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Elevated basal serum levels of calcitonin and simultaneous surgery of MEN2A-specific tumors

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Multiple endocrine neoplasia type 2A (MEN2A) is a rare syndrome caused almost by germline *RET* mutation, and characterized by medullary thyroid carcinoma (MTC), in combination or not with pheochromocytoma (PHEO), hyperparathyroidism (HPTH), cutaneous lichen amyloidosis (CLA), and Hirschsprung's disease (HD). The basal serum calcitonin (Ctn)/carcinoembryonic antigen (CEA) levels are significantly correlated with the MTC stage. Metachronous surgery of MEN2A-specific tumors is a routine procedure. We aimed to explore the clinical significance of pro-gastrin-releasing peptide (proGRP) in MTC with elevated Ctn and simultaneous surgery of MEN2A-specific tumors. We retrospectively investigated 8 *RET* mutation carriers of 2 Chinese pedigrees with MEN2A. Clinical profiles, imaging examinations, preoperative and postoperative biochemical data, surgical procedures, and follow-up records were evaluated. Three patients showed levels of elevated Ctn but normal proGRP. Among them, one patient (FAIII-6) in Family A (one for *RET* C634R mutation), diagnosed with bilateral MTC, left PHEO, bilateral HPTH, and CLA, classified as MEN2A-related CLA subtype, underwent successfully simultaneous adrenal-sparing surgery (ASS), total thyroidectomy (TT), and parathyroidectomy, while TT of the other two patients (FBII-3 and FBIII-7) diagnosed with bilateral MTC in Family B (all for *RET* C618R mutation) were performed. Unexpectedly, the absence of neck lymph node MTC metastasis was indicated by histopathological examination. Postoperatively, all had consistently "undetectable" or normal levels of Ctn/CEA during follow-up. Patients with normal proGRP, despite high levels of Ctn, might have no regional lymph node MTC metastasis, and neck dissection should be avoided. Moreover, simultaneous surgery for coexistent PHEO and either MTC or HPTH is an approach of choice to use as an alternative treatment pattern. Recognition of MEN2A-related CLA and subsequently early screening of *RET* mutation may be favorable for timely management of MEN2A-specific tumors.

Key words: multiple endocrine neoplasia type 2A, medullary thyroid carcinoma, pheochromocytoma, cutaneous lichen amyloidosis, calcitonin, pro-gastrin-releasing peptide

Multiple endocrine neoplasia type 2 (MEN2), divided into MEN2A (OMIM: 171400; ~95%) and MEN2B (OMIM: 162300; ~5%), is a neuroendocrine cancer syndrome characterized by an autosomal dominant inheritance pattern, which was caused by *RET* proto-oncogene (OMIM: 164761) and other two genes mutations of *MET* (OMIM: 164860) and *ESR2* (OMIM: 601663; c.948delT), with approximately 98% and <2%, respectively [1–4]. Generally, MEN2A is clinically manifested as medullary thyroid carcinoma (MTC), in combination or not with pheochromocytoma (PHEO), hyperparathyroidism (HPTH), cutaneous lichen amyloidosis (CLA), and Hirschsprung's disease (HD) [2]. As one of four variants of MEN2A, MEN2A-related CLA accounted for only approximately 9% (18/199) of MEN2A patients, and almost all families were associated with codon 634 mutations (96.4%, 54/56) in exon 11 of *RET* [5–8]. With exception of two patients, a V804M mutation (1.8%, 1/56) in exon 14 and a C611Y mutation (1.8%, 1/56) in exon 10 were also involved [6, 7]. The other variant of MEN2A, familial MTC (FMTC; OMIM: 155240) is characterized by the presence of MTC in at least four family members who develop neither PHEOs/HPTH nor other manifestations of MEN2A [2].

Based on the revised guidelines of the American Thyroid Association (ATA-2015), integrating screening for *RET* mutation and basal serum levels of calcitonin (Ctn)/carcinoembryonic antigen (CEA) can guide the management of

MTC, particularly, which could be beneficial for individualized surgical excision of MEN2-related MTC [2, 9]. Recently, pro-gastrin-releasing peptide (proGRP) showed a significant correlation with levels of Ctn/CEA [10]. As a potential tumor marker, combining proGRP levels may be additionally helpful in the precision evaluation of MTC [10–13]. Furthermore, metachronous surgery for adrenal-sparing surgery (ASS), prior to thyroidectomy and parathyroidectomy, currently is a routine procedure in almost all MEN2 patients [2] but otherwise, only four studies [14–17] describe the simultaneous operation, a few of which have been reported to be successful.

In the present study, we investigated 2 southeastern Chinese Han families diagnosed with MEN2A. Three of them showed the levels of elevated preoperative Ctn (37.54–3,600.80 pg/ml; normal males, <8.4 pg/ml; females, <5.0 pg/ml), but normal proGRP (<65 pg/ml). Subsequently, 1 underwent successfully simultaneous ASS, total thyroidectomy (TT), and parathyroidectomy, while the other 2 were subjected to TT alone. Unexpectedly, the absence of neck lymph node MTC metastasis was indicated by histopathological examination. Postoperatively, all had consistently "undetectable" or normal levels of Ctn/CEA during a follow-up. Moreover, the challenge associated with simultaneous surgery of MEN2A-specific tumors remains to be clarified.

Patients and methods

Participants. The approval of the Ethics Committee (reference number: 20200331/01/01/002) was obtained from the 903rd PLA Hospital (Hangzhou, China). In this study, we examined 2 Chinese pedigrees (Figures 1 FA and FB, Table 1) containing 8 individuals with MEN2A from Zhejiang Province in December 2016 and September 2018, respectively. Written informed consent was provided by the participants (or parent or legal guardian).

Clinical approach. We obtained clinical profiles, imaging examinations, preoperative and postoperative biochemical data, surgical procedures, and follow-up records. According to the published criteria, the patients (III-6 in FA; II-3, II-5, III-7, III-8, III-11, IV-14, and IV-17 in FB; Figure 1 and Table 2) were subjected to a biochemical and clinical examination [2, 7]. The biochemical tests evaluated the levels of Ctn, CEA (normal, <5.0 ng/ml), proGRP, parathyroid hormone (PTH; normal, 12–88 pg/ml), serum calcium (normal, 2.08–2.6 mmol/l), 24 h urinary catecholamine (normal dopamine, 53–493 μg/24 h; normal epinephrine, <19.94 μg/24 h; normal norepinephrine, 10–80 μg/24h). Doppler ultrasound (US), computed tomography (CT), emission CT (ECT) scans, magnetic resonance imaging (MRI), and single-photon emission CT (SPECT) were performed.

***RET* mutation analysis.** According to the manufacturer's instructions (Qiagen, Hilden, Germany), the genomic DNA was extracted from the peripheral blood. Germline the entire *RET* protein-coding region testing was further performed by targeted genes and next-generation sequencing (targeted DNA-HiSeq sequencing) as described previously [1, 8]. In addition, we performed the PCR amplification for the entire exon of the *RET* proto-oncogene, followed by direct bidirectional sequencing for DNA using an ABI Prism 3700 automatic sequencer (Perkin-Elmer) [7, 8]. The presence of *RET* mutation information by targeted DNA-HiSeq sequencing was validated by Sanger sequencing.

Histopathological analysis. Diagnosis of MTC or C-cell hyperplasia (CCH) was further confirmed by histopathology. According to the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) classification system (7th edition), tumor staging was performed [18]. PHEO, HPTH, and CLA were diagnosed based on clinical and histopathological examinations [7].

Results

Diagnostic and clinical data from the 2 pedigrees in this series. Family A. The proband (FAIII-6, Figure 1, Table 1) was diagnosed with bilateral PHEO (left: 6.0 cm; right: 3.7 cm) when she detected hypertension in 2002. Blood pressure returned to the normal range after bilateral metachronous

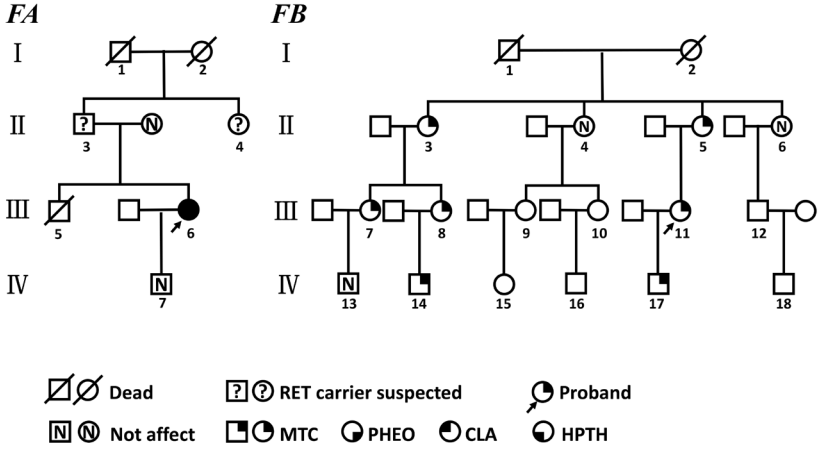

Figure 1. The pedigrees of two families with MEN2A and the *RET* (C634R, FA; C618R, FB) mutations. Squares and circles, male (M) and female (F) family members, respectively. Abbreviations: MTC-medullary thyroid carcinoma; PHEO-pheochromocytoma; HPTH-hyperparathyroidism; CLA-cutaneous lichen amyloidosis. Note: Except for the deceased (FAI-1, FAI-2, FAIII-5, FBI-1, and FBI-2) and rejectors (FAII-3 and FAII-4), all the numbered participants underwent *RET* screening or/and clinical evaluation.

Table 1. Clinical features of *RET* mutation carriers performing surgery.

Patient	*RET* mutation	Tumor size	Surgical treatment	Histology	LN+/resected[a]	TNM
FAIII-6	C634R	Left PHEO (3.3 cm), bilateral MTC (2.5 cm), HPTH (1.5 cm)	Left laparoscopic ASS, TT+cervical LND, and PD	Left PHEO, bilateral MTC, and HPTH	0/27	T2aN0M0 (MTC)
FBII-3	C618R	Bilateral MTC (0.8 cm)	TT+cervical LND	Bilateral MTC	0/2	T1N0M0
FBII-5	C618R	Bilateral MTC (3.0 cm)	TT+cervical LND	Bilateral MTC	43/80	T4aN1bMx
FBIII-7	C618R	Bilateral MTC (1.2 cm)	TT+cervical LND	Bilateral MTC	0/22	T1N0M0
FBIII-8	C618R	Bilateral MTC (4.3 cm)	TT+cervical LND	Bilateral MTC	18/75	T3N1bMx
FBIII-11	C618R	Right MTC (1.0 cm)	RT+cervical LND	Right MTC	1/2	T2N1aM0

Abbreviations: T-primary tumor; N-regional lymph nodes; M-distant metastases; ASS-adrenal-sparing surgery; TT-total thyroidectomy; LND-lymph node dissection; PD-parathyroidectomy; RT-right thyroidectomy; PHEO-pheochromocytoma; MTC-medullary thyroid carcinoma; HPTH-hyperparathyroidism
Notes: [a]LN+ includes positive lymph nodes proven on histopathology; resected includes lymph node resected.

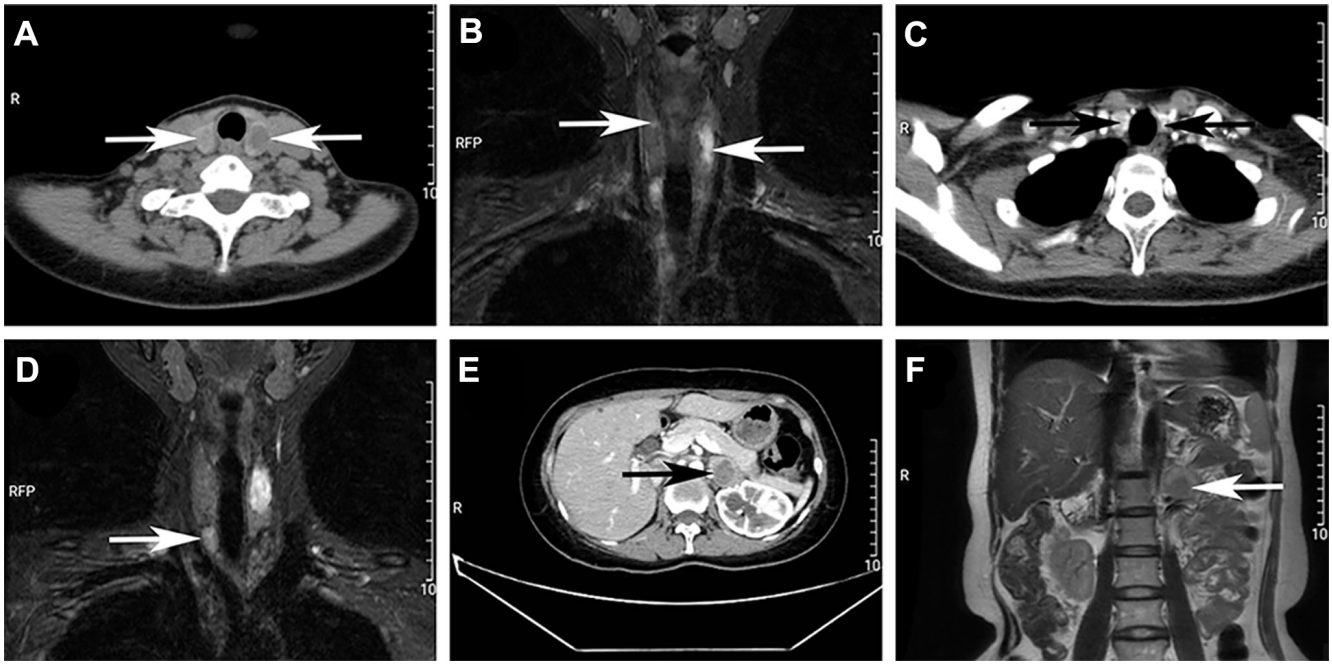

Figure 2. The imaging examination results of the proband (FAIII-6) with MEN2A-specific tumors. A, B) Neck computed tomography (CT)/magnetic resonance imaging (MRI) revealed multiple hypoechoic nodules (left: 2.5 cm; right: 0.6 cm) in bilateral thyroid lobes. C, D) Neck CT/MRI indicated two hypoechoic nodules (left: 0.6 cm; right: 1.5 cm) in lateral lower regions of bilateral thyroid lobes. E, F) Abdominal CT/MRI showed a left adrenal mass (3.3 cm).

PHEO resections in another hospital in Shanghai. In 2007, right PHEO resection in a hospital in Wenzhou was due to a recurrence of the right PHEO (3 cm). The proband had not consistently received hormone replacement therapy postoperatively. In 2018, the proband presented the level of slightly elevated CEA (17.76 ng/ml) for 3 months. Subsequently, pertinent biochemical examination revealed the levels of Ctn (1,498.00 pg/ml), CEA (18.33 ng/ml), and PTH (130.9 pg/ml). Neck US/CT/MRI, respectively, indicated multiple hypoechoic nodules (left: 2.5 cm; right: 0.6 cm) in both thyroid lobes (Figures 2A, 2B), enlarged lymph nodes (1.3 cm) in the bilateral neck, and two hypoechoic nodules (left: 0.6 cm; right: 1.5 cm) in lateral lower regions of bilateral thyroid lobes, the latter, which may originate from the parathyroid glands (Figures 2C, 2D). Further biochemical testing showed a slightly abnormal level of 24 h urinary catecholamine: epinephrin (34.5 μg/24 h), norepinephrine (44 μg/24 h), and dopamine (535.5 μg/24 h), but plasma values were all within normal limits. Abdominal US/CT/MRI indicated a left adrenal mass (3.3 cm) (Figures 2E, 2F). ECT/CT/MRI revealed no evidence of bone, lung, or liver metastasis.

One month later, the proband was admitted to our hospital for further treatment. Upon physical examination, the interscapular area of the left back corresponding to dermatomes T2-T6 level presented with dry, brown hyperpigmented, scaly changes, and multiple papules lesion of approximately 7.0 cm × 5.0 cm × 4.0 cm. Scarlet scratches and mossy-like changes

could be seen on the shoulder and upper back (Figure 3), established a history of repeated itching and scaling in the scapular region for 22 years. Glucocorticoid ointment can relieve symptoms, but symptoms recurred after a withdrawal. The proband was diagnosed with bilateral MTC, left PHEO, bilateral HPTH, and CLA, classified as the MEN2A-related CLA subtype. Moreover, the proband's medical history included extracorporeal shock wave lithotripsy and ureteroscopic lithotripsy, because of recurrent kidney stones in 2017 and 2018, respectively. With this information, additional tests were ordered. Biochemical examination showed elevated levels of Ctn (3,600.80 pg/ml) and CEA (18.20 ng/ml), but the serum levels of proGRP (63.22 pg/ml) and calcium (2.26 mmol/l) were within normal limits. Germline *RET* testing of this proband revealed the presence of the C634R (c.1900T>C) mutation. Based on clinical and molecular genetic diagnosis, the proband successively underwent laparoscopic left ASS after treatment with α-blockers, bilateral TT with modified neck dissection, and bilateral lower pole parathyroidectomy in a single procedure. Left PHEO, bilateral MTC, and nodular hyperplasia of bilateral lower pole parathyroid glands were verified (Figure 4), but none of neck lymph node MTC metastasis (LN+/resected, 0/27; T2aN0M0) was indicated by histopathological examination, as well as the levels of Ctn and CEA respectively decreased to "undetectable" or normal limits during follow-up 9 months, and the levels of proGRP were consistently within the reference range. During this period, the proband received hormone replacement therapy with hydrocortisone and Euthyrox. Furthermore, activated vitamin D and calcium tablets were taken to maintain normal serum calcium levels.

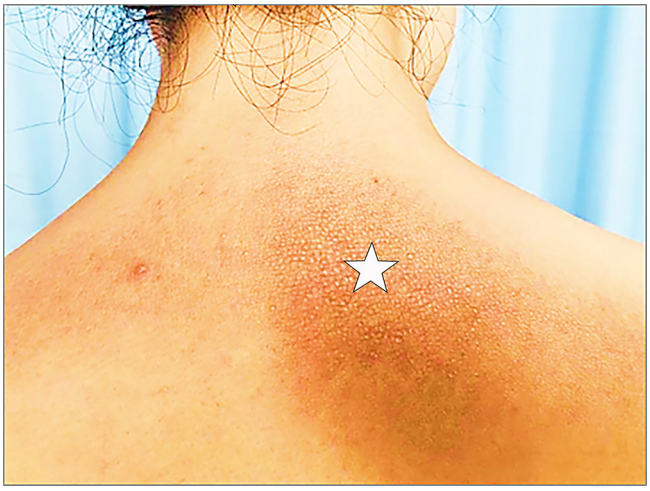

Figure 3. The clinical manifestations of cutaneous lichen amyloidosis (CLA; white asterisk). Brown hyperpigmentation, dry, scaly, and thickened papule skin with moss-like changes on the interscapular region of the left upper back.

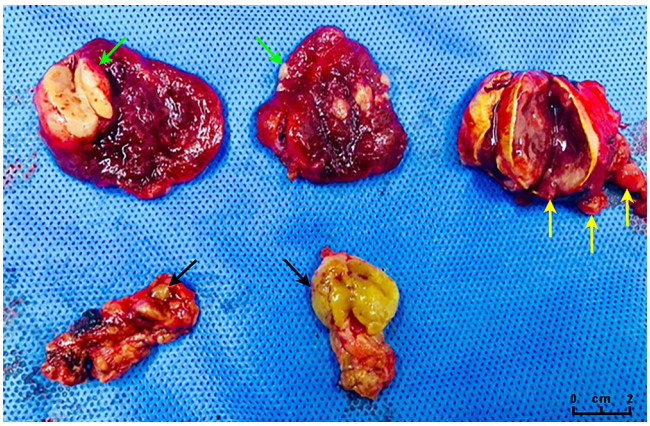

Figure 4. The pathological specimens of the proband (FAIII-6). Left pheochromocytoma (PHEO; yellow arrow), bilateral medullary thyroid carcinoma (MTC; green arrow), and hyperparathyroidism (HPTH; black arrow).

In addition to the proband's mother, her son (FAIV-7) tested negative for screening of C634R and MTC, also in good health. The proband's father (FAII-3) had been suffering from uremia for many years and his sister (FAII-4) had a history of MTC surgery, who should be highly suspected of carrying *RET* germline mutation, but they rejected further evaluation of *RET* testing. Moreover, her older brother (FAIII-5) died of leukemia.

Family B. The proband (FBIII-11, Figure 1, Table 1) was diagnosed with left MTC when she detected a nodule (2.7 cm) of the left thyroid lobe in 2013. Subsequently, a left thyroidectomy was performed in a hospital in Haining (Jiaxing, China). Her initial pathology report was available for confirmation of left-sided MTC. In 2016, she further underwent a right thyroidectomy due to a nodule of the right thyroid lobe by US/CT exam in another hospital in Hangzhou. Right-sided MTC (1.0 cm) was confirmed histopathologically (LN+/resected, 1/2; T2N1aM0). Germline *RET* testing of this proband revealed the presence of the C618R mutation.

For further diagnosis, in addition to the proband, 15 family members agreed to participate in biochemical testing, imaging studies, and *RET* screening. Among them, 9 family members exhibited normal Ctn levels and US images, but 6 (II-3, II-5, III-7, III-8, IV-14, and IV-17) were found to carry the *RET* C618R mutation. Of these 6 C618R carriers, 2 (II-3 and III-7) presented the levels of a slightly elevated Ctn (37.54 and 41.78 pg/ml, respectively), but normal CEA (2.71 and 0.97 ng/ml, respectively) and proGRP (52.55 and 29.81 pg/ml, respectively), while the other 2 (II-5 and III-8) showed the levels of markedly elevated Ctn (>2,000 and 2,568.00 pg/ml, respectively), CEA (201.07 and 311.89 ng/ml, respectively), and proGRP (1,562.65 and 1,206.95 pg/ml, respectively). Subsequently, the 4 (II-3, II-5, III-7, and III-8) underwent bilateral TT with modified neck dissection. The histopathological examinations revealed bilateral MTC (Table 1). Among them, 2 (II-5 and III-8) presented neck lymph node MTC metastasis (LN+/resected, 43/80, T4aN1bMx; and 18/75, T3N1bMx, respectively), but for

Table 2. Individual values (proGRP, Ctn, CEA, Size, TNM classification) of patients with MTC.

Patient	proGRP (pg/ml)	Ctn (pg/ml)	CEA (ng/ml)	Size (mm)	TNM	Reference
1	401.0	1,500	23.7	38	T2N0M0	
2	9,400.0	8,540	180.0	80	T4N1M1	
3	2,672.0	17,900	500.0	50	T4N0M0	
4	836.9	11,600	83.8	14	T2N1M0	
5	710.0	13,232	102.1	35	T2N0M0	
6	573.3	12,959	222.2	44	T3N0M0	
7	568.6	7,910	182.0	65	T2N0M0	
8	4,258	8,590	17.2	40	T2N0M0	Ide et al. [10]
9	80.9	1,506	14.1	30	T2N0M0	
10	45.7	91	13.2	12	T2N0M0	
11	45.1	741	8.6	12	T2N0M0	
12	43.6	1,980	23.2	14	T2N1M0	
13[a]	35.5	957	4.8	25	T2N0M0	
14[a]	27.7	110	1.3	7	T1N0M0	
15[a]	27.6	1,410	65.6	17	T2N0M0	
16[a]	63.22	3,600.80	18.20	25	T2N0M0	
17[a]	52.55	37.54	2.71	8	T1N0M0	
18[a]	29.81	41.78	0.97	12	T1N0M0	Current
19	1,562.65	> 2,000	201.07	30	T4aN1bMx	
20	1,206.95	2,568.00	311.89	43	T3N1bMx	
21	–	17.81	–	10	T2N1aM0	

Abbreviations: proGRP-pro-gastrin-releasing peptide; Ctn-calcitonin; CEA-carcinoembryonic antigen; T-primary tumor; N-regional lymph nodes; M-distant metastases. Notes: Patient 13[a]–18[a], the levels of elevated Ctn and normal ProGRP. Normal range: (Patient 1-15) proGRP <40 pg/ml; Ctn 15–86 pg/ml; CEA <5 ng/ml. (Patient 16–21) proGRP <65 pg/ml; Ctn <5 pg/ml; CEA <5 ng/ml

Table 3. Summary of 5 MEN2 patients with successive simultaneous adrenalectomy, thyroidectomy and parathyroidectomy.

Patient	MEN2 type	*RET* mutation	Diagnosis	Surgical treatment	Postoperative outcome	Reference
1	2A	634	MTC, bilateral PHEO, and HPTH	Bilateral adrenalectomies, TT+LND, and subtotal PD	Uneventful recovery and no evidence of recurrence	McIntyre et al. [14]
2	2A	C634R	MTC, left PHEO, right cPHEO/PGL, and PH	Bilateral adrenalectomies, TT+cervical LND, and PD	Good recovery and no signs of recurrence 3 years post-operation	Efared et al. [15]
3	–	–	Bilateral PHEO and HPTH	Bilateral laparoscopic adrenalectomies, preventive TT, and PD	–	Spinelli et al. [16]
4	2A	–	MTC, bilateral PHEO, and HPTH	Bilateral adrenalectomies, TT, and PD	Right lung MTC metastasis 7 years after the initial surgery	Spapen et al. [17]
5	2A	C634R	MTC, left PHEO, and HPTH	Left laparoscopic ASS, TT+cervical LND, and PD	Recovery and no signs of recurrence 9 months post-operation	Current study

Abbreviation: MEN2-multiple endocrine neoplasia type 2; MTC-medullary thyroid carcinoma; PHEO-pheochromocytoma; HPTH-hyperparathyroidism; cPHEO/PGL-composite pheochromocytoma/paragangliomas; LNM-lymph node metastasis; PH-parathyroid hyperplasia; TT-total thyroidectomy; LND-lymph node dissection; PD-parathyroidectomy; ASS-adrenal-sparing surgery

the other 2 (II-3 and III-7), none of neck lymph node MTC metastasis (LN+/resected, 0/2 and 0/22, respectively; all for T1N0M0) was indicated, as well as the levels of Ctn, CEA, and proGRP were all within the normal range or "undetectable" levels. The remaining 2 (IV-14 and IV-17) had no abnormality of biochemical and imaging examinations and chose a watchful waiting. Moreover, all 6 *RET* C618R carriers exhibited no evidence of PHEO, HPTH, CLA, HD, or other endocrine tumors during the follow-up 30 months.

Discussion

In the present study, 8 *RET* mutation carriers of 2 Chinese pedigrees were presented with MEN2A (1 MEN2A-related CLA, C634R and 1 FMTC, C618R, respectively). The three of them showed markedly or slightly elevated pre-Ctn, but normal proGRP. Unexpectedly, their post-operative Ctn levels, all decreased to "undetectable" or normal, which might imply being biochemically cured. The results provide

a novel perspective for the individualized management of MEN2A-specific tumors.

Almost all patients with CCH/MTC presented high levels of Ctn [2]. Conversely, non-secretory Ctn accounted for only 0.83% (7/839) of patients with advanced MTC [2, 19]. That is, in patients with advanced MTC, normal or low levels of both Ctn and CEA, or a lower level of Ctn out of proportion to a marked elevation in the level of CEA may indicate a poorly differentiated MTC [2]. Nonetheless, such patients were clinically rare, and the exact pathophysiology of Ctn negative MTC is still not clearly understood [20, 21]. Furthermore, the tumor burden of primary and metastatic MTC together determines the levels of Ctn and is positively correlated with the Ctn value. There was convincing evidence on tumor burden. When the largest tumor diameter was less than 1.0 cm, more than 1.0 cm, and 2.0 cm, the average Ctn value was 201, 2,995, and 9,918 pg/ml, respectively [22]. When levels of Ctn were more than 20 pg/ml, the risk of MTC metastasis increased. Initial Ctn levels exceeding 40, 150, and 500 pg/ml, respectively, were associated with lymph node MTC metastasis to the central and lateral (ipsilateral) neck, the central (contralateral) neck, the lateral (contralateral) neck, and the upper mediastinum [2]. In this study, of these 8 carriers, 1 with C634R (FAIII-6) presenting Ctn levels exceeding 3,000 pg/ml was likely to have extensively metastatic disease, which was unlikely to be cured in spite of aggressive surgery [23, 24]. Preoperative Ctn level, combined with imaging examination, is a routine method to evaluate whether there is the presence of MTC and the extent of lymph node MTC metastasis. In addition, more than 50% of patients with MTC have high levels of CEA, while CEA levels above 30 ng/ml could highly predict incurability of MTC with surgery [23, 25]; CEA levels greater than 100 ng/ml may signify wide lymph node and distant MTC metastases [23]. Both Ctn and CEA are important monitoring indicators for post-operative management and prognosis prediction of MTC. Particularly, patients with normal post-Ctn/CEA levels were said to be "biochemically cured", and that 10-years survival was 97.7% [2, 26]. In this study, 3 patients (FAIII-6, FBII-3, and FBIII-7) had no evidence of neck lymph node MTC metastasis (LN+/resected, 0/27, 0/2, and 0/22, respectively; all for N0 stage) postoperatively, and all of Ctn levels decreased to "undetectable" or normal limits, and consistently normal levels of proGRP (63.22, 52.55, and 29.81 pg/ml, respectively) follow-up 9, 30 and 30 months, respectively, implying that 3 patients (FAIII-6, FBII-3, and FBIII-7) had been biochemically cured with an excellent prognosis, yet pre-operatively had the levels of elevated Ctn (3,600.80, 37.54, and 41.78 pg/ml, respectively, Table 2). Consequently, proGRP, which seems to be a helpful and additional marker for the evaluation of the MTC stage in addition to Ctn/CEA, should be considered [10].

Regarding proGRP, it was widely distributed in neuroendocrine cells of the non-gastric antrum, nerve fibers, brain, and lung [10, 12]. Accumulating evidence suggested that proGRP levels also had a significant correlation with MTC but not with non-MTC (follicular thyroid carcinoma [FTC] and papillary thyroid carcinoma [PTC]) [10, 11, 13]. Ide et al. [10] reported that proGRP levels above the cut-off value accounted for 80% (12/15; 9/12 stage I and II, 3/12 stage III and IV) of MTC patients with elevated Ctn levels, and 92.31% (12/13; 9/12 stage I and II, 3/12 stage III and IV) with elevated CEA levels. On the contrary, when proGRP levels had normal limit value, the elevated levels of Ctn (957–1,410 pg/ml; 3/3 stage I and II) and CEA (65.6 ng/ml; 1/1 stage I and II) accounted for 100% (3/3) and 33.33% (1/3), respectively. The proGRP concentrations were correlated with MTC tumor size (disease stage, Table 2). In another study involving tumor markers of MTC (including 9 advanced MTC [stage IVA and IVC] and 13 no evidence of disease [NED]), Parra-Robert et al. [11] analyzed the sensitivity of Ctn, CEA, and proGRP, individually and in combination. The results showed: i) similar to Ctn (88.9%), sensitivity related predominance in terms of proGRP was observed as indicated by the CEA-to-proGRP ratio of approximately 1:1.4 (62.5%:88.9%); ii) if CEA or proGRP is intended to be served as a complement to Ctn, respectively, the ratio of sensitivity was approximately 1:1.12 (88.9%:100%). Further, Ide et al. [10] analyzed 15 MTC patients. Among 12 MTC patients presented high levels of proGRP, with 75% (9/12) non-advance MTC (N0 stage) and 25% (3/12) advanced MTC (N1 stage). In contrast, the remaining 3 MTC patients had normal proGRP levels, with TNM stage T2N0M0 (patient 13), T1N0M0 (patient 14), and T2N0M0 (patient 15), respectively (all for N0 stage; Table 2). The results reflected that patients with the levels of normal proGRP, in spite of high levels of Ctn, had no regional lymph node MTC metastasis, similarly to our series (FAIII-6, FBII-3, and FBIII-7) with the concentrations of elevated Ctn but consistently normal proGRP, pathologically in T2aN0M0 (FAIII-6, patient 16), T1N0M0 (FBII-3, patient 17), and T1N0M0 (FBIII-7, patient 18) stage (all for N0 stage; Table 2). Therefore, the appropriate extent of neck dissection requires validation but could be currently avoided. However, the unique phenomenon still remains unclear and needs to further clarify, but otherwise should be emphasized to identify its potential important clinical significance-normal proGRP levels, whether there is MTC metastasis or not. Of the remaining 5 patients, 2 with elevated levels of Ctn, CEA, and proGRP, were pathologically classified as T4aN1bMx (FBII-5, patient 19) and T3N1bMx (FBIII-8, patient 20) (Table 2), while the other 3 patients (FBIII-11, FBIV-14, and FBIV-17) lacked detailed data.

To our knowledge, only four cases for simultaneous adrenalectomy, thyroidectomy, and parathyroidectomy were previously published (Table 3) [14–17]. Reportedly, outcomes of simultaneous surgery in two cases were successful, lacking the details of the other two. McIntyre et al. [14] considered that a young male patient well with adequate α- and β-blockers was relatively suitable for simultaneous adrenalectomy, TT, and parathyroidectomy.

Efared et al. [15] reported that a female patient simultaneously underwent bilateral adrenalectomy, TT with cervical lymphadenectomy, and parathyroidectomy. The patient recovered well and showed no signs of recurrence 3 years after surgery. In the presence of successive laparoscopic left ASS, TT with modified neck dissection, and bilateral lower pole parathyroidectomy in a single procedure, the proband (FAIII-6) recovered well 9 months after the operation. These results suggest that ASS followed by thyroidectomy and parathyroidectomy in a single procedure could be a surgical approach of a choice to use as an alternative and successful treatment pattern in experienced centers [14, 15]. It should be noted, however, that the proband (FAIII-6) developed recurrence of right and left PHEO 5 and 16 years after initial bilateral PHEO resections, respectively. We speculate that the recurrence of PHEO in the proband (FAIII-6) may be caused by new PHEO development or residual PHEO after surgery. In order to prevent Addison's disease, more than one-third of one adrenal gland was proposed to be retained in patients who need surgery for bilateral adrenal tumors or have previously lost an adrenal gland [27, 28]. However, if the amount of adrenal tissue cannot be achieved, total adrenalectomy was recommended to minimize the risk of recurrence [28]. In an international retrospective study on MEN2-related PHEO, the recurrence rate of PHEO in the same adrenal gland after ASS was 2.61% (4/153) [27]. Usually, the patients with unilateral PHEO develop into contralateral PHEO within 10 years [2, 29]. After subtotal adrenalectomy (that is, ASS), the risk of PHEO recurrence within 20 years is 20% [2]. Moreover, the frequency of benign PHEO was 99.64% (561/563) contrasting with 0.36% (2/563) in malignant PHEO [27]. Subtotal adrenalectomy to preserve adrenal cortical function is an alternative procedure for the treatment of MEN2-related PHEO. Nonetheless, a cautious approach is likely warranted in the management of individuals with the recurrence of PHEO, such as the proband (FAIII-6) in this study who underwent ASS for re-operation of the left PHEO. As for patients with MEN2A-related HPTH, surgical excision is considered only if parathyroid glands are visibly enlarged [2]. Therefore, the proband (FAIII-6) was subjected to bilateral lower pole parathyroidectomy. And for the management of concomitant symptoms – recurrent kidney stones, the lithotripsies were also successfully performed.

It is interesting that, while inquiring about the medical history of the proband (FAIII-6), we accidentally found that the presence of CLA and associated itching in the interscapular region was earlier than other endocrine symptoms of MEN2A-related CLA. Unfortunately, the proband (FAIII-6)'s diagnosis of MEN2A-related CLA was delayed 22 years [7, 8, 30]. Recently, Qi et al. [7, 8] revealed that the mean age was 29.5 (range, 5–60) years at diagnosis of CLA and that CLA may be a relatively early clinical characteristic of MEN2A-related CLA. Besides, it is an unexpected phenomenon that the male-to-female ratio of 1:3.6 presented a high dominance in female patients with CLA. Moreover, the proportion of CLA in the *RET* mutation carriers was 45.1% (55/122) in 20 MEN2 families with CLA, and data from 51 individuals with CLA displayed prevalence for MTC, PHEO, and HPTH of 96.1% (49/51), 47.1% (24/51), and 13.7% (7/51), respectively [7], despite the diverse evidence in a few specific families with CLA [7, 8, 30, 31]. These findings suggest that CLA might represent a common, early, and 'premonitory' clinical symptom of MEN2A-related CLA families, which may imply that improving early recognition of CLA in MEN2A-related CLA facilitate timely screening of *RET*, diagnosis of MEN2A-specific tumors, and subsequent resection of MTC or/and PHEO to improve outcomes [7, 8, 14, 30–32].

In conclusion, our study highlights the importance of monitoring proGRP for patients with MTC. Despite high levels of Ctn, the normal levels of proGRP may suggest no cervical lymph node MTC metastasis, offering a possibility of avoiding neck dissection. For coexistent PHEO, and either MTC or HPTH, simultaneous surgery may be an approach of choice to serve as an alternative treatment pattern. CLA located in the interscapular region, furthermore, may imply earlier clinical symptoms of most patients with MEN2A-related CLA. Timely screening for *RET* in these patients with MEN2A-related CLA might facilitate early diagnosis and personalized treatment of MEN2A-specific tumors.

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