# Multiple endocrine neoplasia 2A with RET mutation p.Cys380Arg (GRCh38): A case report

**Luu Thuy Quynh, Pham Thi Hong Nhung,**
**Nguyen Thi Thuy, Vu Thi Loan,**
**Nguyen Thi Thuy Linh, Le Bich Nhan,**
**Nguyen Van Manh, Le Minh Thang,**
**Nguyen Thi Kieu Trinh, Ngo Thi Phuong**

*108 Military Central Hospital*

**Summary**

MEN 2A is a complex autosomal dominant inherited syndrome characterized by pheochromocytoma (PC), medullary thyroid carcinoma (MTC) and primary parathyroid hyperplasia (PHP). We report a case of 26 years old female offended with bilateral pheochromocytoma, medullary thyroid carcinoma and primary hyperparathyroidism due to multiple endocrine neoplasia type 2A confirmed by RET gene mutation p.Cys380Arg according to the GRCh38 (Genome Reference Consortium Humain Build 38 Organism - 2013) classification. The mutation with a change of amino acid 380 from Cys (Cysteine) to Arg (Arginin) is found in more than one-half of kindreds with MEN2A.

*Keywords*: Multiple endocrine neoplasias 2A, RET gene.

## 1. Background

The MEN (Multiple endocrine neoplasias) syndromes were first described in the early 1900s. They contain a group of heterogeneous disorders characterized by a predisposition for tumors involving two or more endocrine glands. MEN syndromes were classified into two principal categories: MEN 1 and MEN 2.

MEN2 (formerly MEN2A) has a prevalence of 1 in 30.000 individuals [1]. It is comprised of medullary thyroid carcinoma (MTC) in 95% of patients, pheochromocytoma (PC) in ~50% of patients, and primary hyperparathyroidism (PHP) in 20-30% of patients [2]. MTC may present with symptoms of calcitonin excess, including diarrhea and flushing. PC can present with the class triad of palpitation, headache, and diaphoresis. PC is more likely to be bilateral. PHP in MEN2A is usually asymptomatic and diagnosed concurrently with the diagnosis of MTC.

MEN2 are caused by mutations in the RET (rearranged during transfection) proto-oncogene, a 21-exon gene located on chromosome 10q11.2 that encodes a membrane-bound tyrosine kinase cell surface receptor [3].

Screening for MEN2A is aimed at the early detection of MTC, PC, and PHP.

Treatment for MEN2A for each manifestation depends on location, risk of recurrence or malignancy, hormone excess, and surgical morbidity. MEN2 patients with RET mutation require total thyroidectomy and complete resection of any local or regional metastases. In Vietnam, MEN syndromes are sporadic. Genetic testings are now available, so it is simpler to get the diagnosis of MEN and to explore the risk for mutations for the first-degree relatives of the patient. However, there are

***Received**: 31 October 2022, **Accepted**: 14 December 2022*
***Correspondence to:** Luu Thuy Quynh - Department of Endocrinology, 108 Military Central Hospital*
***Email:** quynhluuthuy@gmail.com*

two difficulties in treating patients who have bilateral pheochromocytomas. Firstly is the patient's suspicion of the bilateral adrenalectomy. Secondly is the rarity of mineral corticosteroids for patients who accept surgery.

## 2. Case presentation

A female patient, aged 26 years, with undiagnosed hypertension before, was admitted to the endocrinology department with complaints of paroxysmal palpitation and headache for the last four months. There is no history of endocrine disorders in the patient or other family members. The patient had no abnormal physical examinations. No other symptoms like facial flushing, blurred vision or sweating were observed. The thyroid examination was regular and euthyroid. Significantly, the blood pressure (BP) was high during paroxysmal palpitation (Systolic BP varies from 140-205mmHg, and diastolic BP varies from 50-90mmHg), and heart rate (HR) ranged from 120-140 beats per minute.

Laboratory tests were within normal limits, including complete blood count, fasting blood sugar, liver function test, serum electrolytes, serum creatinine, eGFR, chest X-rays, and ECG reports. Based on symptoms like high blood pressure, headache, and racing heart in a young nonsmoker female patient, we suspected a provisional diagnosis of secondary hypertension due to pheochromocytoma. The patient was done biochemical investigations to confirm this diagnosis.

**Table 1. Plasma and urinary values for catecholamines and their metabolites**

| Test | Result | Reference range |
|---|---|---|
| Plasma dopamine | 19pg/mL | 0-100pg/mL |
| Plasma adrenaline | 495pg/mL | 0-600pg/mL |
| Plasma noradrenaline | 88.33pg/mL | 0-100pg/mL |
| 24hour urine dopamine | 410mcg/24hour | 0-600mcg/24hour |
| 24hour urine adrenaline | 148mcg/24hour | 0-20mcg/24hour |
| 24hour urine noradrenaline | 123.76mcg/24hour | 0-90mcg/24hour |

The immunology assay of 24 hours urine adrenaline was found to be very high ( > 7 times average). This result favored a diagnosis of pheochromocytoma. Then, abdominal CT scan confirmed the presence of a bilateral adrenal tumor (Figure 1).

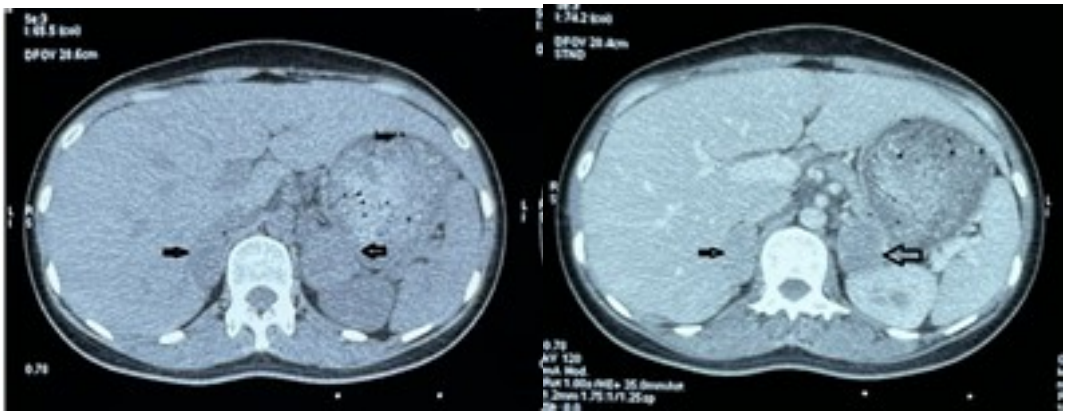

**Figure 1.** Computed tomography of the abdomen

An abdominal CT scan identified bilateral adrenal tumors and no renal artery stenosis. The right adrenal mass (arrow) measured 16 × 33mm with an unenhanced CT attenuation of 35HU; the relative percentage washout (RPW) was 36.7%, and the absolute percentage washout (APW) was 50%. The left adrenal mass

(open arrow) measured 28 × 32mm with an unenhanced CT attenuation of 45HU; the RPW was 9%, and the APW was 28%. The CT attenuation was > 10HU and diagnostic of a lipid-poor adrenal mass. Both had slow contrast washout (< 50% at 10 minutes).

The imaging and the biochemical findings suggested the diagnosis of pheochromocytoma. To investigate the multiple endocrine neoplasias, the patient underwent thyroid ultrasound and cervical CT scan, which confirmed the presence of thyroid nodules and lymph node metastases of stations IV and VI on both sides, suggestive of malignancy such as hypoechoic, irregular borders, microcalcifications (Figure 2, Figure 3, Figure 4).

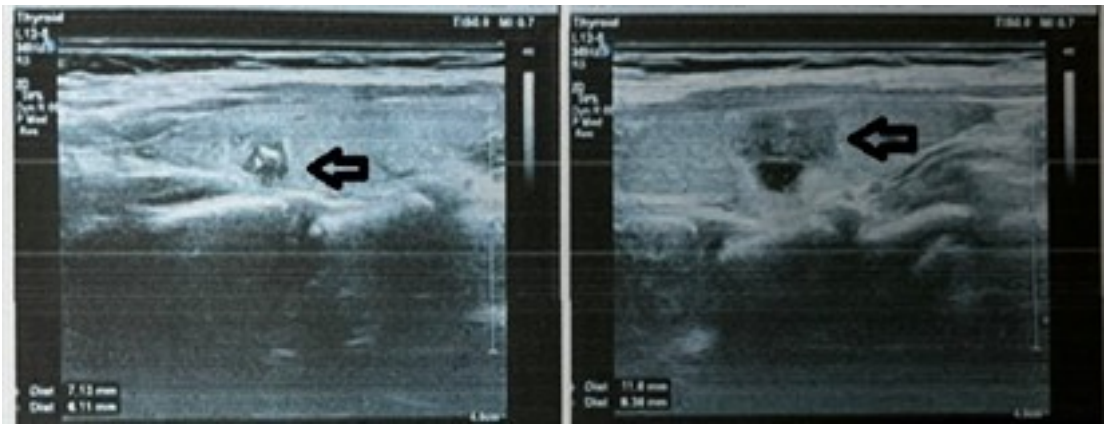

**Figure 2.** Ultrasound of thyroid nodule
The left and right lobes of the thyroid gland were at risk of malignancy with TIRADS 5

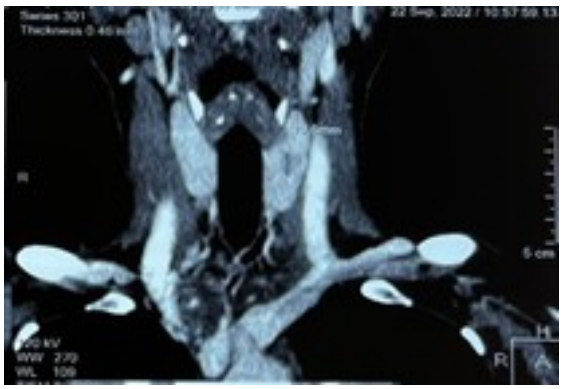

**Figure 3.** Computed tomography of thyroid nodule
The left lobe of the thyroid had a low attenuation nodule (maximum diameter of 10.6mm for nodule).

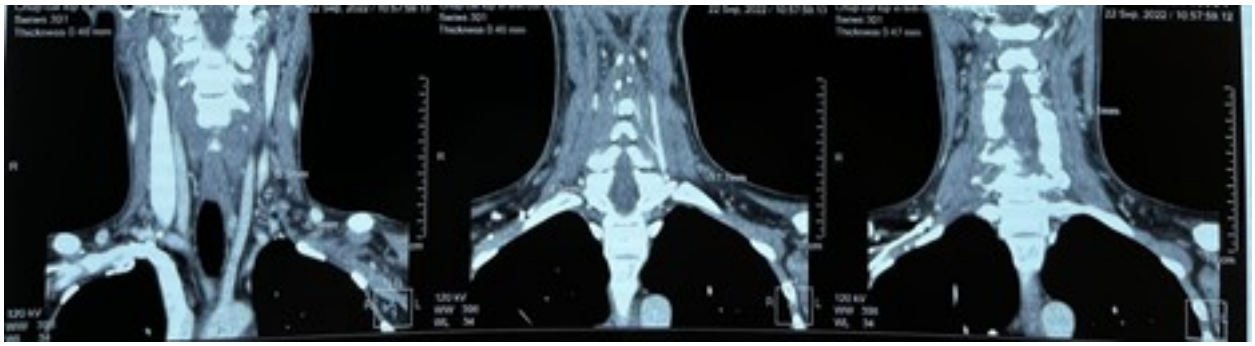

**Figure 4.** Computed tomography of malignant cervical lymph nodes
On both sides, there were many cervical lymph nodes of stations II, III, IV, and V.

Because of the suspicion of MTC in the MEN category, the Fine Needle Aspirarion (FNA) of the thyroid nodules, the serum calcitonin and carcinoembryonic antigen (CEA) were investigated. The results of FNA biopsy of thyroid nodules and lymph nodes was MTC (Figure 5). The diagnosis of MTC was also confirmed by serum calcitonin of 959pg/ml (normal range from 0 to 9.82pg/ml) and the CEA values of 17ng/ml (normal range from 0 to 5ng/ml).

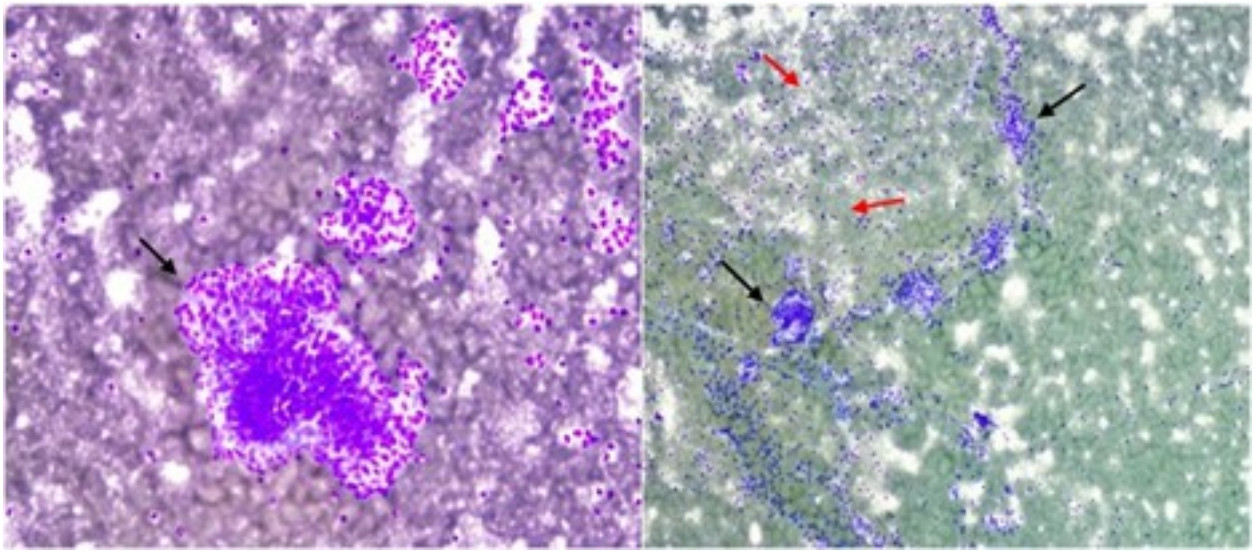

**Figure 5.** Thyroid nodules and lymph nodes cytology by FNA

**Figure 5A.** Cytology images of the right lobe thyroid tumor show that the tumor cell has ovoid or elongated nuclei, wide cytoplasm with inconspicuous margins and basophilic nuclei with no characteristics of papillary nuclei.

**Figure 5B.** Cytology images of the carcinoma metastasis to the lymph node: The lymphoid cells with round small nuclei, in cohesive clusters (black arrow) or isolated individual cells (red arrow).

Screening for PHP with serum calcium levels corrected for albumin elevated was 2.6mmol/l, and normal serum phosphate was 1.17mmol/l. Parathyroid hormone (PTH) measurement confirmed the diagnosis of PHP with high level of PTH was 107.2pg/ml. CT scan of parythyroid gland and parathyroid scintigraphy using 99mTc-MIBI were performed (Figure 6, Figure 7).

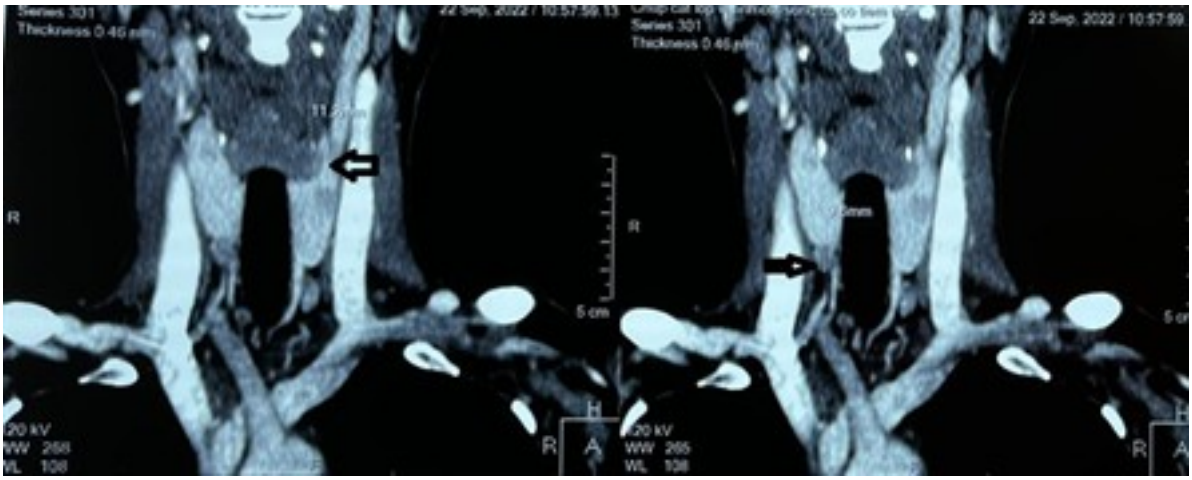

**Figure 6.** Computed tomography of left *(1st)* and right *(2nd)* parathyroid gland

A contrast CT scan of the neck showed parathyroid hyperplasia. The left superior parathyroid gland measured 11.2 × 8.3mm and the right inferior parathyroid glands measured 9.6 × 6.9mm (bigger than the normal pea-sized).

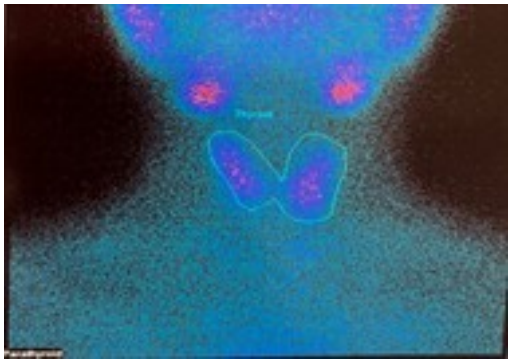

**Figure 7.** Parathyroid 99mTc-MIBI scan: Parathyroid gland not visible

Given the age of disease onset and pathological confirmation of the diagnosis of a chromaffin tumor, the suspicion of MEN 2A syndrome was raised and genetic testing to identify RET gene mutations was performed. Genetic testing confirmed a c.1138T>C (p.Cys380Arg) heterozygous pathogenic mutation in exon 10 of the RET gene (Figure 8).

MEDICAL GENETICS INSTITUTE
MOLECULAR GENETICS LABORATORY
GENE SOLUTIONS JSC
[redacted]
AOSC VLAM 1.0480 ISO 15189:2012
GENE SOLUTIONS Diagnostics & Cures

**GENETIC ANALYSIS REPORT**

**PATIENT INFORMATION**

| | | | | | |
|---|---|---|---|---|---|
| **Name:** | [redacted] Pham | **Sex:** | Female | **DOB:** | 1996 |
| **Patient ID:** | GABO63 | **Sample:** | Blood | **Phone number:** | 0343891202 |
| **Sample collecting date:** | Aug 5th, 2022 | **Sample collecting place:** | Department of Endocrinology-Hospital 108 | | |
| **Test:** | Diagsure | **Doctor:** | Hong-Nhung Thi Pham | | |
| **Clinical information:** | Dx: Multiple Endocrine Neoplasia Type 2A (MEN2A). 1. Pheochromocytoma. 2. Medullary thyroid cancer. 3. Parathyroid Tumor. Targeted panel: NT-05 Hyperparathyroidism and NT-11 Premature Ovarian Failure. | | | | |

**RESULT**

| Gene | Inheritance | Zygosity | Position | Alteration | Consequence | Phenotype | Mutation Classification in Clinvar |
|---|---|---|---|---|---|---|---|
| *RET* | Autosomal dominant | Heterozygous | chr10: 43114500 | NM_001355216.1: c.1138T>C (NP_001342145.1: p.Cys380Arg) | Missense variant | Multiple endocrine neoplasia IiA | Pathogenic |

**INTERPRETATION**

**1. One pathogenic variant is found in *RET* gene (autosomal dominant, heterozygous). This variant has been reported in the literature in multiple individuals affected with Multiple Endocrine Neoplasia Type 2A.**
**2. Combination of other clinical features and subclinical tests is suggested.**
**3. Consultation with your physician is required.**

**Figure 8.** Genetic analysis report

This patient was diagnosed MEN2A with PHEO, MTC and PHP. We planned for surgical bilateral adrenalectomy, total thyroidectomy with cervical lymph nodes dissection and resection of left uper and right inferior parathyroid glands. The patient was prepared for the surgery by cardiologists. She

was first given doxazosin (an alpha blocker), starting with 4mg/day and increasing up to 8mg then metoprolol (a beta blocker) 50mg/day starting from the seventh day to control the tachycardia. Blood pressure (BP) was controlled with systolic BP 100-120mmHg and diastolic BP 70 to 50mmHg). Adequate hydration and salt loaded diet was given to prevent intra-operative sudden drop in blood pressure.

The genetic testing for mutations in the RET gene is recommended for all her family members.

**3. Discussion**

Multiple endocrine neoplasias (MEN) are characterized by the occurrence of tumors involving more than two endocrine glands in a single patient. Up to now, three major types of MEN have been recognized and referred to as type 1 (MEN1), type 2A (MEN2A), and type 2B (MEN2B). MEN2A represents 95% of all MEN2, while MEN2B represents 5% of the remaining cases [3]. Multiple endocrine neoplasia 2A (MEN2A) is a rare autosomal-dominant genetic syndrome. The main components of MEN2A are medullary thyroid carcinoma (MTC), pheochromocytoma (PHEO), and primary hyperparathyroidism (PHP), with an incidence of about 1/30.000 individuals. The morbidity and mortality are mainly due to medullary thyroid carcinoma. RET gene sequencing is the gold standard for the diagnosis of MEN. The most frequent mutations are in exons 10 and exons 11. In a MEN2 patient, testing should be performed for immediate family members specific to the RET mutation [4].

In this report, our patient was diagnosed with MEN2A presented by PHEO, MTC, and PHP. The first step in this approach was to diagnose PHEO. The patient had the first clinical manifestation of PHEO (symptoms of adrenaline release) consistent with previous studies and the literature. The urinary values for catecholamines and their metabolites were higher seven times than usual. On computed tomography (CT), the typical appearance of a pheochromocytoma is a mass with an unenhanced density more significant than 10HU, bilateral avid contrast enhancement due to a rich capillary network, and delayed washout [5]. With our patient, abdominal CT scan characteristics fulfilled criteria with unenhanced attenuation values > 10HU (35HU for the right and 45HU for the left, both had slow contrast washout (< 50% at 10 minutes).

The second step was the diagnosis of MTC, confirmed by an FNA biopsy of thyroid nodules and lymph nodes. She had bilateral cervical lymph node metastases of stations II, III, IV, V and VI, so the predictive survival was poor [6]. The development of MTC in MEN2A is almost certain [3], even with microscopic MTC. Lymph node metastases decrease the patient's prospects of cure from 95% (with 0 metastases) to 31% to 57% (with 1-10 lymph node metastases) [7].

The next step was to diagnose PHP by elevated calcium levels corrected and parathyroid hormone levels [8]. In our patient, sestamibi scans were negative. The sensitivity of sestamibi scanning has been documented as ranging from 70% to 85%, depending on the source and the inclusion criteria. The rate of false negatives depends on various factors, including the size of the adenoma, multiglandular disease, and the location of hyperplastic tissue [9]. Studies have acknowledged the problem of negative sestamibi scans in a patient with lower preoperative PTH, and it is recommended to undergo further imaging, such as computed tomography. Our patient had a PTH result not too high, 107.2pg/ml, but the cervical CT has localized the parathyroid hyperplasia.

In one cohort study of Alysandra et al, in the USA, between March 2001 and April 2006, 578 consecutive patients with HPT underwent parathyroidectomy by a single surgeon, preoperative sestamibi had been done in 458 patients and 90 (20%) of which had negative results. This study showed that patients with a negative sestamibi generally had minor parathyroid glands at the time of surgery than the average gland weight reported in most other studies. Therefore, even in a negative sestamibi scan, most patients with primary HPT are still candidates for minimally invasive

parathyroidectomy [10]. Fortunately, in this case, the complication accompanying PHP not present, such as hypercalciuria and osteoporosis.

Regarding diagnosis, MEN2A should be suspected in individuals with one or more specific endocrine tumors: Medullary thyroid carcinoma, pheochromocytoma, or primary hyperparathyroidism. Our patient had had enough of these endocrine disorders, so the diagnosis of MEN2A was raised.

The patient was tested for the RET gene and found heterozygous mutation c.1138T>C (p.Cys380Arg). The RET gene is located on chromosome 10. According to the GRCh38 classification (Genome Reference Consortium Humain Build 38 Organism - 2013), the RET gene is located at position 43114500. This c.1138T>C (p.Cys380Arg) mutation is a form of the gene. point mutation, dominant, heterozygous, codon 1138 changes from T to C, resulting in a 380 amino acid change from Cys (Cysteine) to Arg (Arginine). Due to the GRCh38 and GRCh37 gene classification updates, the mutation we found is also the c.1900T>C (p.C634R) mutation according to the GRCh37 classification. As previously documented, codon 1900 T to C resulted in an amino acid change from 634 Cys to Arg. Therefore, we use mutant c.1138T>C (p.Cys380Arg) according to the latest classification for easy tracking. The c.1138T>C (p.Cys380Arg) mutation accounted for 85% of all mutations identified in MEN2A and the Cysteine to Arginine substitution at that site c.1138T>C (p.Cys380Arg) was found. in more than half of the genes with MEN2A [13]. Several cases of MEN2A have been described with mutations in the tyrosine kinase domain in protein 790 or 804 [11]. In 2020, Nguyen Hai Ha et al. reported a case of MEN2A in a Vietnamese family with the c.1138T>C mutation (p.Cys380Arg) similar to our patient [12]. Searching the NCBI bank and using the VARSOME bioinformatics tool, we found that this is a highly pathogenic polymorphic PS5 (Pathogen Strong 5), such as the American Society for Medical Genetics (ACMG)) recommended. The frequency of the polymorphism is f =0.0000121 in the population. For the East Asian population, the allele frequency is 0.000054, meaning that in 1 million people, there are 54 allele carriers, which is not a small percentage.

Treatment for pheochromocytoma is urgent surgical resection; however, blood pressure must be controlled preoperatively with α-blockers to prevent a hypertensive crisis. After the surgical bilateral adrenalectomy, the patient needs total thyroidectomy, cervical lymph node dissection, and resection of the left upper and right inferior parathyroid glands. MTC cells do not concentrate radioactive iodine, and the effect of chemotherapy is also limited. In advanced MTC, tyrosine kinase inhibitors have yielded promising results in phase II trials. Vandetanib was the first drug to be approved for the treatment of advanced MTC in 2011 [13].

## 4. Conclusion

MEN2A should be suspected in individuals with one or more specific endocrine tumors: medullary thyroid carcinoma, pheochromocytoma, or parathyroid adenoma/hyperplasia. RET gene sequencing is the gold standard for the diagnosis of MEN. Patients with MEN2A should be screened regularly and managed by a multidisciplinary team. Genetic testing for mutations in the RET gene is recommended, especially for first- or second-degree family members of the patient.

## References

1. Eng C (2019) *Multiple Endocrine Neoplasia Type 2*. 1999 Sep 27 [Updated 2019 Aug 15]. In: Adam MP, Everman DB, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2023.
2. Wells SA, Pacini F, Robinson BG, Santoro M (2013) *Multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma: An update*. J Clin Endocrinol Metab 98(8): 3149-3164.
3. Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C et al (2001) *Guidelines for diagnosis and therapy of MEN type 1 and type 2*. J Clin Endocrinol Metab 86(12): 5658-5671.

4. Ramundo V, Milone F, Severino R, Savastano S, Di Somma C, Vuolo L et al (2011) *Clinical and prognostic implications of the genetic diagnosis of hereditary NET syndromes in asymptomatic patients.* Horm Metab Res Horm Stoffwechselforschung Horm Metab 43(11): 794-800.
5. Blake MA, Kalra MK, Maher MM, Sahani DV, Sweeney AT, Mueller PR et al (2004) *Pheochromocytoma: An imaging chameleon*. RadioGraphics 24(1): 87-99.
6. American Thyroid Association Guidelines Task Force, Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF et al (2009) *Medullary thyroid cancer: Management guidelines of the American Thyroid Association*. Thyroid Off J Am Thyroid Assoc 19(6): 565-612.
7. Guyétant S, Dupre F, Bigorgne JC, Franc B, Dutrieux-Berger N, Lecomte-Houcke M et al (1999) *Medullary thyroid microcarcinoma: A clinicopathologic retrospective study of 38 patients with no prior familial disease.* Hum Pathol 30(8): 957-663.
8. Alevizaki M, Saltiki K (2015) *Primary Hyperparathyroidism in MEN2 Syndromes.* Recent Results Cancer Res Fortschritte Krebsforsch Progres Dans Rech Sur Cancer 204: 179-186.
9. Slitt GT, Lavery H, Morgan A, Bernstein B, Slavin J, Karimeddini MK et al (2005) *Hyperparathyroidism but a negative sestamibi scan: A clinical dilemma.* Am J Surg 190(5): 708-712.
10. Lal A, Chen H (2007) *The negative sestamibi scan: is a minimally invasive parathyroidectomy still possible?* Ann Surg Oncol 14(8): 2363-2366.
11. RET Proto-Oncogene - an overview | ScienceDirect Topics [Internet]. [cited 2022 Oct 30]. Available from: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/ret-proto-oncogene.
12. Nguyen Hai Ha, Nguyen Thi Thanh Hoa, Pham Thi Dung, Nguyen Huy Binh, Nguyen Dang Ton (2020) *Genetic testing identifies the potential risk of multiple endocrine neoplasia in a vietnamese family.* Vietnam Journal of Biotechnology.
13. Thornton K, Kim G, Maher VE, Chattopadhyay S, Tang S, Moon YJ et al (2012) *Vandetanib for the treatment of symptomatic or progressive medullary thyroid cancer in patients with unresectable locally advanced or metastatic disease.* Clin Cancer Res; 18(14).