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Case Report

Noncardiogenic Pulmonary Edema as the Chief Manifestation of a Pheochromocytoma: A Case Report of MEN 2A with Pedigree Analysis of the RET Proto-Oncogene

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OKADA, Y., SUCHI, M., TAKEYAMA, H., HODGSON, M.E., KATO, T. and MANABE, T. *Noncardiogenic Pulmonary Edema as the Chief Manifestation of a Pheochromocytoma: A Case Report of MEN 2A with Pedigree Analysis of the RET Proto-Oncogene.* Tohoku J. Exp. Med., 1999, **188** (2), 177-187 —— Pheochromocytomas are rare neoplasias of the adrenal medulla which generally present with paroxysmal or sustained hypertension. Cardiogenic pulmonary edema is a common feature of these tumors, but few cases have been described with noncardiogenic pulmonary edema. We report a pheochromocytoma with the principle manifestation of noncardiogenic pulmonary edema and characterize a genetic lesion associated with the disorder. A 30-year-old man was admitted with abdominal pain and breathlessness. x-Ray examination of the chest revealed a massive, diffuse infiltration of the left lung without cardiomegaly. No paroxysmal blood pressure fluctuations or heart failure were evident during the entire course, and the infiltrate and dyspnea resolved in three days without inotropic or diuretic agents. Serum norepinephrine and epinephrine levels were elevated twenty and fifty times above normal, respectively. The patient was ultimately diagnosed with multiple endocrine neoplasia type 2A (MEN 2A). Mutations in the RET proto-oncogene have been described recently in patients with MEN 2A. Mutation analysis of selected RET exonic sequences identified a germline mutation at codon 634 in exon 11 of the RET proto-oncogene. The mutation introduces a transition encoding a non-conservative substitution from TGC (Cys) to CGC (Arg) and creates a novel restriction site recognized by *Hha*I. We further screened for this mutation among four of the proband's relatives by *Hha*I restriction analysis. One asymptomatic family member was identified who subsequently elected prophylactic total thyroid removal. Histological examination of this specimen confirmed the presence of medullary thyroid carcinoma. —— pheochromocytoma; noncardiogenic pulmonary edema; multiple endocrine neoplasia type 2A; RET proto-oncogene; presymptomatic diagnosis

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Multiple endocrine neoplasia type 2A (MEN 2A) is a rare familial cancer syndrome (Sipple 1961; Catalona et al. 1971; Chong et al. 1975; Raue et al. 1994). All patients with MEN 2A develop medullary thyroid carcinoma by the third or fourth decades of life. Thirty percent to fifty percent of MEN 2A patients harbor a pheochromocytoma, and approximately 30% suffer from hyperparathyroidism. The diagnosis of a pheochromocytoma in MEN 2A cases is generally made during annual screenings among inherited kindreds, or more commonly, when medullary thyroid carcinoma leads to a systemic investigation for MEN 2A. Only rarely are pheochromocytoma-related symptoms the initial clinical manifestations.

Pheochromocytomas are tumors derived from adrenal chromaffin cells. The associated excess catecholamine production can give rise to various symptoms including paroxysmal or sustained hypertension and other symptoms related to adrenergic stimulation such as palpitation, headache, excessive sweating, and even sudden death (Gifford et al. 1993). Catecholamine induced hypertension and cardiomegaly occasionally result in cardiogenic pulmonary edema (Blom et al. 1987; Zimmermann-Hoshi et al. 1990; Lorz et al. 1993). However, pulmonary edema without indicated heart failure is an exceedingly rare condition in association with a pheochromocytoma (De Leeuw et al. 1986). In this paper, we report a case of pheochromocytoma in which the cardinal manifestation was noncardiogenic pulmonary edema.

Germline mutations of the RET proto-oncogene have been identified in patients with MEN 2A (Donis-Keller et al. 1993; Mulligan et al. 1993; Hofstra et al. 1994), and the RET gene product is expressed at high levels in medullary thyroid carcinomas and in pheochromocytomas (Santoro et al. 1990). The MEN 2A disease locus has recently been assigned by linkage analysis to chromosome 10q11.2, the same position wherein the RET proto-oncogene was mapped (Mole et al. 1993). Indeed, fully 97% of all patients with MEN 2A demonstrate mutations within the RET gene at one of five cysteine codons (609, 611, 618, 620 and 634) located in exons 10 and 11. We have similarly confirmed a germ-line mutation at codon 634 of the RET gene in our patient. A family study revealed co-inheritance by an asymptomatic sister, who elected prophylactic total thyroidectomy. Because histological findings of the removed thyroid confirmed the presence of medullary thyroid carcinoma, we feel strongly that the DNA testing and the preventive thyroidectomy significantly benefited this family.

Case Report

A 30-year-old man was admitted with sudden onset, severe abdominal pain and breathlessness. He was afebrile. Although he was tachycardic (heart rate 145 beats/minutes), tachypneic (respiratory rate 40/minutes) and cyanosed, blood pressure was normotensive (131/71 mmHg) with normal jugular venous pressure. Chest x-ray examination showed diffuse infiltration of the left lung absent cardiomegaly indications (Fig. 1). Sinus tachycardia was detected by

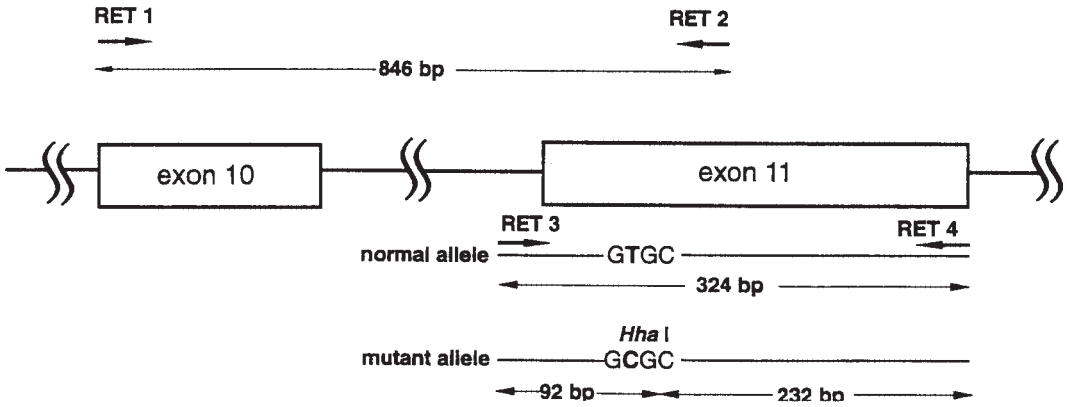

Fig. 1. Chest roentogenogram upon admission following symptom onset. Massive diffuse infiltration is observed within the middle and lower left pulmonary segments.

TABLE 1. *Laboratory data*

	Normal controls	On admission	Before surgery		Normal controls	On admission	Before surgery
WBC ($\times 10^3$)	4.3–8.4	14.1	6.6	CPK (IU/liter)	29–187	109	56
RBC ($\times 10^6$)	4.27–5.55	5.52	5.22	BUN (mg/ml)	8.0–20.0	10	11
Hb (g/100 ml)	12.5–16.7	17.2	15.6	CRE (mg/ml)	0.6–1.3	1	0.7
PLT ($\times 10^4$)	14.0–32.2	34	23.6	Na (mEq/liter)	135–147	139	142
TP (g/ml)	6.5–8.2	7.5	6.6	K (mEq/liter)	3.5–5.1	3.2	4.8
Alb (g/ml)	3.8–5.8	5	4.4	Ca (mg/ml)	8.5–10.2	8.2	9.5
GOT (IU/liter)	10–40	15	14	Cl (mEq/liter)	98–108	98	104
GPT (IU/liter)	6–40	17	21	P (mg/ml)	2.4–4.6	n.d.	4.7
LDH (IU/liter)	230–460	334	268	CRP (mg/ml)	0–0.6	10.1	0.3
γ-GTP (IU/liter)	0–50	10	20	PTH (ng/ml)	0.20–1.00	n.d.	0.47
ALP (IU/liter)	60–230	139	112	A/G rate		1.94	1.8
Amy (IU/liter)	50–180	102	161				

n.d., not determined.

WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PLT, platelet; TP, glutamic pyruvic transaminase; LDH, lactate dehydrogenase; γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; Amy, amylase; CPK, creatine phosphokinase; BUN, blood urea nitrogen; CRE, creatine; Na, natrium; K, kalium; Ca, calcium; Cl, chloride; P, phosphorus; CRP, C reactive protein; PTH, parathyroid hormone; A/G rate, albumin globulin ratio.

electrocardiogram with no evidence of myocardial infarction on admission. As shown in Table 1, serum LDH and CPK levels were within control reference ranges. The patient was treated with nasal O_2 and antibiotics over 11 days for possible bacterial pneumonia, since white blood count and CRP were elevated. Results from blood and sputum cultures were negative for microorganisms. The pulmonary infiltrates and dyspnea resolved over three days without cardioactive or diuretic treatment. An abdominal CT scan several days following admission

TABLE 2. *Levels of catecholamines and their metabolites*

	Normal controls	Before surgery	After surgery
Plasma			
Adrenaline (ng/ml)	0–0.10	5.00	0.30
Noradrenaline (ng/ml)	0.07–0.31	6.07	0.05
Dopamine (ng/ml)	0–0.10	0.28	0.1
Urine			
Vanillyl mandelic acid (mg/day)	1.3–5.3	n.d.	3.8
Homo vanillic acid (mg/day)	1.6–6.9	n.d.	7.4
Metanephrine (mg/day)	0.01–0.3	n.d.	0.39
Normetanephrine (mg/day)	0.05–0.40	n.d.	0.19

n.d., not determined.

revealed a $5 \times 3 \times 5$ cm solid, noncalcified, left adrenal mass. Serum epinephrine, norepinephrine, and dopamine were 6.07 ng/ml, 5.00 ng/ml and 0.28 ng/ml, respectively (Table 2).

For continued evaluation, the patient was referred to our hospital 36 days post-onset. On admission to our hospital, the patient underwent cardiac function tests, i.e., echocardiogram, treadmill test, Holter 24-hour electrocardiogram. These tests revealed no evidence of cardiac dysfunction. Adrenal scintigraphy with ^{131}I-metaiodobenzylguanidine demonstrated intense uptake in the suprarenal region. Urinary excretion of catecholamine metabolites was increased (Table 2), and high serum calcitonin levels (184 μg/ml) were noted. In addition, bilateral thyroid tumors were detected by cervical CT scan and ultrasonography. As noted in Table 3, parathyroid hormone levels fell within the normal range. Laboratory data and radiological findings also assisted in the diagnosis of MEN 2A.

Total thyroidectomy and left adrenectomy were performed 75 days following admission and consecutively under continuous anesthesia. The patient was premedicated with intramuscular mitazolam, pentazocine, and atropine sulfate. Anesthesia was induced by mitazolam and suxamethonium chloride and was maintained with a mixture of nitrous oxide: oxygen (4 : 2) plus isofluoram. Notably the patient experienced one intra-operative hypertensive event (3 minutes) in which blood pressure reached 200/100 mmHg. This episode was easily controlled following phentolamine mesilate administration. The removed adrenal tumor was encapsulated and $4 \times 7 \times 3$ cm in dimension. Macroscopic thyroid observation identified a 3×3 mm neoplastic lesion situated within the upper right lobe, and no enlargement of the parathyroids was noted. Histological examination confirmed the adrenal tumor as a pheochromocytoma and the thyroid mass as a medullary thyroid carcinoma.

Materials and Methods

Subjects

Five individuals (3 males and 2 females) of a MEN 2A family were analyzed for the RET mutation. The proband and his father were known to have medullary thyroid carcinoma by histological examination prior to DNA analysis.

DNA extraction and mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes as described (Baas et al. 1984). DNA fragments spanning exons 10 and 11 of the RET gene were amplified by PCR (Saiki et al. 1988) using the following primers: RET1 5′-GCAGCATTGTTGGGGGACA-3′, RET2 5′-GACAGCAGCACCGAGACGAT-3′, RET3 5′-GCATACGCAGCCTGTACCC-3′, and RET4 5′-AAGCTTGAAGGC-ATCCACGG-3′ (Fig. 2). Fifty μl reaction mixtures consisted of 1$\times$PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 6.5 mM $MgCl_2$, 0.1 mg/ml gelatin), 0.2 mM nucleotide triphosphates (dNTPs), 2 units recombinant *Taq* DNA polymerase, (TaKaRa *Taq*, TaKaRa Shuzo, Tokyo), and 1 μM oligonucleotide primers. Amplification conditions for the RET1 and RET2 primers were 40 cycles of 94°C for 1 minutes, 57°C for 1 minutes, and 72°C for 1 minutes 30 seconds, followed by a single extension at 72°C for 5 minutes in a thermal cycler (Model 480, Perkin Elmer Cetus, Norwalk, CT, USA). With the RET3 and RET4 primers, 72°C cycle extensions were performed for 40 seconds. Amplified product DNA was subcloned into the pCR II vector (Invitrogen, Calsbad, CA, USA), and six indepen-

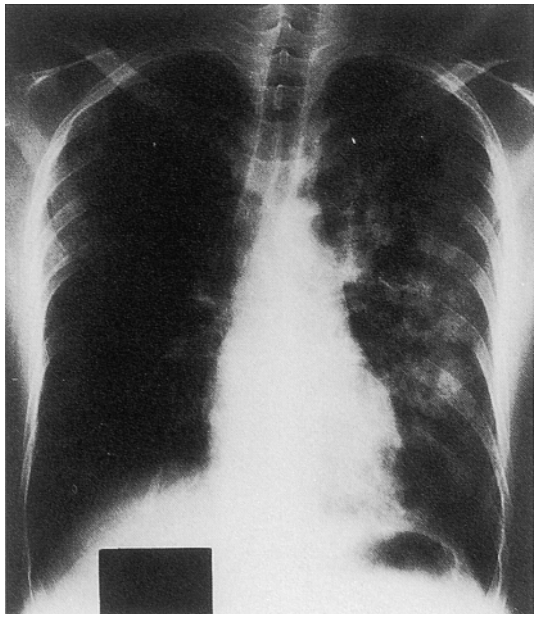

Fig. 2. Schematic illustration of the C634R mutation identification in the RET protooncogene. PCR amplification of genomic DNA was performed using primer sets of RET1/RET2 and RET3/RET4. The mutant allele contains a novel *Hha*I site enabling cleavage of the 324 bp amplification product into 232 bp and 92 bp fragments.

dent clones were sequenced by the dideoxy-chain termination method (Sanger et al. 1977) using synthetic oligonucleotide primers and Sequenase 2.0 (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Reaction products were resolved by electrophoresis on a 5% denaturing Long Ranger Hydrolink gel (FMC Bio-Products, Rockland, ME, USA).

Screening for the C634R mutation

A 324 bp DNA fragment, containing codon 634 in exon 11, was amplified from genomic DNA isolated from four additional family members and from a sample of the adrenal neoplastic tissue using primers RET3 and RET4. PCR conditions were 30 cycles of 94°C for 1 minutes, 59°C for 1 minutes, and 72°C for 40 seconds followed by one cycle of 72°C for 5 minutes. Reaction mixtures were 50 μl and included 0.75 mM $MgCl_2$ and 10% DMSO. Amplified products were phenol: chloroform (1 : 1) extracted, ethanol precipitated, and resuspended in 10 μl TE buffer (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA). From each PCR sample, 1 μl was digested with 10 units of *Hha*I (TaKaRa Shuzo), and size fractionated by electrophoresis in 1.2% agarose gels. Restriction fragments were visualized by ethidium bromide staining and UV illumination.

Results

Sequence analysis of the PCR-amplified genomic DNA fragments from the proband revealed a T to C transition in exon 11 of the RET proto-oncogene (Fig. 3). Three of six independent subclones contained the base change, indicating the heterozygous status of the proband. The transition results in a cysteine (TGC) to arginine (CGC) substitution at codon 634 (C634R) and introduces a novel *Hha*I

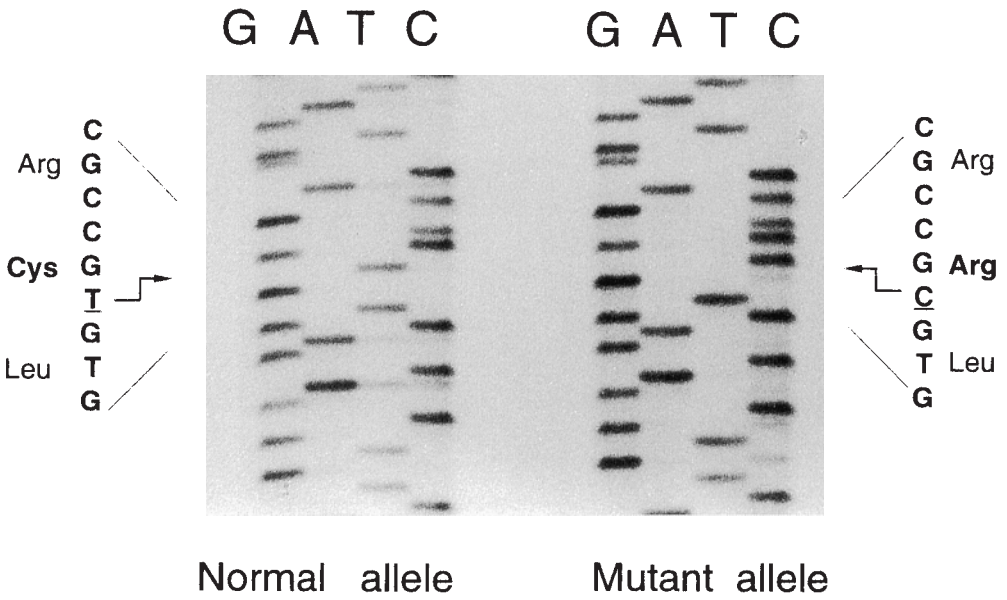

Fig. 3. Identification of the C634R mutation in the proband. Representative sequence from normal and mutant subclones is shown. Arrows indicate the position of the T to C transition at codon 634.

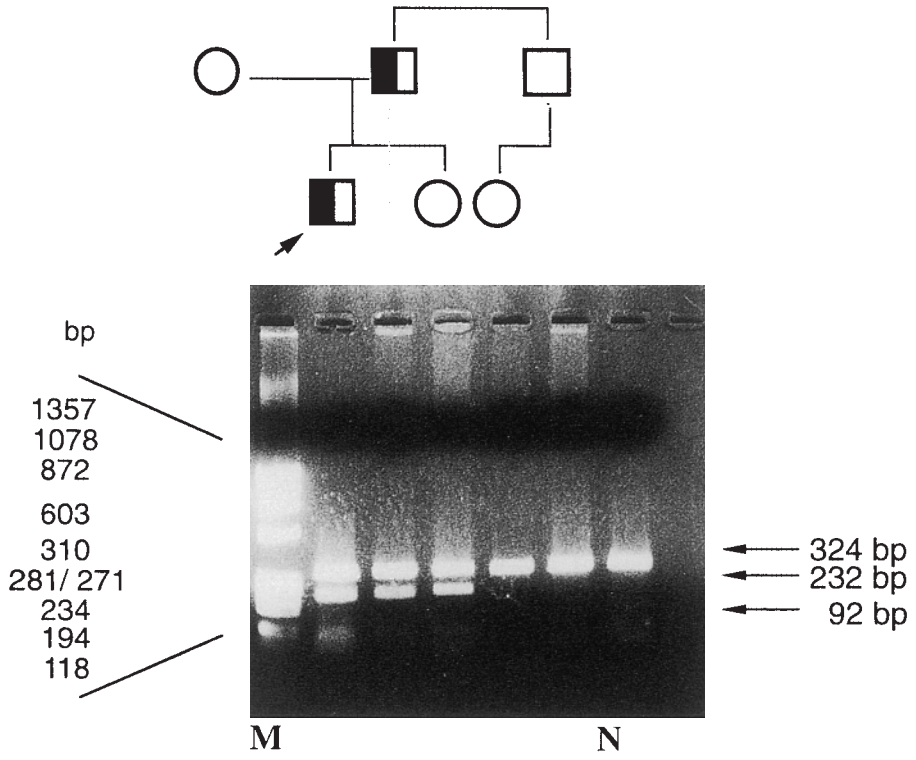

Fig. 4. Screening of the RET proto-oncogene C634R mutation. Agarose gel electrophoresis of *Hha*I digested PCR-amplified genomic DNA from the proband and family members is depicted. Amplified DNA (324 bp) containing the mutation is digested yielding 232- and 92-bp fragments. Lane M is 0.5 μg of ψx174 *Hae*III-digested size marker; lane N represents an unrelated normal individual.

cleavage site.

Four additional family members were screened for the mutation, by *Hha*I restriction analysis. Digestion of the 324 bp amplified PCR products containing the mutation yields fragments of 92 bp and 232 bp (Fig. 2). The proband's father, who had a prior thyroid tumor removal, and one asymptomatic sister were also identified as heterozygous for the C634R mutation (Fig. 4). Six months following this determination, the sister underwent preventive total thyroidectomy. Histological examination of the thyroid revealed the presence of medullary thyroid carcinoma.

PCR amplification of genomic DNA isolated from the surgically-removed pheochromocytoma and subsequent *Hha*I digestion revealed that the tumor was coposed of cells containing both the 634C (CGC) and the 634R (TGC) alleles (data not shown).

Discussion

Pheochromocytomas are characterized by a highly heterogeneous clinical symptomology (Gifford et al. 1993). Although typical cases present with paroxysmal hypertension, headache, palpitations, and diaphoresis resulting from elevated catecholamines, these tumors are often associated with mild and nonspecific

manifestations. Cardiogenic pulmonary edema is also common among pheochromocytoma cases, sharing features similar to that of catecholamine-induced myocarditis. The prognosis is generally poor for pheochromocytoma-related acute cardiac pulmonary edema; in one report, four of five patients died within 24 hours of onset (Sardesai et al. 1990). Noncardiogenic pulmonary edema, on the other hand, is an extremely rare manifestation in association with a pheochromocytoma, and all but six cases appear in the literature since 1966 (Joshi and Manni 1993). Although this condition is considered less life-threatening than pulmonary edema of cardiac origin, two of the six reported cases ended in fatal courses.

Despite the lack of pulmonary hemodynamic measurements and other catheterization-dependent values in the present case, the clinical data strongly suggest that our patient suffered acute noncardiogenic pulmonary edema. The patient was young and had no previous history of myocardial dysfunction, hypertension, or hypotension. The onset of respiratory distress was extremely sudden, and serum CPK and LDH levels did not become subsequently elevated. Moreover, no left ventricular enlargement or abnormalities were evident upon chest x-ray analyses or echocardiography, and recovery was complete in three days without inotropic agent or diuretic administration. We cannot, however, fully exclude the possibility of a transient myocardial contractility deficiency.

The mechanism of noncardiogenic pulmonary edema in pheochromocytoma patients remains unclear. Catecholamine surges elaborated from the neoplasm may illicit pulmonary edema in a manner similar to neurogenic pulmonary edema following cerebral damage (Theodore and Robin 1975). In such cases, a volumetric shift of blood from systemic to pulmonary circulation occurs coincident with pulmonary vasodilatation and lymphatic constriction. The elevated capillary hydrostatic pressures and interstitial oncotic pressures under these conditions impair fluid clearance ending in congestive accumulation. In addition, pulmonary hypertension can lead to microvascular hemorrhage. Our patient complained of severe abdominal pain similar to abdominal angina with nausea and vomiting. These symptoms may stem from gastrointestinal ischemic changes conducive to bacterial and endotoxin translocation. This precarious circumstance is a major risk factor in the development of adult respiratory distress syndrome (ARDS) and could explain the respiratory distress in our patient.

The present case of pheochromocytoma in combination with medullary thyroid carcinoma lead to the diagnosis of MEN 2A, a dominantly inherited cancer syndrome. Mutations in the RET proto-oncogene have been correlated with MEN 2A as well as familial medullary thyroid carcinoma (Santro et al. 1990; Mole et al. 1993; Hofstra et al. 1994). PCR amplification and dideoxy sequencing of the RET gene exons 10 and 11 from the patient identified a T to C transition at codon 634 (exon 11). This base change, which results in a cysteine to arginine substitution, is the most frequent mutation observed among MEN 2A

subjects (Mulligan et al. 1994). The mutation was further confirmed within the tumor mass, which was composed of cells containing both 634C and 634R alleles. This mutation has also been reported to have a high association with pheochromocytoma development and hyperparathyroidism (Schuffenecker et al. 1994). Consistent with these observations, our patient harbored a pheochromocytoma but did not manifest parathyroid hyperfunction.

The father of the proband who had previously undergone thyroid tumor removal and a sister who was being monitored for high serum calcitonin levels were identified as heterozygous for the C634R mutation by *Hha*I restriction analysis. Following consultation, the sister underwent a preventive total thyroidectomy. Death in MEN 2A patients is most often associated with metastasis of medullary thyroid carcinoma. Thyroid removal therefore eliminates the primary risk in this group. Because microscopic examination of the excised thyroid disclosed minute foci of medullary thyroid carcinoma, we believe that our mutation screening efforts and clinical management were beneficial for this family.

It remains unclear what role important physiologic modulators such as atrial natriuretic peptide (Di Nardo and Peruzzi 1992), proadrenomedullin peptide (Kitamura et al. 1994), and calcitonin gene-related peptide (Amara et al. 1982) may have in our patient's condition. The last may be particularly significant in MEN 2A due to its established relationship with thyroid tumors. Adrenomedullin, a novel hypotensive peptide with strong vasodilating activity, may similarly be integral to the pathophysiology observed in pheochromocytoma-related pulmonary edema. Recently isolated from a human pheochromocytoma (Kitamura et al. 1993), adrenomedullin shares two notable features consistent with adrenal medulla and pulmonary involvement. First, the adrenomedullin secretion profile corresponds with circulating norepinephrine levels, and second, 25% of adrenomedullin elimination occurs by passage through the lungs (Nishikimi et al. 1994). Unfortunately, we were unable to obtain values for these peptides during the episode of pulmonary edema. Further evaluation of circulating hormones and other substances is necessary to aid our understanding of noncardiogenic pulmonary edema accompanying pheochromocytoma.

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