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A Vietnamese MEN2A syndrome patient with C634G germline mutation of the *RET* proto-oncogene

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ABSTRACT

Multiple endocrine neoplasia type 2A (MEN 2A) is a rare, autosomal dominant disease. It is characterized by complete penetrance of medullary thyroid carcinoma (MTC), lower prevalence of pheochromocytoma, hyperparathyroidism, and sometimes cutaneous lichen amyloidosis. Here, we reported a rare case of a 39-year-old man from Vietnam with pheochromocytoma as the first symptom of MEN 2A. An abdominal computed tomography (CT) revealed large, bilateral adrenal masses. Additional biochemical investigations presented a significant increase of both the urinary catecholamines and serum calcitonin. The patient then underwent a bilateral adrenalectomy, and histopathological examinations confirmed suspicions of MTC. Genetic testing indicated a nucleotide substitution located in exon 11 of the *RET* proto-oncogene (c.1900T > G, p.Cys634Gly), which was reported as a pathogenic mutation of MEN2A. Furthermore, this identical mutation was also detected in several family members of the patient. His sibling has the mutation, but was originally diagnosed with

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papillary thyroid carcinoma and had a total thyroidectomy. The mutation was also found in the patient's daughter and his sibling's daughter, which emphasizes their high risks of thyroid cancer. Therefore, this case in Vietnam draws attention to the importance of genetic counselling in C634G carriers, as well as strict follow-up appointments to reduce morbidity and mortality since the mutation is classified as a high-risk group within the MTC guidelines.

1. Introduction

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal endocrinopathy characterized by medullary thyroid carcinoma (MTC) and/or its precursors: C-cell hyperplasia, pheochromocytoma, and parathyroid adenoma or hyperplasia [1]. This inherited cancer syndrome results from germline activating mutations in the *RET* proto-oncogene, encoding a single-pass transmembrane receptor tyrosine kinase (RTK) [2]. Most of the *RET* mutations were detected in the cysteine-rich extracellular domain, which was demonstrated to form a ligand-independent receptor dimerization and cross-phosphorylation. This process consequently leads to constitutive activation of its downstream signals [3]. Currently, MEN 2 is sub-classified into three distinct syndromes based on clinical appearance: MEN 2A, MEN 2B and familial medullary thyroid carcinoma [4]. MEN 2A, or Sipple syndrome, constitutes for approximately 80% of hereditary MEN 2 cases [5]. These patients develop multifocal, bilateral MTC (nearly 100% penetrance), pheochromocytoma (42% penetrance), and hyperparathyroidism (10–30% penetrance) [6].

In this study, we report a diagnosis of MEN 2A in a patient who presented bilateral pheochromocytoma as a primary diagnosis and was subsequently screened for the *RET* germline mutation C634G.

2. Case description

A 39-year-old male patient (P1) visited a local hospital with a description of trembling hands and feet, muscle cramps, and hip pain. Bilateral adrenal tumors were identified by an abdominal contrast-enhanced computed tomography (CT) scan, revealing enhanced masses on both sides of the adrenal glands (diameter of 3.3 × 4.3 cm for the right adrenal tumor and 6.3 × 6.7 cm for the left one) (Fig. 1A). Subsequent biochemical assessment of the patient's adrenal function detected that an excretion of catecholamine in his plasma and 24-h urine were elevated (Table 1). However, during this same time period, biochemical examinations including quantitating of total protein, albumin, total cholesterol and triglyceride, as well as immunological testing of free thyroxine, thyroid stimulating hormone, C-peptide, cortisol, and adrenocorticotropic hormone were normal. Additionally, no definitive evidence of movement disorder in his heart valves were disclosed by the Doppler echocardiography. The patient's serum calcitonin level, however, was apparently elevated at 40.94 pg/mL (reference range for male < 10.2 pg/mL). Therefore, the patient was requested to examine his bilateral thyroids due to MTC suspicion. Indeed, the thyroid scintigraphy imaging provided localization of abnormal radiopharmaceutical accumulations (Fig. 2A). The histological verification indicated that both the left and the right lobes contained small tumors, one was pale yellow with a diameter of 0.2 x 0.3 × 0.5 cm and the other tiny one was white. Furthermore, the thyroid biopsy with H&E staining

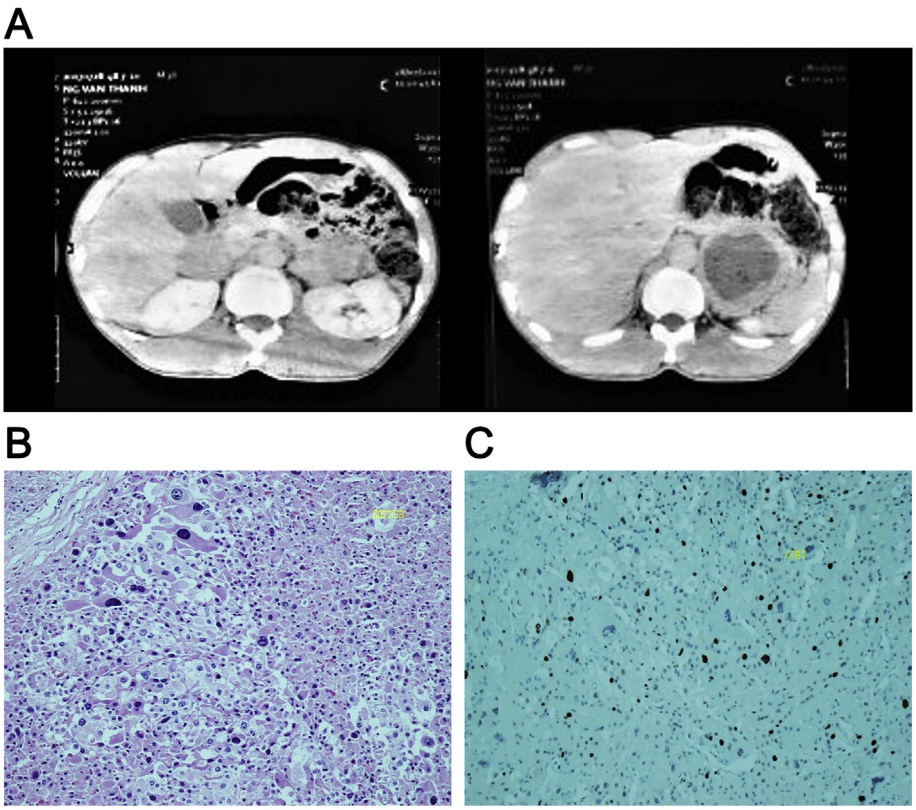

Fig. 1. (A) Contrast-enhanced computed tomography of the patient's abdomen shows right and left adrenal tumors. **(B)** Pathological diagnosis of the left adrenal mass is pheochromocytoma (hematoxylin and eosin stain, x 40). **(C)** Ki-67 immunohistochemical study of pheochromocytoma (left adrenal gland).

Table 1
Dynamic levels of plasma and 24-h urinary excretion of catecholamines in the patient.

Test (units)	Plasma (pg/mL)	Reference range	Urinary (μg/24h)	Reference range
Dopamine	74.63	0–100	437.2	0–600
Adrenaline	204.21	0–100	197.0	0–20
Noradrenaline	633.33	0–600	248.1	0–90
Aldosterone	98.30	40–310		

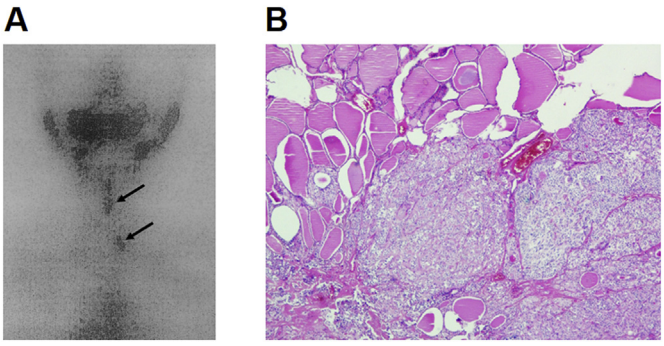

Fig. 2. (A) ^{99m}Tc thyroid scintigraphy presents localization of abnormal uptake in the thyroid gland of the patient (arrows). **(B)** Light microscopy of thyroid biopsy with H&E staining shows tumor cells concentrated into groups.

also showed that tumor cells concentrated into groups and follicular gland was hardly observed (Fig. 2B). Finally, selective screening for the RET proto-oncogene at exons 5, 8, 10, 11, and 13–16 was performed using genomic DNA extracted from the peripheral blood leukocytes of the patient. A heterozygous, pathogenic germline mutation c.1900T > G was detected at exon 11 of RET gene (Gene ID: 5979; OMIM 164761) by direct sequencing using an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). This transversion substituted glycine with cysteine at the codon 634 (p.Cys634Gly) (Fig. 3A). This confirmed that the index patient P1 has MTC with the subtype MEN 2A, and the treatment via both adrenalectomy and thyroidectomy was necessary.

The patient P1 initially underwent surgical resection of the bilateral adrenal tumors. The postoperative pathology was more confidently confirmed by histological examination (Fig. 1B) and immunohistochemical staining with the proliferation marker, Ki-67 (Fig. 1C). The histopathological results showed that the section of tumors was yellowish with some umber regions, and they consisted of irregular shape cells with large cytoplasm and abundant basophilic grains. It was noticeable that the expression of Ki-67 in the nucleus of cells was associated with malignancy of tumors and prognostic assessment for patient. Two months after the first surgery, major functional parameters of his kidneys, such as renal perfusion and glomerular filtration, were reported in normal reference ranges through the radioisotope renography using ^{99m}Tc-diethylenetriaminepentacetate (DTPA) as a radiolabelled pharmaceutical agent.

The index patient then undertook a thyroid surgery. After the thyroidectomy, the Congo red staining of the tumor specimens revealed that cancer cells were aggregated into clusters, stromal fibrous tissue was proliferated, and a phenomenon of amyloidosis occurred in many regions (Fig. 4A). The immunohistochemistry also exhibited strong, positive tests to calcitonin and TTF1 (Fig. 4B and C). Fortunately, no evidence of bone lesion was observed when a bone scintigraphy, using ^{99m}Tc-methylene diphosphonate (MDP), was performed.

The P1's younger sister (P2) was diagnosed with a papillary thyroid carcinoma, with lymph node metastasis, and underwent surgery to remove her thyroid. Her genetic testing result was also positive for the *RET* C634G mutation. Since P1 and P2 harbor the C634G germline mutation, all their family members were asked for *RET* mutation screening, regardless of a lack of clinical symptoms. Indeed, the identical mutation in exon 11 of the *RET* gene was identified in one daughter of each patient, P1 and P2 (Fig. 3B). Hence, the daughters are being followed-up carefully as pre-symptomatic MEN 2A cases.

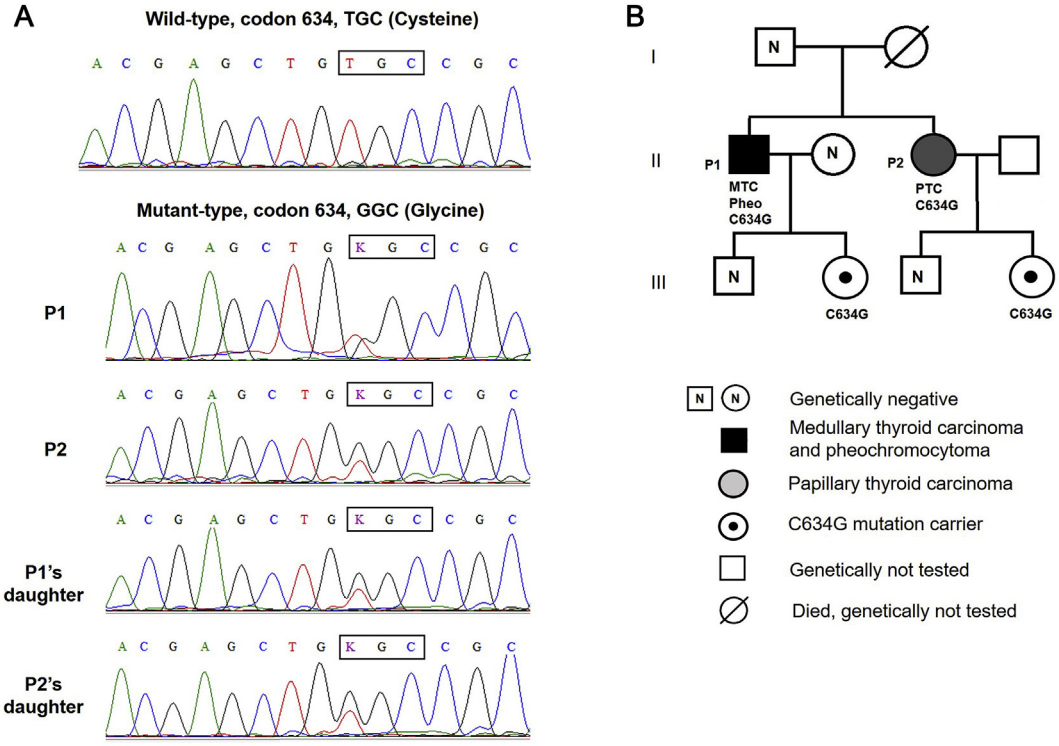

Fig. 3. Sequencing chromatograms **(A)** illustrating the missense TGC → GGC mutation at codon 634 in exon 11 of the *RET* proto-oncogene detected in the proband (P1 patient) and his relatives. The pedigree of the family **(B)**.

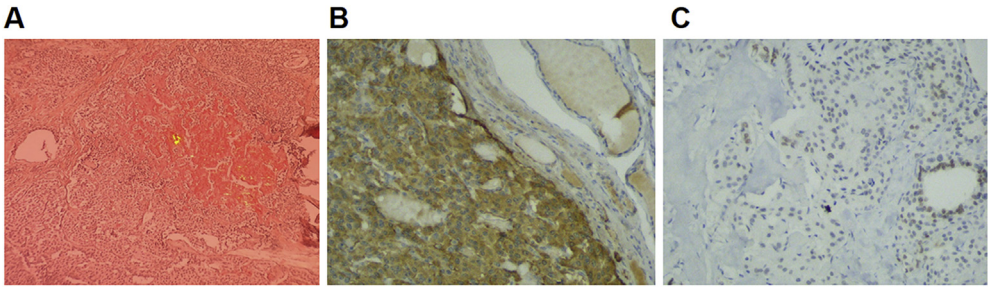

Fig. 4. Pathological images of the thyroid lesion in the patient. **(A)** Phenomenon of cutaneous lichen amyloidosis is revealed (congo red, x 10). Immunohistochemistry of thyroid shows strong positivity for **(B)** calcitonin (brown staining with polyclonal rabbit anti-human antibody, 1:400, Dako), and **(C)** TTF1 (brown staining with monoclonal mouse antibody, clone 8G7G311, 1:100, Leica). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Discussion

MEN 2A is an inherited, autosomal dominant disorder which is caused by alterations in the *RET* proto-oncogene. In this case, the patient P1 was identified to have pheochromocytoma before he was suspected to have thyroid carcinoma. The genetic analysis then revealed a causative mutation of MTC with a substitution of cysteine for glycine at the codon 634 in exon 11 of the *RET* proto-oncogene. In MEN 2A, the onset of pheochromocytoma is usually concomitant or subsequent to MTC [7]. However, 13–27% of MEN 2A cases have pheochromocytoma as the first symptom of the syndrome [8]. It is observed that screening, following the age-related development of MEN 2 associated pheochromocytoma, may be warranted from the age of 10 in carriers of *RET* mutations in codons 918, 634, and 630, and from age 20 in the remaining codons [9]. The genotype-phenotype correlation of the cysteine codon 634 mutations with MEN 2A are associated with the highest penetrance of pheochromocytoma. The penetrance has also been found to increase with age, being 25% by age 30, 52% by age 50, and 88% by age 77 [10]. Additionally, cutaneous lichen amyloidosis is a rare disorder that occurs almost exclusively in MEN 2A patients with the *RET* codon 634 mutation [11].

Pathogenic *RET* variants influencing the cysteine codons of the extracellular domain (codons 609, 611, 618, 620, 630, and 634) account for 98% of all mutations associated with MEN 2A [2,12]. The disruption of a cysteine by such mutations may allow the partner cysteine to become available for aberrant disulfide bonding with other mutant *RET* molecules. This process induces constitutive receptor dimerization, and hence activation, which leads to overregulation of the extracellular signals for processes as diverse as cell growth, differentiation, survival, and programmed cell death [3]. According to the American Thyroid Association guideline, the current risk categories for hereditary MTC are classified based on *RET* mutations associated with increasing aggressiveness of the MTC, consisting of highest risk, high risk, and moderate risk. The *RET* codon C634 variants (C634F/G/R/S/W/Y) are categorized into the high risk level [11] and the recommendations for the family members who genetically test positive are: prophylactic thyroidectomy before the age 5, annual albumin-corrected calcium or ionized serum calcium measurements (with or without serum intact parathyroid hormone) beginning at the age of 8, and annual plasma free metanephrines and normetanephrines or 24-h urine collection for metanephrines and normetanephrines beginning by the age 20 [13]. In this particular case, the replacement of a cysteine 634 residue with glycine was detected not only in the proband, but also in his younger sister and each of their daughters. This finding indicated that the family carries an inherited type of MTC (Fig. 2). However, different members of a family carrying the same *RET* variation can display different levels of MTC. This may be due to diverging levels of genetic penetrance or the presence of genetic modifiers [13].

This is the first observed case of MEN 2A with the clinical feature of pheochromocytoma caused by C634G *RET* germline mutation in Vietnam. Despite the fact that the mutations in codon 634 are the most frequently reported, only a few cases with C634G variations were described in several nations such as Germany, the Netherlands [14], Italy [15], and India [16]. However, these reports mainly consisted of patients with hereditary MTC or MEN 2A without accompanying pheochromocytoma. Similar circumstances of MEN 2A caused by the genetic mutation at the codon 634, accompanied with multifocal MTC, pheochromocytoma, and hyperparathyroidism were rarely presented in 76-year-old and 42-year-old women in Slovenia [17], an 80-year-old man in France [18], and a 42-year-old man in North America [13]. For such patients, pheochromocytoma should be removed before a thyroidectomy is performed, since an undetected pheochromocytoma could be a reason for an intraoperative fatal, hypertensive crisis. Prophylactic thyroidectomy is believed to be an effective treatment for *RET* mutation carriers. Recommendations for the time point of a prophylactic thyroidectomy are based on the patient's genotype-phenotype correlations [7]. Due to MTC being malignant, the prognosis of patients with MEN 2A was closely related to the early detection and treatment of MTC. Indeed, molecular diagnostic testing in which *RET* genetic screening is the key tool has been a mainstay of the clinical management of MTC for many years. Furthermore, a crucial benefit is that the comprehensive analysis of molecular alterations in MTC allows for rapid identification of mutations. The acceleration of this process allows for patients to be quickly classified into different risk levels [11], providing them with appropriate therapeutics for each specific case.

Conflicts of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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