Supplemental figure 1: Large and bilateral tumors observed in the RET C634Y/Y791F mutation carriers. **Top:** CT scan showed bilateral large adrenal masses of patient F1-II.1, the index-case of family 1. Pathologic data revealed bilateral adrenal pheochromocytomas measuring 14 x 11 cm and weighting 640 g at the right and 8 x 6 cm weighing 90 g at the left. **Bottom:** Patient F3-II.1, the index-case of family 3, developed bilateral pheochromocytoma measuring 8.0 x 6.5 x 6.0 cm at the left and 4.5 x 2.9 cm at the right.
**SUPPLEMENTAL DATA**

**Clinical approach**

The diagnosis of MTC was made when the serum levels of basal or stimulated calcitonin (calcium test) were higher than 100 pg/ml. Cervical ultra sound was conducted to identify thyroid nodules and investigate compromised lymph nodes. Suspected thyroid nodules were biopsied mainly in the index cases before knowledge of MTC or MEN 2A in these families. Immunohistochemistry for calcitonin was performed in all cases. Total thyroidectomy, cervical lymphadenectomy and cervical thymectomy (with resection of lymph nodes) were performed in the cases followed by us at the Head and Neck Division of our institution. RET mutation-positive carriers were submitted to thyroid surgery independent of calcitonin serum levels according to the recommendation of the Consensus of the international MEN workshop 1999 (ref - 3). Patients were considered biochemically cured when serum levels of basal and stimulated calcitonin were undetectable (<2 pg/mL; immunochemiluminometric assay, DPC, CAL. Pheochromocytoma was diagnosed by abnormal and high levels of serum or urinary cathecolamines (epinephrine, norepinephrine and dopamine) and/or their metabolites (vanylmandelic acid and metanephrine). Abdominal MRI or CT and MIBG scintigraphy were provided in order to identify radiological characteristics of pheochromocytoma (localization, size, extension, bilaterality and metastasis). When pheochromocytoma was present, adrenalectomy was performed either by lombotomy or endoscopy after a period with alpha-blocker (prazosin 8-10 mg/daily). Short term cure criteria were established when all tumoral tissue was removed, including bilateral pheochromocytoma, and the biochemical markers were persistently normal after surgery. Patients have been followed up once a year after surgery. Hyperparathyroidism was excluded based on repeatedly normal serum levels of calcium and PTH.
Index case 1 (F1-II.1)

A 34 year-old woman presented with fever, weight loss (14 kg) and a large axillary mass (11 cm) that was diagnosed as a nodular sclerotic form of type 1 Hodgkin lymphoma by lymph node/bone marrow biopsy. There were also multiple enlarged lymph nodes in the retroperitoneum (up to 6.0 cm) and a hepatic nodule due to lymphoma metastasis. She also presented palpitations, sweating and repeated peaks of high blood pressure (140 X 90) caused by physical effort. CT scan showed bilateral large adrenal masses presenting typical features of pheochromocytoma. Serum and urinary epi- (1.818 pg/mL, n.v = 0.75 pg/mL; 328 pg/mL; n.v= 0.5-20 pg/mL) and nor-epinephrines (8.265 pg/mL, 40-268 pg/mL; 1.060 µg/mL, n.v. = 14-80 µg/mL) and urinary metanephrines/dopamine (45.2 µg/mg, n.v = 0.05-1.2 µg/mg / 2.096 µg/mg, n.v = 65-400 µg/mg), as well as 24h-urinary vanillylmandelic acid (149.000 µg/mL, n.v = 1.100-7.100 µg/mL) were exceedingly high. Prazosin was used (10 mg/day) to control the signs and symptoms of the excessive alpha-adrenergic activity. She was subsequently submitted to lumbar, bilateral adrenalectomy and extensive resection of several enlarged retroperitoneal lymph nodes. Pathologic data revealed bilateral adrenal pheochromocytomas measuring 14 x 11 cm and weighting 640 g at the right and 8 x 6 cm weighing 90 g at the left (Supplemental figure, above). No lymphatic or capsular invasion was observed. Lymphonodomegalies were due to Hodgkin lymphoma. Genetic screening identified the double C634Y/Y791F mutation in the \textit{RET} gene. Basal calcitonin was 162/246 pg/mL (n.v., < 5.0 pg/mL). Thyroid ultrasound showed a thyroid hypoechoic calcified nodule measuring 1.8 x 1.3 x 1.4 cm. Immunocytochemistry from the thyroid biopsy was positively reactive for calcitonin and a total thyroidectomy associated with extensive cervical resection was performed. Pathologic data showed two MTC tumors (0.6 and 1.2 cm) and multifocal C-cell hyperplasia. There were metastatic cervical lymph nodes due to lymphoma but no metastasis of MTC in the 18 examined cervical lymph nodes. Six months after surgery, basal calcitonin values remained undetectable (< 2 pg/mL; n.v. for controls = < 5.0 pg/mL; and for total thyroidectomized patients = < 2.0 pg/mL), as well as the biochemical markers for the adrenal medulla. The patient was on chemotherapy for lymphoma and died at 36 years of age due to complications secondary to lymphoma.

Index case 2 (F2-II.1)
This female patient was referred to us with the diagnosis of pheochromocytoma at the age of 28 years. She reported sporadic paroxysmal crises with duration up to 15 min. in the last two months. Twenty-four hour monitoring showed high blood pressure. Serum epinephrine (1684 pg/mL; n.v., 0-75 pg/mL) and norepinephrine (6,386 pg/mL; n.v., 40-268 pg/mL) were markedly high. Urinary epinephrine (277 µg; n.v., 0.5-20 µg), norepinephrine (586 µg; n.v., 14-80 µg), metanephrine (17.4 µg/mg; n.v., 0.05-12 µg/mg), and 24h VMA (96.8 mg; n.v., < 12 mg) were also elevated. Abdominal CT scan revealed a heterogeneous unilateral left adrenal mass with hypersignal at T2, defined limits, measuring 9.8 x 8.8 x 8.9 cm compressing the kidney and no local lymph node enlargement was presented. The tumor resection was performed by lumbar approach and it measured 9.5 x 9.0 x 8.5 cm and weighed 295 g with no macro- or microscopic evidence for malignancy. In a follow up of 54 months, biochemical results and imaging studies showed absence of tumor recurrence. Initially, no thyroid nodule was detected and the basal calcitonin was within the normal range. Due to the positive RET mutation test, another screen of the thyroid was soon after performed and showed increased basal (16-30 pg/mL; n.v., < 5.0 pg/mL) and calcium-stimulated serum levels of calcitonin (384 pg/mL; n.v., < 40 pg/mL). At age 30 y, total thyroidectomy was performed and two white/yellowish thyroid nodules, measuring 5 mm at the right and 3 mm at the left lobe, were found. Pathologic data confirmed MTC in both tumors and no cervical lymph node metastasis. Three and half years after thyroid surgery, basal and stimulated calcitonin levels were normal (< 2 pg/mL).

**Index case 3 (F3-II.1)**

This patient was diagnosed in another institution with bilateral pheochromocytoma at age 27 years. Abdominal CT revealed a mass of 8.4 cm in the left adrenal gland with a cystic component and a mass of 4.5 cm with a cystic component of 7.8 cm in the right adrenal. Subsequently, a bilateral adrenalectomy was conducted and pathologic findings confirmed pheochromocytomas measuring 8.0 x 6.5 x 6.0 cm at the left and 4.5 x 2.9 cm at the right with local invasiveness (Supplemental Figure, bottom). During the last 12 months follow up, serum and urinary biochemical data remained normal. MTC was diagnosed due to elevated serum calcitonin levels and a positive biopsy for MTC. At age 29, total thyroidectomy associated with an extensive cervical lymphadenectomy was performed. Cervical lymph nodes were compromised. Pathological data revealed two nodules at the right (the largest, 0.6 cm) and one in the left thyroid lobe (0.9 cm).
There were cervical lymph nodes metastases. Nine months after surgery, baseline calcitonin values were persistently high (22 pg/mL.; NL= < 2 pg/mL, for total thyroidectomized cases).

**Index case 4 (F4-I.7)**

A 63 y-old woman was referred to our hospital and molecular biology laboratory to perform \textit{RET} mutation analysis. She had been submitted to thyroidectomy at age 54 y and pathologic data at that time showed two thyroid nodules with 3.0 and 1.8 cm of “PTC-follicular variant”. Four years after this first thyroid surgery, she was diagnosed with, and operated on for, a pheochromocytoma (6.0 x 3.0 x 1.5 cm) in the left adrenal. During the clinical screening, a MTC was identified and an extensive cervical lymph nodes resection was performed and MTC lymph node metastasis was detected. We asked for pathological revision of her first thyroid surgery and a MTC with positive calcitonin immunochemistry was recognized. Recently, a contralateral pheochromocytoma (4.9 x 2.6 cm) was documented and she will be soon submitted to adrenalectomy. Calcitonin and catecholamine values remain persistently high and she reports episodes of palpitation, excess sweating and weakness.