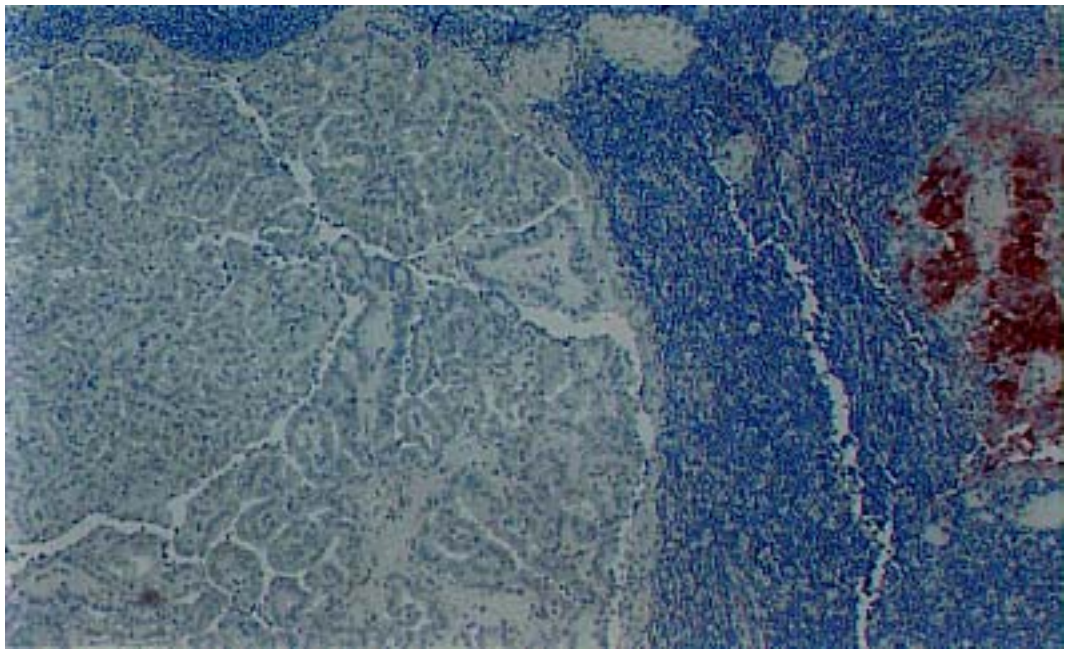

Fig. 2. Cervical lymph node. The nodal architecture was completely destroyed by the presence of two tumor types: on the left, PTC was negative for calcitonin stain; on the right, MTC stained for calcitonin. (Calcitonin stain, 400×, original magnification.)

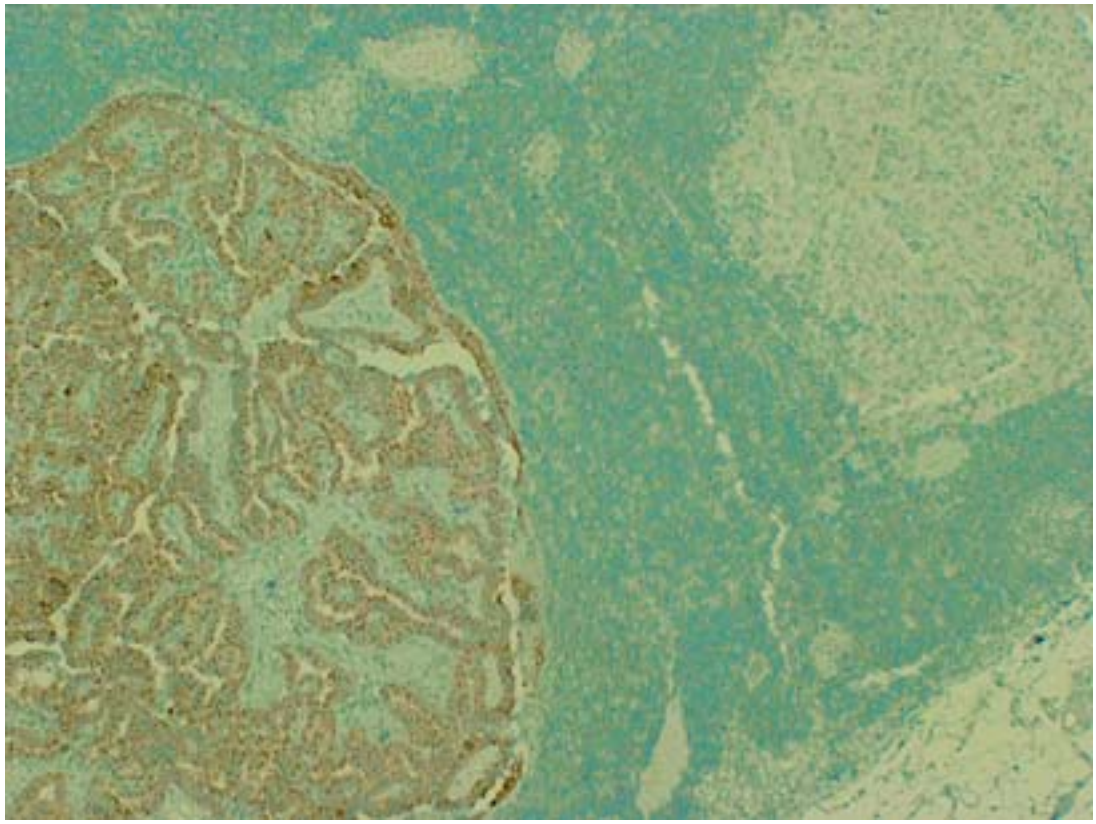

Fig. 3. Cervical lymph node. PTC metastasis (on the left) stained positively to thyroglobulin, whereas MTC (on the right) was negative for thyroglobulin stain. (Thyroglobulin stain, 400×, original magnification.)

roid lobe, growing next to MTC; no other PTC areas were detected within the gland.

The immunostaining results were identical in the primary thyroid tumors and in the lymph node metastases, showing the presence of two distinct cell populations. MTC cells showed intense staining for CT and CH, and a negative reaction for TG and CK19 (Fig. 2). In contrast, PTC stained for CK19 and TG (Fig. 3), and was negative for CT and CH.

DNA analysis of peripheral blood leukocytes for *RET* proto-oncogene germline mutations showed a point mutation (Val→Met) in exon 14 at codon 804. The same genetic analysis was performed on the patient's sister and son. *RET* proto-oncogene mutations were not found.

Discussion

In this article, a unique case of concurrent lymph node metastases of MTC and PTC in an woman with *RET* proto-oncogene germline mutation is reported. A few reports in the literature describe the simultaneous occurrence of MTC and PTC [5–12], in both the thyroid alone and in extrathyroidal sites as well; in no case has an association with *RET* proto-oncogene germline mutations been described.

It is well known that MTC and PTC have different histogeneses, the former being of neuroectodermal derivation, and the latter of endodermal origin. Hence, the occurrence of both forms of tumor, with different cells of origin, in the same patient is more likely to be coincidental rather than caused by a common pathogenetic mechanism. However, a number of reports have described thyroid malignancies that exhibit histological and immunohistochemical features of both medullary and follicular cells [6,13,14]; this led to the introduction of a new entity termed "mixed medullary-follicular carcinoma" in the WHO classification of thyroid neoplasms in 1988 [4].

The definition itself of mixed thyroid tumors still remains controversial. Some authors [7] suggest that tumors exhibiting only one cell type identifiable at light microscopy but dual differentiation at immunohistochemistry be regarded as true mixed tumors, whereas tumors with histological evidence of both follicular and medullary components be regarded as composite tumor. On the other hand, it has been clearly established that a conclusive diagnosis of mixed follicular-medullary carcinoma can be made only if metastases exhibiting dual differentiation similar to the tumor of origin are demonstrated [15]. Various hypotheses (the "stem cell theory," the "divergent differentiation theory," the "collision theory," the "field effect hypothesis," the "hostage theory") have been postulated about the pathogenesis of mixed thyroid tumors [16]. The results of most recent studies [16] provide strong evidence that the follicular and medullary components in mixed medullary-follicular carcinomas exhibit a different pattern of mutations, allelic losses, and clonal composition, and therefore are not derived from a single progenitor cell.

In our case, MTC and PTC were spatially distinct tumors, although they were detected in the same thyroid lobe, next to one another. Each tumor contained cytological, histological, and immunohistochemical features peculiar to only one line of cell differentiation, in both the primary focus and the nodal metastatic deposits. No collision phenomenon was present [9]. Therefore, our case is best defined as "concurrent" (neither "mixed" nor "composite") papillary and medullary thyroid carcinomas. Previous papers reported MTC and PTC in the same thy-

roid lobe [5], but the characteristic of our case is that the two tumors metastasized to the same lymph node, and no other separate nodal PTC metastases were demonstrated. This finding may suggest a common preferential lymphatic drainage from both neoplasms: it is conceivable that MTC may carry some unknown trophic factor triggering neoplastic transformation of contiguous follicular epithelial cells.

Another possible pathogenetic mechanism may be represented from a common tumorigenic stimulus determining the development of both MTC and PTC. The familial occurrence of papillary thyroid microcarcinomas has been identified [17]: interestingly, these familial forms of papillary carcinomas show an unfavorable behavior: in our case, a microscopic PTC produced cervical lymph node metastasis. Quite recently, the association of familial forms of MTC and PTC was found in 30% of patients undergoing thyroidectomy for MTC [18]. In these situations, genetic analyses excluded the possible germline involvement of *RET* proto-oncogene. On the contrary, in our case a point mutation in exon 14 of *RET* proto-oncogene was detected: such mutation is usually found in FMTC [3]. The negative screening tests for pheochromocytoma and parathyroid disease excluded a MEN 2 syndrome. These results support a diagnosis of FMTC, although the patient's immediate relatives had negative tests.

The genetic background of this patient opens a suggestive scenario on the pathogenesis of concurrent MTC and PTC in our case. In fact, a very recent study by Brauckhoff et al. [12] described 104 patients with *RET* proto-oncogene germline mutations who underwent prophylactic surgery at their center. They found a 9.1% frequency of PTC in all patients with *RET* germline mutation in codon 790, 791, and 804, but in none of the patients with mutations not affecting such codons (p = 0.0015); the authors supposed a potential pathogenetic role of exon 13 and 14 *RET* mutations with regard to the development of PTC. Unfortunately, owing to the microscopic size of the tumor, we were not able to test for a *RET/PTC* translocation in the PTC of our patient: absence of such mutation would strengthen the plausibility of a pathogenetic role for the *RET* proto-oncogene mutation.

In conclusion, we reported a unique case of concurrent lymph node metastases of MTC and PTC in a patient with *RET* proto-oncogene germline mutation in codon 804, peculiar to FMTC. Although it is likely that the coexistence of the two malignancies in the thyroid of the same patient is coincidental, we suggest that *RET* mutation might play a pathogenetic role in the development of both tumors, and not only in MTC.

References

1. Heshmati HM, Gharib H, van Heerden JA, Sizemore GW. Advances and controversies in the diagnosis and management of medullary thyroid carcinoma. Am J Med 103:60–69, 1997.
2. Murray D. The thyroid gland. In: Kovacs K, Asa S, eds. Functional endocrine pathology, vol. 1. Boston: Blackwell, pp. 293–374, 1991.
3. Eng C, Clayton D, Schuffenecker I, et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA 276:1575–1579, 1996.
4. Hedinger C, Williams ED, Sobin LH. The WHO histological classification of thyroid tumors: a commentary on the second edition. Cancer 63:908–911, 1989.
5. Lamberg BA, Reissel P, Stenman S, et al. Concurrent medullary and papillary thyroid carcinoma in the same thyroid lobe and in siblings. Acta Med Scand 209:421–424, 1981.
6. Albores-Saavedra J, de la Mora TG, Gould E. Mixed medullary-papillary carcinoma of the

thyroid: a previously unrecognized variant of thyroid gland. Hum Pathol 21:1151–1155, 1990.
7. Apel RL, Alpert LC, Rizzo A, LiVolsi V, Asa SL. A metastasizing composite carcinoma of the thyroid with distinct medullary and papillary components. Arch Pathol Lab Med 118:1143–1147, 1994.
8. Matias-Guiu X, Caxas A, Costa I, Cadezas R, Prat J. Compound medullary-papillary carcinoma of the thyroid: true mixed tumour. Histopathology 25:183–185, 1994.
9. Pastolero GC, Coire CI, Asa SL. Concurrent medullary and papillary carcinomas of the thyroid with lymph node metastases. A collision phenomenon. Am J Surg Pathol 20:245–250, 1996.
10. Shimizu M, Hirokawa M, LiVolsi VA, et al. Combined "mixed medullary-follicular" and "papillary" carcinoma of the thyroid with lymph node metastasis. Endocr Pathol 11:353–358, 2000.
11. Shiroko T, Yokoo N, Okamoto K, et al. Mixed medullary-papillary carcinoma of the thyroid with lymph node metastases: report of a case. Surg Today 31:317–321, 2001.
12. Brauckhoff M, Gimm O, Hinze R, Ukkat J, Brauckhoff K, Dralle H. Papillary thyroid carcinoma in patients with RET proto-oncogene germline mutation. Thyroid 12:557–561, 2002.
13. Hales M, Rosenau W, Okerlund MD, Galante M. Carcinoma of the thyroid with a mixed medullary and follicular pattern. Morphologic, immunohistochemical, and clinical laboratory studies. Cancer 50:1352–1359, 1982.
14. Ljungberg O, Ericsson UB, Bodenson L, Thorell J. A compound follicular-parafollicular cell carcinoma of the thyroid: a new tumor entity? Cancer 52:1053–1061, 1983.
15. LiVolsi VA. Mixed thyroid carcinoma: a real entity? Lab Invest 57:237–239, 1987.
16. Volante M, Papotti M, Roth J, et al. Mixed medullary-follicular thyroid carcinoma. Molecular evidence for a dual origin of tumor components. Am J Pathol 155:1499–1509, 1999.
17. Lupoli G, Vitale G, Caraglia M, et al. Familial papillary thyroid microcarcinoma: a new clinical entity. Lancet 353:637–639, 1999.
18. Fugazzola L, Cerutti N, Mannavola D, et al. Multigenerational familial medullary thyroid cancer (FMTC): evidence for FMTC phenocopies and association with papillary thyroid cancer. Clin Endocrinol (Oxf) 56:53–63, 2002.