Endocrine, *vol. 28, no. 2, 193–198, November 2005* 0969–711X/05/28:193–198/$30.00

A *RET* Mutation with Decreased Penetrance in the Family of a Patient with a "Sporadic" Pheochromocytoma

Seth M. Arum,[1] Patricia L. M. Dahia,[2] Katherine Schneider,[3] and Lewis E. Braverman[1]

[1]Department of Medicine, Section of Endocrinology, Diabetes, and Nutrition, Boston University Medical Center, Boston MA; and the [2]Departments of Cancer Biology; and [3]Population Sciences, Dana-Farber Cancer Institute, Harvard Medical School, Boston MA

We present the case of a 38-yr-old man with a sporadic, multifocal pheochromocytoma and paraganglioma who was discovered to carry a Y791F germline mutation in exon 13 of the *RET* proto-oncogene. This mutation was found in his 65-yr-old mother and his 86-yr-old maternal grandmother. Neither of them had either biochemical evidence of pheochromocytoma or medullary thyroid carcinoma. The patient had a prophylactic thyroidectomy, which revealed mild C-cell hyperplasia. This case brings to discussion several issues: (1) the benefit of screening patients with apparently sporadic pheochromocytomas for genetic mutations; (2) the management of patients and families with "lower-risk" *RET* mutations; and (3) the possibility that lower-penetrance *RET* mutations may contribute to the list of causes of familial pheochromocytomas.

Key Words: Pheochromocytoma genetics; MEN2; RET mutation.

Introduction

Pheochromocytomas (pheos) are rare tumors of chromaffin cells, typically arising from the adrenal medulla and usually associated with excessive catecholamine production *(1)*. Although there are several familial syndromes associated with pheos, most often they are sporadic, with no family history of the tumors. Germline mutations in one of several genes are often found in families with pheos *(2–4)*. Although germline mutations are less frequently found in patients with sporadic pheos, they may still occur in up to 24% of cases *(5)*. This has lead to some debate regarding the circumstances in which it would be appropriate to test patients with sporadic pheos for germline mutations *(5–7)*. We present a patient with a sporadic pheo in which genetic testing revealed a mutation with potential effects across several generations.

Received July 16, 2005; Revised August 30, 2005; Accepted September 6, 2005.

Author to whom all correspondence and reprint requests should be addressed: Lewis E. Braverman, MD, 88 East Newton St, Evans 201, Boston, MA 02118. E-mail: lewis.braverman@bmc.org

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal-dominant syndrome that includes neoplastic growth of the adrenal medullary chromaffin cells (pheos) as well as the calcitonin-producing C-cells of the thyroid (medullary thyroid carcinoma, MTC). Mutations in the *RET* proto-oncogene were first associated with the MEN 2 syndromes in 1993 *(8)*. The *RET* proto-oncogene encodes a tyrosine kinase receptor that is involved in the normal development of neural crest tissue *(9)*. The majority of families with MEN 2A have mutations in exons 10 or 11 in the extracellular domain of the *RET* tyrosine kinase receptor *(10,11)*. Mutations in exons 13 and 14 in the intracellular domain of the receptor are less frequently found in families with the MEN 2 phenotype *(9,11,12)*. These mutations may have variable penetrance or a less aggressive course than the more common extracellular-domain mutations *(9,13)*. As a result, consensus panels have been unable to reach an agreement regarding the management of patients with these exon 13 and 14 mutations *(14)*.

Although *RET* mutations have been found in some patients with sporadic pheos *(5)*, there have not been confirmed cases of *RET* mutations in families in which pheo is the sole manifestation ("pheo-only" families). One theory is that the high penetrance of MTC in families with *RET* mutations would make it unlikely to have a germline mutation inherited without evidence of MTC in prior generations—hence making it unprecedented to find a *RET* mutation in a pheo-only family. We believe that the present case, while not a pheo-only family *per se*, suggests that 791 *RET* mutations in exon 13, in addition to a low-penetrance thyroid phenotype, can confer susceptibility to pheo, which would mimic a pheo-only syndrome in affected individuals. Thus, it may still be reasonable to test for less penetrant *RET* mutations in pheo-only families.

Results and Discussion

Masses were removed surgically in July, 2002 with resolution of symptoms and normalization of catecholamine and metanephrine levels. Pathology was consistent with a pheochromocytoma and a paraganglioma. There were no pathologic characteristics of malignancy.

Genetic Testing of the Index Patient

His family history was notable for several maternal family members with HTN, some fairly severe. Should the patient be tested for genetic mutations? While some recommend that anyone with a sporadic pheo should undergo genetic screening *(5)* and others suggest that anyone under the age of 50 yr should be screened *(6)*, there are some who feel that unilateral, adrenal pheos should only be screened for mutations in patients who present under the age of 21 yr *(7)*. Because this patient had multifocal, extraadrenal pheos, he is more likely to harbor a germline mutation and was tested. However, a mutation in the *RET* proto-oncogene would be unlikely because it is unusual for *RET* mutations to present with extraadrenal disease *(5)*. Nevertheless, a Y791F *RET* mutation was identified, resulting from a T to A substitution in exon 13. This region corresponds to the proximal intracellular domain of the receptor.

In the mid 1990s, several reports found mutations in exons 13 and 14 in the intracellular domain of the receptor in families with the clinical MEN 2A phenotype *(12)*. It was not until 1998 when the first reports of families with the Y791F mutation in exon 13 were described by Berndt et al. *(9)*. Although this mutation could cause lethal MTC, there also seemed to be variable penetrance, or at least a less aggressive course with this *RET* mutation *(9,13)*. Most carriers of mutations in exons 10 and 11 develop C-cell hyperplasia (CCH), a benign precursor of MTC, by the age of 5, which has lead to recommendations for prophylactic thyroidectomy for all carriers of these mutations at this young age *(14)*. However, there is no agreement regarding the benefit of a prophylactic thyroidectomy in patients carrying the Y791F mutation. Therefore, more information was needed regarding the patient's thyroid C-cells.

Clinical Evaluation

Serum calcium concentration was 9.6 mg/dL. Intact (1-84) PTH concentration was 33 pg/mL. Baseline serum calcitonin level was 5 pg/mL (normal <8). Neither physical exam nor an ultrasound of the neck revealed any evidence of a thyroid nodule. However, it was unclear whether C-cell hyperplasia could be present. A normal basal serum calcitonin concentration does not eliminate the possibility of either CCH or MTC. However, a positive stimulation test would have affected his management. An IV calcium infusion test was carried out. Serum calcium level rose from 9.6 to 10.4 mg/dL. Serum calcitonin concentrations peaked 3 min after the infusion to 135 pg/mL, a normal response (<200 pg/mL) *(15)*. Unfortunately, false-negative results are not uncommon. A recent report described the use of various stimulation tests in predicting the presence of MTC in asymptomatic *RET* mutation carriers *(16)*. Of 27 carriers with negative stimulated serum calcitonin levels, six patients (22%) had node-negative MTC and one (4%) had node-positive MTC. Thus, a normal calcium infusion test does not rule out the presence of MTC or CCH.

Decision Regarding Prophylactic Thyroidectomy

As seen in Table 1, there have been 67 cases reported with the Y791F mutation. Of specific interest are the 53 patients with this mutation in whom the definitive pathology of their thyroids was clear, either by clinical course or surgical pathology. There were six Y791F carriers reported who had no C-cell disease after thyroidectomy (11%), the oldest being 39 yr of age *(17,18)*. On the other hand, there have been 13 patients with fully developed MTC (25%), the youngest being 21 yr of age *(9)*. There have also been two premature deaths, at ages 45 and 52, from metastatic MTC in families with the Y791F mutation *(9,19)*. The vast majority (34 of 53 or 64%) of carriers of the Y791F mutation who have had prophylactic thyroidectomies had CCH, the youngest being 6 yr of age *(19)*. Thus, given the prevalence of C-cell disease (89% including CCH and MTC) as well as the potential for life-threatening cancer, we believe it is reasonable to suggest that these patients have a prophylactic thyroidectomy by an experienced surgeon.

Age at time of thyroidectomy is a more difficult question. Although CCH has been found in a child as young as 6 yr old with the Y791F mutation, there have been no cases of MTC found in anyone under the age of 21. For this reason, we feel it is appropriate to evaluate patients through their teenage years with serum calcitonin levels and calcium infusions if necessary.

Pathology

The patient chose to have a thyroidectomy. There were no complications. Histology and immunohistochemical staining for calcitonin and synaptophysin revealed focal mild CCH (Fig. 1). There was also an incidental finding of an occult 4 mm micropapillary carcinoma.

His findings were consistent with the previous literature in that the majority of these carriers do have some degree of CCH. Of interest, papillary thyroid carcinomas (PTC) have been previously reported in patients with *RET* 791 mutations *(18)*. A direct role of this mutation in the pathogenesis of PTC has not been established.

Genetic Testing of the Family (Fig. 2)

His parents were first tested, although it was possible that they were both negative and that this was a *de novo* mutation. This would have eliminated the need to test the rest of the family.

- His mother (III-3) was found to be positive for the Y791F mutation and was referred to a local endocrinologist for further evaluation of an adrenal mass as well as to rule out MTC. She has had repeated plasma free metanephrines and 24-h urine collections that did not reveal any catecholamine excess. Her baseline serum calcitonin level was 2 pg/mL (normal level <4) and her serum calcium was normal. She has not had a calcitonin stimulation or a thyroidectomy to date.
- His maternal grandmother (II-4) was also found to be positive for the Y791F mutation. Her baseline serum calcitonin

Table 1
Literature Review of Y791F *RET* Mutations[b]

Reference	#Affected/Families	Path Results	Comment
Berndt '98 *(9)*	3 Families 8 Affected	3 had MTC 1 had presumed CCH (high calcitonin/no MTC) 4 did not have surgery	• 21 yr old with MTC • Father died at age 45 from MTC
Neuman '02 *(5)*	1 Affected No family data	No path results	• No clinical data given
Brauckhoff '02 *(18)*	1 Family 3 Affected	1 had 5 mm PTC but no CCH 2 had CCH	• Normal C-cells at age 39 • PTC
Colombo-Benkmann '02 *(19)*	1 Family 5 Affected	1 had MTC 3 had CCH 1 did not have surgery	• 6-yr-old with CCH • 71-yr-old carrier with calcitonin of 40 pg/mL—surgery was declined • Paternal uncle died at age 52 from MTC • **1st Case with pheo**
Fitze '02 *(13)*	1 Family 2 Affected	1 with MTC (recurrent) 1 with normal thyroid	• Normal path at age 29 • Index had a somatic M918T mutation
Gimm '02 *(17)*	6 Families 17 Affected	4 with MTC 9 with CCH 4 with normal thyroids	• Normal path at age 39 • **Two cases with pheo**
Machens '03 *(20)*	6 Families 15 Affected	1 with MTC 14 with CCH	• No normal thyroids
EUROMEN '03 *(11)*[a]	3 Families 5 Affected	All 5 with CCH	• All subjects <20 yr old with prohylactic thyroidectomy
Vierhapper '04 *(21)*	4 Affected No family data	No path results	• Two had mildly abnormal pentagastrin stims • Two with normal basal calcitonin
Jindrichová '04 *(22)*	3 Families 7 Affected	All 7 with MTC	• MTC was an inclusion criteria • One Family (three subjects) had 791 and 918
Totals	67 Cases Reported 63 excluding 918 duals 53 had definitive path (clinical course or path)	13/53(25%) had MTC 6/53(11%) no C-cell disease 34/53 (64%) had CCH	• **NO hyperparathyroidism** • **Only three cases with Pheo**

[a]Machens A, personal communication— detailed path results are not in the reference.
[b]Review of the previous reports of subjects with a Y791F mutation in the *RET* proto-onco gene. Note: There is a high prevalence of C-cell disease (25% had MTC and 64% had CCH).

was found to be <1 pg/mL. Her plasma metanephrines and serum calcium levels were both normal. Prophylactic thyroidectomy has not been recommended.
- His brother's child (V-2) was tested and is negative for this mutation.
- His sister (IV-4) was negative for this mutation and, therefore, her son (V-4) was not tested.
- His maternal uncle (III-6) has refused testing to this point, but his two adult sons will be informed about the importance of being tested.

His grandmother's siblings and/or their children will be contacted to find other affected family members. It is clear that this mutation may be passed on for several generations with seemingly low penetrance, as is evident by his 86-yr-old grandmother with no clinical or biochemical evidence of endocrine tumors. However, it may eventually declare itself with a potentially life-threatening manifestation as it did in our patient with the pheos. There may be some unexpected findings in distant relatives.

Conclusions

We have presented a case of a man with a sporadic, multifocal pheochromocytoma in whom a Y791F *RET* mutation was discovered.

1. There have been arguments for several years about when to offer genetic testing to patients with "sporadic" pheos *(23)*. We agree with Bryant et al. *(6)* that it would be appropriate to carry out genetic testing on all patients under the age of 50 with apparently sporadic pheos—patients presenting over the age of 50 rarely have germline mutations *(5)*. If a mutation is found, it could have a tremendous impact on the health and longevity of other members of the family.

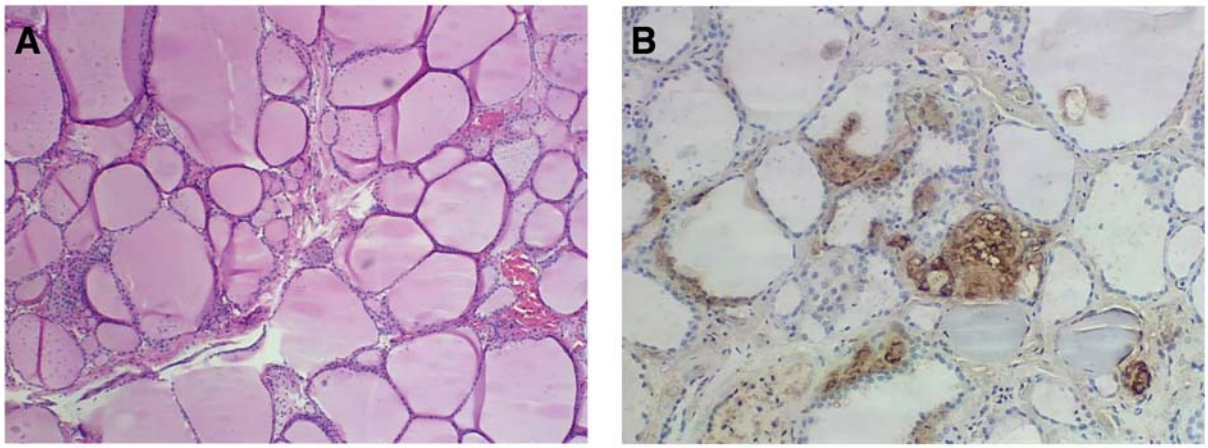

Fig. 1. Histologic slides of the index case's thyroidectomy showing mild, focal C-cell hyperplasia. (**A**) H&E staining at ×100 magnification. (**B**) Immunohistochemical stain for calcitonin at ×400 magnification.

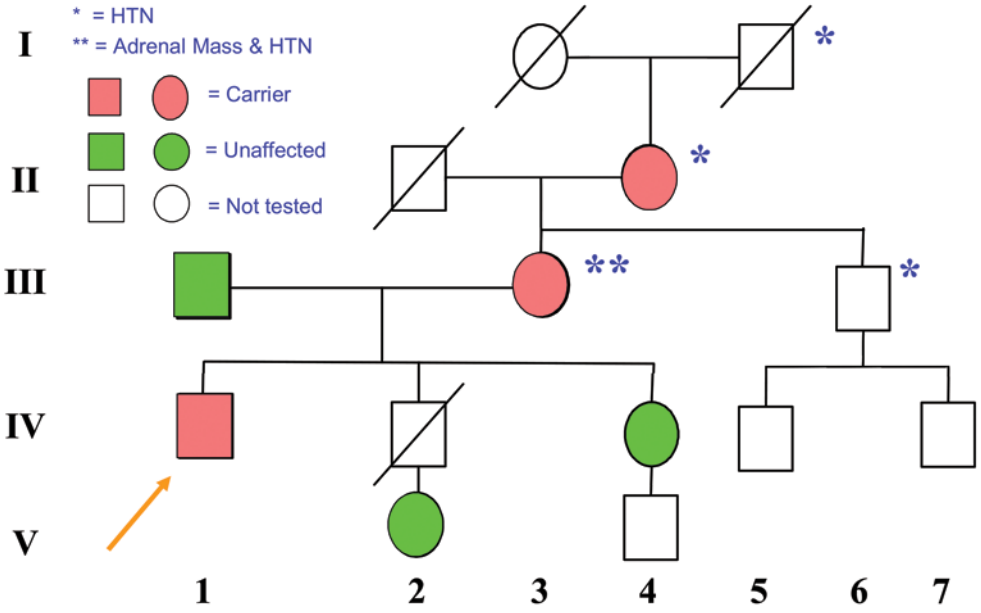

Fig. 2. Results of the family's genetic screening. Pertinent family history is marked.

2. The 2001 consensus statement on the management of MEN 1 and MEN 2 syndromes was unable to come to an agreement regarding the "least high risk" 790/791 carriers *(14)*. In reviewing the literature, it seems clear that these mutations do predispose to less aggressive tumors with older age of onset. However, it is also evident that a significant percentage of carriers do develop clinically significant MTC with potential premature mortality. Because the vast majority of carriers do have C-cell abnormalities on pathologic evaluation, it seems appropriate to recommend prophylactic thyroidectomies for any carrier of this unusual *RET* mutation. However, unlike the more common, high-risk mutations found in exons 10 and 11, it is reasonable to postpone a thyroidectomy until the late teen years or older—assuming no biochemical evidence of C-cell disease.

3. For decades, there have been families in which several generations were affected by pheos without other manifestations of the various syndromes. Mutations in the *VHL*, *SDHB,* and *SDHD* genes have been discovered in some of these families, but there have not been confirmed cases of *RET* mutations in pheo-only families after long-term surveillance *(2–4)*. However, the earlier studies did not sequence all exons, which, as is now known, may contain mutations causing the MEN 2 phenotype. The absence of biochemical evidence of a pheo in other family members and the detection of focal C-cell hyperplasia in the index patient does not substantiate the diagnosis of a "pheo-only" family in our case. However, at a minimum, the disease presentation in this family suggests that, in addition to a low-penetrance thyroid phenotype, 791 *RET* mutations can confer susceptibility to pheo, which would mimic

Table 2
Results of the Original 24-h Urine Collection[b]

Norepinephrine 1825 μg/d (<100)[a]	Normetanephrines 4290 μg/d (<540)
Epinephrine 113 μg/d (<24)	Metanephrines 685 μg/d (<230)
Dopamine 411 μg/d (<480)	VMA 28.5 mg/d (<10)

[a]Numbers in parentheses are the upper limit of normal.
[b]Results of the initial 24-h urine collection for catecholamines and metanephrines.

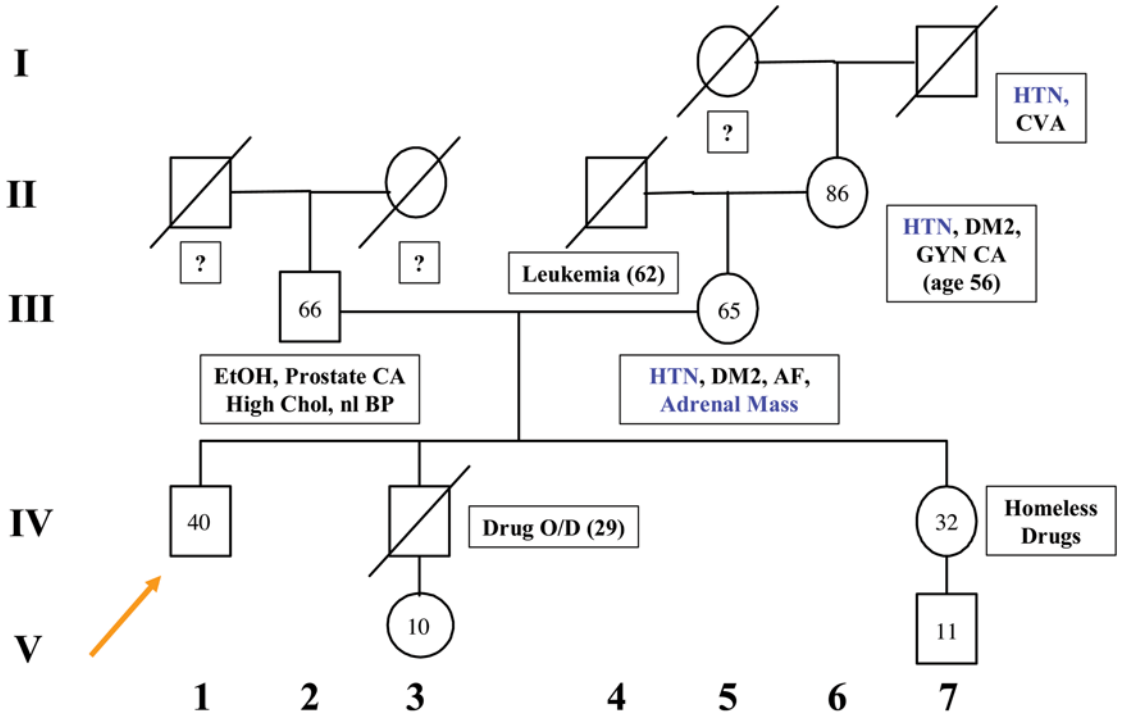

Fig. 3. Family pedigree. The index case (IV-A) is represented by the arrow. A diagonal line indicates deceased. Pertinent medical history is noted in the boxes along side the family member and the question marks indicate lack of clinical information. Current age is noted and the age at the time of death is noted alongside the pertinent medical history. **Note**: The index patient's mother (III-E) had HTN and an adrenal mass, and HTN was also found in several generations on the mother's side of the family.

a pheo-only syndrome in affected individuals. Screening large cohorts of pheo patients, which includes a comprehensive analysis of the *RET* gene (exons 10,11,13,14,15, and 16), will be required to determine whether families with exclusive pheo presentation are part of the clinical spectrum of *RET* mutations.

Patients and Methods

Case History

A 38-yr-old Caucasian man presented in June, 2002 with recent onset of palpitations, sweats, headaches, insomnia, and hypertension (HTN). A 24-h urine collection revealed a predominantly noradrenergic elevation in his catecholamines and metanephrines (Table 2). Serum calcitonin, calcium, and intact (1-84) PTH levels were normal, and there were no clinical features suggestive of von Hippel-Lindau syndrome or neurofibromatosis. An abdominal MRI revealed a 5.5 cm left adrenal mass in addition to a 2.8 cm left paraaortic mass. Both masses had bright signal intensity on T2 consistent with chromaffin tissue.

The patient's medical history was significant for a 10-yr history of mild secondary hypogonadism of undetermined etiology and subclinical hypothyroidism for which he took 10 g topical testosterone gel and 150 μg levothyroxine daily.

Family history (Fig. 3) was significant for a 65-yr old mother (III-5) with HTN, type 2 diabetes mellitus, atrial fibrillation, and an "adrenal mass." This had apparently been evaluated with a urine collection, which "ruled out" a pheochromocytoma. His father (III-2) has prostate cancer and elevated cholesterol, but normal blood pressure. The patient had a younger brother (IV-3) who died at age 29 from a drug overdose. That brother had a child (V-3). His younger sister (IV-7) is a homeless person. She had one son (V-7) who lived with his grandmother, the patient's mother (III-5). The patient's maternal grandmother (II-6) had HTN and her father (I-7) was hypertensive and died from a CVA. There was no history of other endocrine tumors.

Biochemical Assays

Serum calcium levels were measured using standard techniques on a Bayer Advia 2400 analyzer (Bayer Diagnostics, Tarrytown, NY). Serum calcitonin was determined with an immunochemiluminometric assay (Nichols Institute, San Juan Capistrano, CA). Serum intact (1-84) PTH levels were measured using a two-site immunochemiluminometric assay [Advantage Bio-Intact PTH (1-84) assay; Nichols Institute, San Clemente, CA]. Urine catecholamines and metanephrines were measured using HPLC with electrochemical detection (Nichols Institute, San Juan Capistrano, CA).

Genetic Testing

Genetic testing was carried out through an IRB-approved research protocol, after signed informed consent was obtained. Germline DNA was used to sequence known pheochromocytoma susceptibility genes [*RET, von Hippel-Lindau (VHL), Succinate dehydrogenase–subunits B and D (SDHB, SDHD*)] using standard procedures *(3,24)*. Results were confirmed by an independent screen at a CLIA certified laboratory (Quest Diagnostics, San Juan Capistrano, CA). The index patient's family was then tested through the same commercial laboratory.

Calcium Infusion Protocol

An intravenous bolus of 10% calcium gluconate, 2 mL/kg, was infused over 1 min. Serum calcium and calcitonin levels were measured at baseline and at 1, 3, 5, and 10 min after the infusion.

Acknowledgments

The authors thank Varsha Manucha, MD for her help with the photomicrographs of the pathology. We thank Robert F. Gagel MD, MACE for his advice regarding calcium infusion protocols. We thank Raymond Fenwick, PhD for his assistance with the genetic testing. We would also like to thank Andreas Machens, MD for his invaluable suggestions with regard to patient management and literature review. P.L.M.D. is the recipient of a Claudia Adams Barr Investigator Award.

References

1. Lenders, J. W. M., Pacak, K., Walther, M. M., et al. (2002). *JAMA* **287,** 1427–1434.
2. Crossey, P. A., Eng, C., Ginalska-Malinowska, M., et al. (1995). *J. Med. Genet.* **32,** 885–886.
3. Astuti, D., Latif, F., Dallol, A., et al. (2001). *Am. J. Hum. Genet.* **69,** 49–54.
4. Astuti, D., Douglas, F., Lennard, T. W. J., et al. (2001). *Lancet* **357,** 1181–1182.
5. Neumann, H. P. H., Bausch, B., McWhinney, S. R., et al. (2002). *N. Engl. J. Med.* **346,** 1459–1466.
6. Bryant, J., Farmer, J., Kessler, L. J., Townsend, R. R., and Nathanson, K. L. (2003). *J. Natl. Cancer Inst.* **95,** 1196–1204.
7. Young, W. F. Jr. and Kaplan, N. M. (2004). Up to Date; version 13.1.
8. Mulligan, L. M., Kwok, J. B. J., Healey, C. S., et al. (1993). *Nature* **363,** 458–460.
9. Berndt, I., Reuter, M., Saller, B., et al. (1998). *J. Clin. Endocrinol. Metab.* **83,** 770–774.
10. Machens, A., Gimm, O., Hinze, R., Höppner, W., Boehm, B. O., and Dralle, H. (2001). *J. Clin. Endocrinol. Metab.* **86,** 1104–1109.
11. Machens, A., Niccoli-Sire, P., Hoegel, J., et al. (2003). *N. Engl. J. Med.* **349,** 1517–1525.
12. Bolino, A., Schuffenecker, I., Luo, Y., et al. (1995). *Oncogene* **10,** 2415–2419.
13. Fitze, G., Schierz, M., Bredow, J., Saeger, H. D., Roesner, D., and Schackert, H. K. (2002). *Ann. Surg.* **236,** 570–575.
14. Brandi, M. L., Gagel, R. F., Angeli, A., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 5658–5671.
15. Wells, S. A. Jr., Baylin, S. B., Linehan, W. M., Farrell, R. E., Cox, E. B., and Cooper, C. W. (1978). *Ann. Surg.* **188,** 139–141.
16. Machens, A., Ukkat, J., Brauckhoff, M., Gimm, O., and Dralle, H. (2005). *J. Intern. Med.* **257,** 50–59.
17. Gimm, O., Niederle, B. E., Weber, T., et al. (2002). *Surgery* **132,** 952–959.
18. Brauckhoff, M., Gimm, O., Hinze, R., Ukkat, J., Brauckhoff, K., and Dralle, H. (2002). *Thyroid* **12,** 557–561.
19. Colombo-Benkmann, M., Brämswig, J., Höppner, W., et al. (2002). *World J. Surg.* **26,** 1286–1290.
20. Machens, A., Holzhausen, H.-J., Thanh, P. N., and Dralle, H. (2003). *Surgery* **134,** 425–431.
21. Vierhapper, H., Bieglmayer, C., Heinze, G., and Baumgartner-Parzer, S. (2004). *Thyroid* **14,** 580–583.
22. Jindřichová, Š., Včelák, J., Vlček, P., Neradilová, M., Němec, J., and Bendlová, B. (2004). *J. Endocrinol.* **183,** 257–265.
23. Neumann, H. P. H., Reincke, M., Eng, C., and Conlin, P. R. (2001). *N. Engl. J. Med.* **345,** 547–548 (Correspondence).
24. Aguiar, R. C., Cax, G., Pomeroy, S. L., and Dahia, P. L. M. (2001). *J. Clin. Endocrinol. Metab.* **86,** 2890–2894.