Clinical significance of *RET* mutation screening in a pedigree of multiple endocrine neoplasia type 2A

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Abstract. The clinical characteristics and RET proto-oncogene (*RET-PO*) mutation status of a patient with multiple endocrine neoplasia type 2A pedigree (MEN2A) was analyzed with the aim of preliminarily exploring the molecular mechanisms and clinical significance of the disease. Clinical characteristics of a single MEN2A patient were analyzed. Genomic DNA was extracted from the peripheral blood of the proband and 10 family members. The 21 exons of *RET-PO* were PCR amplified and the amplified products were sequenced. Of the family members, 5 exhibited a C634Y (TGC→TAC) missense mutation in exon 11 of *RET-PO*, among which 2 family members were screened as mutation carriers, while the others did not exhibit clinical symptoms of the mutation. The screening and analysis of *RET-PO* mutations for the MEN2A proband and the family members suggests potential clinical phenotypes and enables assessment of the risk of disease development, thus providing useful information for determining the surgical timing of preventive thyroid gland removal.

Introduction

Multiple endocrine neoplasia type 2 (MEN2), also known as Sipple's syndrome, is associated with medullary thyroid cancer (MTC) and thyroid C cell hyperplasia. It is an autosomal dominant genetic disease caused by a mutation in the RET proto-oncogene (*RET-PO*) on chromosome 10 (1,2), which results in 2 or more endocrine adenomas or hyperplasia in the same patient, either concurrently or successively, and leads to the clinical syndrome characterized by the hyperfunctioning of the affected glands. Based on the clinical manifestation, MEN2 can be divided into MEN2A, MEN2B and familial medullary thyroid carcinoma, among which MEN2A is the most common subtype. MEN2A is characterized by medullary thyroid carcinoma (MTC), pheochromocytoma (PHEO) and hyperparathyroidism. Additionally, a small number of patients exhibit skin lichen amyloidosis or Hirschsprung's disease (3,4). MTC is often the first manifestation of this subtype, with an incidence of close to 100% (5). When admitted, the majority patients have already progressed to MTC or exhibit lymph node metastasis. MTC is the predominant cause of death in patients with MEN2A (6), and it has previously been reported that 50% of patients are at risk of MTC recurrence (7). However, the appearance of MTC or MEN2A may differ among family members. Specifically, basic lesions may be fully or not completely presented, the involved endocrine glands may exhibit lesions that occur at different time intervals (which may be as long as several years), and multiple endocrine glands are occasionally involved and may exhibit simultaneous onset. Currently, patients with MEN2A that exhibit MTC as the early manifestation are often misdiagnosed. Therefore, the present study analyzed the clinical data of one family with MEN2A to investigate the clinical features and the significance of *RET-PO* mutations.

Materials and methods

Clinical data. The family was a typical family with MEN2A, and the family pedigree is presented in Fig. 1. The serum calcitonin (Ct), catecholamine (CA) and carcinoembryonic antigen (CEA) levels of the patients (5 family members: III2, III5, III9, IV1, and IV4) are presented in Table I.

Detection method for RET-PO mutations. Peripheral blood (5 ml) with anticoagulant was extracted from 13 family members, including the 5 patients (1 patient died). Briefly, all samples were anti-coagulated with sodium citrate. White blood cells were isolated from each blood sample, and genomic DNA. Genomic DNA (200 ng) was amplified in a 50 μl reaction containing 10 mmol/l Tris-HCL (pH 8.4), 50 mmol/l KCL, 1.5 mmol/l $MgCl_2$, 0.5 μmol/l each of the specific primers, 100 μmol/l each of dATP, dGTP, dCTP and dTTP, 2 units of Taq DNA polymerase (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The primers for exon 11 were synthesized by Shenzhen Huada Gene Research Institute (Shenzhen, China). The sequences were as follows: Forward,

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5'-ACACCACCCCCACCCACAGAT-3' and reverse, 5'-AAG CTTGAAGGCATCCACGG-3' (273 bp). The thermocycling conditions were as follows: Denaturation at 94C for 5 min; 30 cycles of 94˚C for 50 s, at 58-62˚C for 50 sec, 72˚C for 50 sec; and a final extension at 72˚C for 10 min. The DNA sequencing analysis was conducted in Shenzhen Huada Gene Research Institute.

Imaging and biochemical tests. Imaging tests, such as ultrasound/CT or emission computed tomography, examined the thyroid, adrenal gland, and parathyroid, at minimum. Serum Ct and CEA were analyzed using an automated chemiluminescence immunoassay system (i2000; Abbott Laboratories, Chicago, IL, USA) according to the manufacturer's protocols. The serum CA was detected using the arsenazo III method (8). The tribromoarsenazo was provided by Sigma-Aldrich (St. Louis, MO, USA).

Follow-up. Follow-up was performed once every 3 months within 2 years following discharge the patient, then subsequently once every 6 months for 3 to 5 years, and once every 5 years thereafter. The follow-up indicators measured included serum Ct, CEA, thyroid function series, CA level and radiographic examination. Patients with an *RET* mutation without PHEO were screened initially, followed by examinations of CA levels and imaging annually beginning at 20 years old. The present study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Fudan University (Shanghai, China). Written informed consent was obtained from all participants.

Results

Characteristics of the studied family members. A total of 6 patients (4 males and 3 females) were diagnosed with MEN2A and there was 1 suspected case. Among the 6 patients with MEN2A, 3 exhibited MTC and PHEO simultaneously. Additionally, 1 patient died of hypertensive crisis during thyroid surgery and was considered to have PHEO. The remain 2 patients exhibited MTC only. The detailed clinical data are as follows.

The proband (III2) was a female and the onset age of MEN2A was 28 years. The patient was admitted for 'episodic headache, chest tightness and shortness of breath for 5 years' in 1991 and exhibited a blood pressure of 220/110 mmHg, serum Ct of 637 ng/l, and was positive for blood CA (+). The patient was diagnosed as having PHEO in the right adrenal gland and a right adrenal tumor resection was performed. A total thyroidectomy was performed in 2001; the post-operative pathology demonstrated MTC and the patient was finally diagnosed with MEN2A. Left adrenal PHEO surgery was performed in 2008, and reviewed on 13 October 2014. The serum Ct level was 175.00 ng/l (reference range: Male, <8.40 ng/l; female, <5.00 ng/l), CEA was 6.59 ng/ml (reference range, 0.00-5.00 ng/ml), and blood and urinary CA levels were normal. B-ultrasonic examination of the thyroid (B-US) prompted resection of the bilateral thyroids. The bilateral sides of the neck exhibited lymphadenectasis, with calcification on the left side, however no space-occupying lesion was present in the bilateral adrenal glands. A computed tomography (CT) examination revealed no lung, liver or bone metastasis, and the patient refused further dissection toward the carotid lymph nodes.

The proband's father (II1) died of sudden high blood pressure at 48 years old. According to Mendelian inheritance, MEN2A was suspected. The proband's aunt (II6) underwent thyroid resection at 51 years old and died of intraoperative hypertensive crisis. Her pathological features suggested MTC. The proband's brother (III5) underwent a physical examination at 28 years old that indicated space-occupying masses in the bilateral adrenal glands and PHEO surgery was performed in 2001. A B-US examination revealed a right thyroid mass in 2003, and a right thyroid lobectomy and central lymph node dissection was performed. Furthermore, pathological analysis demonstrated MTC. An examination on 13 October 2014 revealed the following: Serum Ct, <2.00 ng/l; serum CEA, 6.59 ng/ml; and normal blood and urine CA levels. B-US prompted removal of the right thyroid gland and the left thyroid gland exhibited no lumps. No cervical lymph node metastasis was found in the bilateral neck and no space-occupying lesion was found in the bilateral adrenal glands. A CT examination did not detect any lung, liver or bone metastasis.

The proband's cousin (III9) had a MEN2A onset age of 48 years old. He was admitted for 'left neck mass for 1 month' in 2009, and a left thyroid lobectomy and central lymph node dissection was performed. The pathological analysis demonstrated MTC. In 2010, a B-US review revealed a right thyroid mass, thus, a right thyroid lobectomy and central lymph node dissection was performed, and MTC was observed. An examination on 13 October 2014 revealed the following: Serum Ct, <2.00 ng/l; CEA, 1.17 ng/ml; and normal blood and urine CA levels. B-US prompted removal of the bilateral thyroids. No cervical lymph node metastasis was found in the neck, and no space-occupying lesions were detected in the bilateral adrenal glands. A CT examination did not detect any lung, liver or bone metastasis.

The proband's daughter (IV1) had a MEN2A onset age of 23 years old, and an *RET* C634Y mutation was detected via genetic screening in July 2010. A B-US examination demonstrated bilateral thyroid nodules and a CT examination observed a thickened left adrenal gland with the possibility of hyperplasia. A preoperative examination demonstrated the following: Serum Ct, 56.20 ng/l; and blood CA, negative. A total thyroidectomy was performed on 14 September 2010 and MTC was detected. A review on 13 October 2014 demonstrated the following: Serum Ct, 4.10 ng/l; CEA, 3.75 ng/ml; and blood and urine CA levels, normal. B-US prompted a resection of the bilateral thyroid and small lymph nodes were observed in the neck, however no space-occupying lesion was detected in the bilateral adrenal glands. A CT examination did not detect any lung, liver or bone metastasis.

The proband's nephew (IV4) had an onset age of 19 years old and an *RET* C634Y mutation was identified by genetic screening in July 2010. B-US revealed bilateral thyroid nodules (multiple on the right side, including a right central nodule K), posterior tubercles in the *radix nasi* of the right thyroid gland, which may originate from the parathyroid, and a right adrenal gland that exhibited a mixed tumor of the right adrenal region with the possibility of pheochromocytoma plus liquefaction. Preoperative serum Ct was 169.00 ng/l and blood CA was positive. The patient underwent right adrenal PHEO surgery on 25 July 2010,

Table I. Serum Ct, CA and CEA of 5 members in the multiple endocrine neoplasia type 2A pedigree family.

		Preoperative		Postoperative		
Patient	Gender	Ct (ng/l)	CA	Ct (ng/l)	CA	CEA (ng/ml)
III2	Female	637	+	175.00	-	6.59
III5	Male	Untested	Untested	<2.00	-	6.59
III9	Male	Untested	Untested	<2.00	-	1.17
IV1	Female	56.20	-	4.10	-	3.75
IV4	Male	169	+	14.10	-	1.36

Ct, calcitonin; CA, catecholamine; CEA, carcino embryonic antigen.

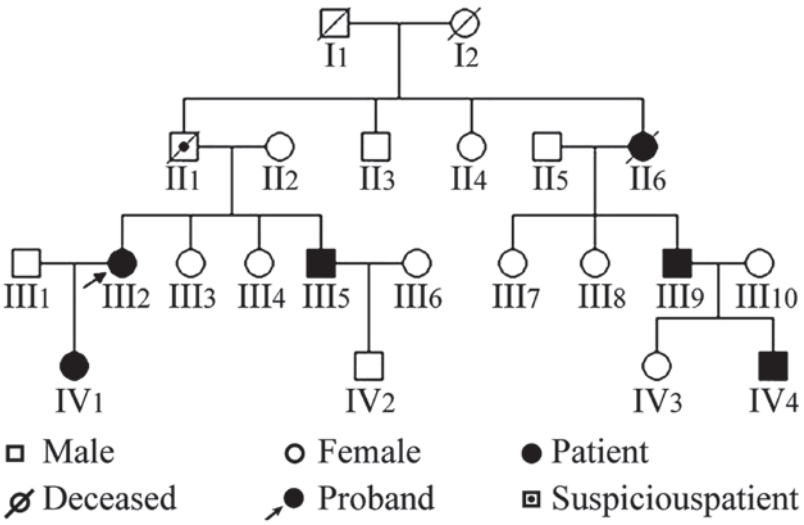

Figure 1. Pedigree chart of the multiple endocrine neoplasia type 2A pedigree family with RET proto-oncogene p.C634Y mutation.

and a total thyroidectomy on 4 August 2010, where MTC was observed. A review on 13 October 2014 revealed the following: Serum Ct, 14.10 ng/l; CEA, 1.36 ng/ml; and blood and urine CA levels, normal. B-US prompted the removal of the bilateral thyroid. No cervical lymph node metastasis was found in the neck and no space-occupying lesion was observed in the bilateral adrenal glands. A CT examination did not detect lung, liver or bone metastasis.

Results of genetic tests. Among the 13 family members, 5 members (III2, III5, III9, IV1, and IV4) exhibited the p.C634Y mutation. This was a heterozygous missense mutation at locus 634 of exon 11 of the *RET* gene resulting in a tyrosine to cysteine (TGC→TAC) substitution in the protein amino acid sequence (Fig. 2). Of these cases, 2 were *RET* mutation carriers (IV1 and IV4), but did not exhibit any clinical symptoms. The remaining family members exhibited no *RET* mutation, or clinical abnormalities based on imaging or Ct levels indicating MTC. Additionally, 1 case (II6) was previously diagnosed as MTC, but *RET* testing could not be performed due to death of the patient. A pedigree analysis (Fig. 1) demonstrated that generations III and IV were mutation carriers, and the transmission was consistent with an inherited disease due to a dominant single gene.

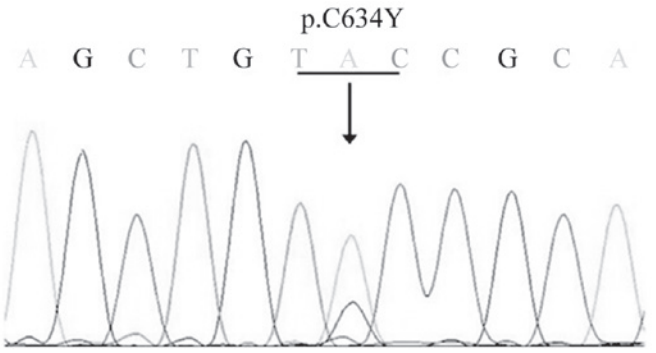

Figure 2. Sequencing of exon 11 of RET proto-oncogene from the multiple endocrine neoplasia type 2A pedigree family.

Discussion

The *RET-PO* gene consists of 21 exons located at chromosome 10q11.2 and encodes a tyrosine kinase receptor. *RET-PO* mutations can lead to the conversion of a cysteine residue to a different amino acid, which may lead to excessive cell proliferation and potentially cancer development. Following the observations in 1993 by Mulligan *et al* (9) and Donis-Keller *et al* (10) that an *RET-PO* mutation leads to

MEN2A, almost all MEN2A cases have been associated with an *RET-PO* mutation (11,12). A total of 93-98% of MEN2A cases exhibit a single-base *RET-PO* mutation in exon 10 (codons 609, 611, 618 and 620) or exon 11 (codon 634), with 87% exhibiting a mutation at codon 634. The most common mutation type is C634R (TGC→CGC), accounting for 52%, followed by C634Y (TGC→TAC), accounting for 25% (13). In the current study, the family of patients exhibited a C634Y mutation based on *RET* testing, which is a common *RET-PO* mutation.

Numerous studies have confirmed that *RET-PO* mutations are the predominant molecular etiology of MTC onset in patients with MEN2A (9,14-16). When *RET* mutations are confirmed, the probability of a patient developing MTC is close to 100% (17). Thus, *RET-PO* detection is a reliable method for diagnosing MEN2A, and is the gold standard for early diagnosis (18). Its accuracy is higher than that of Ct measurement and stimulation tests. In 2009, it was recommended by the American Thyroid Association (ATA) guidelines (3) that all patients pathologically diagnosed with C cell hyperplasia, MTC, MEN2A or autosomal dominant inheritance risk should undergo *RET-PO* testing. Thus, in clinical practice, MTC patients should be carefully examined and genetic testing should be performed as early as possible. If germline *RET-PO* mutations are detected in an individual without sporadic MTC, the patient is likely to be a hereditary MTC proband.

MTC originates from thyroid parafollicular cells, and unlike papillary thyroid carcinoma and follicular thyroid carcinoma, the predominant, and only effective, MTC treatment is to resect the primary tumor lesions and corresponding metastatic lymph nodes as soon as possible (19). There remains controversy regarding the optimal surgical methods for thyroid primary tumors. Additionally, MTC has a low incidence and diverse clinical manifestations, and factors that affect prognosis vary between different studies (20). Some scholars propose that the most appropriate method to treat MTC is total thyroidectomy and central lymph node dissection, based on the theory that each parafollicular cell of the *RET-PO* germline mutation carrier has the potential for cancerization, and as such, all parafollicular cells should be resected (21,22). Moreover, some scholars have previously suggested that a patient with a unilateral lesion only requires thyroid lobe and isthmus resection, and central lymph node dissection on the affected side, and the contralateral gland should be explored or resected (23). For the patients with bilateral lesions, a total thyroidectomy should be performed. The total thyroidectomy may result in increased postoperative complications, including thyroid and parathyroid insufficiency. For these reasons, the 2009 MTC treatment guidelines established by the ATA (3), which described the scope and extent of surgical resection toward typical MTC patients, total thyroidectomy and preventive neck lymph node dissection were recommended to reduce the recurrence of MTC.

Mutations in the *RET* gene are closely associated with clinical manifestations and risk stratification of MTC. Currently, the treatment of hereditary MTC is undergoing a revolution from empirical treatment to individual treatment; the latter emphasizes individualized treatment based on the codon mutation site. In 2010, the North American Association of Neuroendocrine Tumors published the MTC diagnosis and treatment guidelines, which were divided into 3 groups based on the gene mutation according to MTC invasion and high-risk characteristics (24). *RET-PO* mutations 609, 768, 790, 791, 804 and 891 are included in the low-risk group, which rarely develops into cancer before age 10, thus a preventive thyroidectomy before 10 years of age is recommended. *RET-PO* mutations 611, 618, 620 and 634 are included in the middle-risk group, and a thyroidectomy is recommended before 5 years of age. *RET-PO* mutations 883, 918 and 922 are included in the high-risk group; patients in this group exhibit metastasis at 1 year of age, thus, thyroidectomy is recommended within 1 month of reaching 6 months of age (25).

In the present study, 2 MEN2A gene mutation carriers were identified by screening the 21 exons of *RET-PO*, and preventive total thyroidectomy was performed. The postoperative pathologies all confirmed MTC without lymph node metastasis. The postoperative 4-year follow-up did not detect recurrence or metastasis. This suggests that a preventive total thyroidectomy can improve the cure rate before MTC is clinically observed, as lymph node metastasis rarely occurs at this time, and it may also avoid trauma caused by neck dissection. However, total thyroidectomy has higher risks, for example, the incidences of complications, including postoperative recurrent laryngeal nerve injury and permanent hypoparathyroidism, are higher in children than in adults. Accordingly, surgery in children should be performed by experienced pediatric specialists to minimize complications.

PHEO in patients with MEN2A is associated with bilateral adrenal tumors that may occur several years later, with clinical manifestations including persistent or paroxysmal hypertension, however, malignant tumors rarely occur or occur outside of the adrenal glands. Approximately 30-50% of patients are asymptomatic or experience sudden hypertension in thyroid surgeries, and increased blood/urinary excretion of CA and its metabolites may be the only abnormality in the early stage of this disease (26,27). The diagnosis predominantly relies on biochemical and imaging data, and 2 patients in the present study exhibited elevated blood CA levels. If PHEO is present, it should be resected with the application of an α-receptor blocker, followed by the resection of other tumors to avoid induced intraoperative hypertensive crisis and shock. Prior to surgery, preparations including decompression, expansion, and heart rate control should be conducted. Patients with bilateral adrenalectomy should be given hormone replacement to prevent adrenal crisis. The surgery should preserve a certain amount of normal adrenal tissues to reduce the requirement of long-term hormone replacement therapy. Additionally, radical thyroid resection should be performed promptly.

As *RET-PO* mutations in MEN2A are strongly correlated with clinical phenotypes of this disease, mutation screening and analysis for MEN2A probands and family members may suggest potential clinical phenotypes and enable a risk assessment, thus, helping to determine the surgical timing of preventive thyroidectomy, which may prevent or delay the progression of the disease, and maximize clinical benefits. However, in China there remain various difficulties associated with routine *RET-PO* screening and the timing of preventive thyroidectomy cannot be optimized in the short term. Previous investigations and the results of the current study on a typical MEN2A family suggest that family members with MEN2A

should undergo *RET-PO* testing as early as possible, asymptomatic carriers should be regularly followed up, and surgery should be performed when lesions are detected. This course of action is appropriate for use in China.

References

1. Raue F and Frank-Raue K: Update multiple endocrine neoplasia type 2. Fam Cancer 9: 449-557, 2010.
2. Wagner SM, Zhu S, Nicolescu AC and Mulligan LM: Molecular mechanisms of RET receptor-mediated oncogenesis in multiple endocrine neoplasia 2. Clinics (Sao Paulo) 67 (Suppl 1): S77-S84, 2012.
3. American Thyroid Association Guidelines Task Force, Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JF, Pacini F, Ringel MD, *et al*: Medullary thyroid cancer: Management guidelines of the American thyroid association. Thyroid 19: 565-612, 2009.
4. Qi XP, Ying RB, Ma JM, Liu WT, Du ZF, Fei J, Yang CP, Song QZ, Jin HY, Chen ZG, *et al*: Case report: A p.C618S RET proto-oncogene germline mutation in a large Chinese pedigree with familial medullary thyroid carcinoma. Fam Cancer 11: 131-136, 2012.
5. Hibi Y, Ohye T, Ogawa K, Shimizu Y, Shibata M, Kagawa C, Mizuno Y, Kurahashi H and Iwase K: A MEN2A family with two asymptomatic carriers affected by unilateral renal agenesis. Endocr J 61: 19-23, 2014.
6. Sakorafas GH, Friess H and Peros G: The genetic basis of hereditary medullary thyroid cancer: Clinical implications for the surgeon, with a particular emphasis on the role of prophylactic thyroidectomy. Endocr Relat Cancer 15: 871-884, 2008.
7. Roy M, Chen H and Sippel RS: Current understanding and management of medullary thyroid cancer. Oncologist 18: 1093-1100, 2013.
8. Ferguson JW, Richard JJ, O'Laughlin JW and Banks CV: Simultaneous spectrophotometric determination of calcium and magnesium with chlorophosphonazo III. Anal Chem 36: 796-799, 1964.
9. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, *et al*: Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 363: 458-460, 1993.
10. Donis-Keller H, Dou S, Chi D, Toshima K, Lairmore TC, Howe JR, Moley JF, Goodfellow P and Wells SA Jr: Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet 2: 851-856, 1993.
11. Gschwind A, Fischer OM and Ullrich A: The discovery of receptor tyrosine kinases: Targets for cancer therapy. Nat Rev Cancer 4: 361-370, 2004.
12. Machens A, Niccoli-Sire P, Hoegel J, Frank-Raue K, van Vroonhoven TJ, Roeher HD, Wahl RA, Lamesch P, Raue F, Conte-Devolx B, *et al*: Early malignant progression of hereditary medullary thyroid cancer. N Engl J Med 349: 1517-1525, 2003.
13. Pasini B, Ceccherini I and Romeo G: RET mutations in human disease. Trends Genet 12: 138-144, 1996.
14. Qi XP, Chen XL, Ma JM, Du ZF, Fei J, Yang CP, Cheng J, Song QZ, Han JS, Jin HY, *et al*: RET proto-oncogene genetic screening of families with multiple endocrine neoplasia type 2 optimizes diagnostic and clinical management in China. Thyroid 22: 1257-1265, 2012.
15. Mulligan LM, Eng C, Healey CS, Clayton D, Kwok JB, Gardner E, Ponder MA, Frilling A, Jackson CE, Lehnert H, *et al*: Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. Nat Genet 6: 70-74, 1994.
16. Santoro M, Carlomagno F, Romano A, Bottaro DP, Dathan NA, Grieco M, Fusco A, Vecchio G, Matoskova B, Kraus MH, *et al*: Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. Science 267: 381-383, 1995.
17. Sippel RS, Kunnimalaiyaan M and Chen H: Current management of medullary thyroid cancer. Oncologist 13: 539-547, 2008.
18. Sawka AM, Jaeschke R, Singh RJ and Young WF Jr: A comparison of biochemical tests for pheochromocytoma: Measurement of fractionated plasma metanephrines compared with the combination of 24-hour urinary metanephrines and catecholamines. J Clin Endocrinol Metab 88: 553-558, 2003.
19. Milan SA, Sosa JA and Roman SA: Current management of medullary thyroid cancer. Minerva Chir 65: 27-37, 2010.
20. Roman S, Lin R and Sosa JA: Prognosis of medullary thyroid carcinoma: Demographic, clinical and pathologic predictors of survival in 1252 cases. Cancer 107: 2134-2142, 2006.
21. Scollo C, Baudin E, Travagli JP, Caillou B, Bellon N, Leboulleux S and Schlumberger M: Rationale for central and bilateral lymph node dissection in sporadic and hereditary medullary thyroid cancer. J Clin Endocrinol Metab 88: 2070-2075, 2003.
22. Fleming JB, Lee JE, Bouvet M, Schultz PN, Sherman SI, Sellin RV, Friend KE, Burgess MA, Cote GJ, Gagel RF and Evans DB: Surgical strategy for the treatment of medullary thyroid carcinoma. Ann Surg 230: 697-707, 1999.
23. White ML, Gauger PG and Doherty GM: Central lymph node dissection in differentiated thyroid cancer. World J Surg 31: 895-904, 2007.
24. Chen H, Sippel RS, O'Dorisio MS, Vinik AI, Lloyd RV, Pacak K and North American Neuroendocrine Tumor Society (NANETS): The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: Pheochromocytoma, paraganglioma, and medullary thyroid cancer. Pancreas 39: 775-783, 2010.
25. Krampitz GW and Norton JA: RET gene mutations (genotype and phenotype) of multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma. Cancer 120: 1920-1931, 2014.
26. Rodriguez JM, Balsalobre M, Ponce JL, Ríos A, Torregrosa NM, Tebar J and Parrilla P: Pheochromocytoma in MEN 2A syndrome. Study of 54 patients. World J Surg 32: 2520-2526, 2008.
27. Inabnet WB, Caragliano P and Pertsemlidis D: Pheochromocytoma: Inherited association, bilaterality and cortex preservation. Surgery 128: 1007-1011, 2000.