

## RESEARCH

# The *RET* C611Y mutation causes MEN 2A and associated cutaneous lichen amyloidosis

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## Abstract

**Background:** Cutaneous lichen amyloidosis (CLA) has been reported in some multiple endocrine neoplasia type 2A (MEN 2A) families affected by specific germline *RET* mutations C634F/G/R/W/Y or V804M, as a characteristic of the clinical manifestation in 'MEN 2A with CLA', one of four variants of MEN 2A, which was strictly located in the scapular region of the upper back.

**Patient Findings:** This study reports a large south-eastern Chinese pedigree with 17 individuals carrying the MEN 2A-harboring germline C611Y (c.1832G>A) *RET* mutation by Sanger sequencing. One individual presented MEN 2A-related clinical features, including typical CLA in the interscapular region; another individual exhibited neurological pruritus and scratching in the upper back but lacked CLA skin lesions. Both subjects presented with CLA or pruritic symptoms several years before the onset of medullary thyroid carcinoma (MTC) and/or pheochromocytoma. The remaining 15 *RET* mutation carriers did not exhibit CLA; of these, one presented with MTC and pheochromocytoma, nine with MTC only, two with elevated serum calcitonin and three younger subjects with normal serum calcitonin levels. This family's clinical data revealed a later diagnosis of MTC (mean age, 45.9 (range: 23–73) years), a lower penetrance of pheochromocytoma (2/17, 11.8%) and CLA (1/17, 5.9%). However, no hyperparathyroidism and Hirschsprung disease were reported in this family.

**Summary and Conclusions:** This is the first description of a family with MEN 2A-related CLA due to a germline *RET* C611Y mutation, which might exhibit a novel and diversified genotype–phenotype spectrum in MEN 2A.

## Key Words

- ▶ multiple endocrine neoplasia type 2A
- ▶ medullary thyroid carcinoma
- ▶ cutaneous lichen amyloidosis
- ▶ *RET* proto-oncogene
- ▶ C611Y mutation

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## Introduction

Cutaneous lichen amyloidosis (CLA) is a rare skin disease occasionally detected in multiple endocrine neoplasia type 2A (MEN 2A). Initially, the presence of CLA was observed in patients with MEN 2A, who were explicitly

related to codon 634 germline mutations in exon 11 of *RET* proto-oncogene; this mutation locates in the extracellular cysteine-rich region of the RET protein. CLA in MEN 2A is exclusively located in the scapular

region of the upper back, corresponding to dermatomes T2–T6 (1, 2, 3, 4, 5, 6, 7). Reportedly, the following two germline mutations exist in intracellular tyrosine kinase domains: the *RET* V804M mutation within exon 14 in an American female with medullary thyroid carcinoma (MTC) and CLA on the upper back (8) and the *RET* S891A mutation within exon 15 binding *OSMR* variant G513D in a Chinese familial MTC (FMTC) family with cutaneous biphasic amyloidosis comprising the lower legs to thighs, upper back, shoulders, arms and forearms (9).

The recurring extracellular C611Y germline mutation within exon 10 of *RET* has been regularly reported to associate with MTC, pheochromocytoma (PHEO) and hyperparathyroidism (HPT), but the absence of CLA and Hirschsprung disease (3, 4, 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19). This study reports a south-eastern Chinese Han MEN 2A family with CLA, which was caused by the *RET* C611Y germline mutation, offering a novel insight into the genotype–phenotypic spectrum in MEN 2A.

## Subjects and methods

### Participants

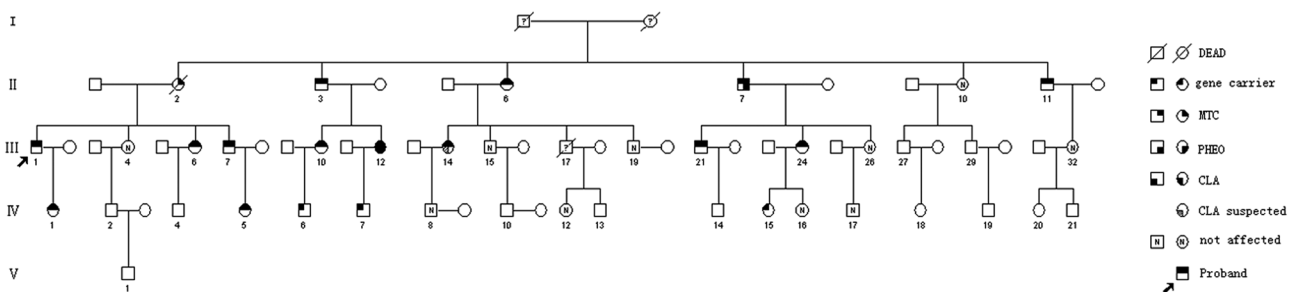
The study was approved by the Ethical Committees of the 117th PLA Hospital. In this study, we examined a five-generation southern Chinese pedigree with MEN 2A from Fujian Province, China, at Jian’ou Municipal Hospital (Fujian, China), the 117th PLA Hospital and Zhejiang Cancer Hospital (Zhejiang, China) between April 1988 and September 2017 (Fig. 1). We obtained written informed consent from all participants and/or their legal guardians, as required by the Ethical Committees of the 117th PLA Hospital.

### Clinical approach

We obtained clinical profiles, surgical procedures, preoperative and postoperative biochemical data, imaging examinations and follow-up records. On the basis of the published criteria, we clinically and biochemically examined 39 members of this family (4, 5, 9, 20). The biochemical examination comprised basal serum calcitonin (Ctn) by the chemiluminescence assay (normal males, <8.4 pg/mL; females, <5.0 pg/mL; Immulite 2000 Immunoassay System; Siemens Ltd), parathyroid hormone (PTH; normal, 15–65 ng/mL), carcinoembryonic antigen (CEA; normal, <5.0 ng/mL) by electrochemiluminescent immunoassays (Elecsys analyzer, Roche Diagnostics GmbH), plasma catecholamine (normal, <100 ng/mL (norepinephrine); <600 pg/mL (dopamine); and <100 ng/mL (epinephrine)) by radioimmunoassay or metanephrin (normal, <62 ng/mL) and normetanephrin (normal, <145 ng/mL) by liquid chromatographic tandem mass spectrometry (AB Sciex 4500MD LC/MS/MS; GenTech Scientific, New York, NY, USA), serum calcium by the arsenazo III method (Hitachi 7180 Biochemistry Analyzer; Hitachi). In addition, we performed slit lamp examination. The imaging examinations comprised thyroid ultrasound (US), CT, adrenal gland and parathyroid gland nuclear MRI, CT and/or emission CT (ECT), if indicated.

### RET mutation analysis

We extracted the genomic DNA from the peripheral blood per the manufacturer’s instructions (Qiagen, Hilden, Germany). In addition, the PCR amplification of each entire exon of the *RET* proto-oncogene was performed, followed by direct bidirectional DNA sequencing with the ABI Prism 3700 automatic sequencer (Perkin-Elmer) (7, 9).



**Figure 1**

Pedigree of the southern five-generation Chinese family members with MEN 2A associated with CLA and the *RET* C611Y mutation. Circles and squares, female (F) and male (M) family members, respectively. CLA, cutaneous lichen amyloidosis; MTC, medullary thyroid carcinoma; PHEO, pheochromocytoma. Slashes, deceased subjects. All the numbered subjects are those who underwent clinical evaluation and/or *RET* screening.

## Histopathological analysis

We confirmed the diagnosis of MTC, PHEO and CLA by a histopathological examination (Leica DM1000 biological microscope; Leica). In addition, we performed the tumor staging according to the American Joint Committee on Cancer (7th edition) tumor–node–metastasis (TNM) classification system (21). The skin biopsy specimen was obtained from the cutaneous lesions of one subject (III:12), fixed in 10% buffered formalin and embedded in paraffin wax. Furthermore, we used haematoxylin–eosin, Congo red and crystal violet (Solaibao, Beijing, China) staining to 4 µm thick sections (1, 6, 7, 22, 23, 24).

## Results

### Clinical features and phenotypic data

#### Patients with MTC and PHEO

Based on five-generation family medical history investigation, the results revealed post-operation histopathological findings showed that of seven patients, 6 (II:2, proband/III:1, III:7, III:10, III:14, IV:5) had only MTC, but another (III:12) presented MTC and right PHEO. (Fig. 1 and Table 1). In 2017, we further performed biochemical testing, imaging studies and/or *RET* screening on all family members, except one (II:2; Table 1). Overall, 22 members exhibited normal Ctn levels and US images, and 17 (II:3, II:6, II:7, II:11, III:1, III:6, III:7, III:10, III:12, III:14, II:21, III:24, IV:1, IV:5, IV:6, IV:7 and IV:15) exhibited the *RET* C611Y mutation. Then, of 11 newly enrolled subjects, 10 underwent surgery, except 1 (III:6, 56 years) who presented with higher elevated Ctn (1326.3 pg/mL) and CEA (20.08 ng/mL) and multicentric hypoechoic nodules (left, 0.8 cm; right, 2.5 cm; T2NxMx) with calcifications in both thyroid lobes. In addition, two subjects (II:3 and II:11) exhibited increased Ctn levels (247.8 and 356 pg/mL) who underwent total thyroidectomy with bilateral level VI lymph node dissection and modified bilateral neck dissection. The histopathological examination revealed bilateral MTC with lymph node metastasis (T1aN1aM0 and T1bN1bM0). Furthermore, five subjects (II:6, II:7, III:21, III:24 and IV:1) exhibited slightly elevated Ctn levels (range, 9.87–41.5 pg/mL); of these, three (II:6, II:7 and IV:1) accepted and underwent total thyroidectomy with bilateral level VI lymph node dissection, and one (II:7) presented with right PHEO (2.6 cm × 1.6 cm × 1.0 cm) and slightly increased serum metanephrin and normetanephrin but lack of clinical symptoms, who underwent laparoscopic right adrenal-sparing surgery 2 weeks before the initial thyroid surgery. The histopathological examination confirmed bilateral MTC

in all (T1aN0M0) and right PHEO in one (II:7). Another two subjects (III:21 and III:24) agreed to undergo scheduled thyroidectomy later (might have had MTC or C cell hyperplasia). Of note, three younger carriers (IV:6, IV:7 and IV:15) presented no abnormal Ctn/CEA values and imaging manifestation and selected a watchful waiting approach. Besides, six patients presented before the *RET* mutation screening as follows: 2 subjects (III:1 and III:12) presented with increased Ctn and CEA (211 and 1417.9 pg/mL; 14.48 and 37.48 ng/mL, respectively) and thyroid nodules (2.8 and 2.5 cm, respectively) with calcifications in left/both thyroid lobes. Fortunately, the ECT examination revealed no distant metastases. Consequently, they underwent left/total thyroidectomy with modified bilateral neck dissection. Histopathology revealed left or bilateral MTC with lymph node metastases (T2N1bM0). Moreover, three subjects (III:7, III:10 and III:14) presented with a slightly elevated Ctn (range, 5.42–48.3 pg/mL) and/or residual thyroid lobes with local enlarged cervical nodules (<1.3 cm). Thus, a much more careful watch is warranted. Notably, one subject (IV:5) still had consistently undetectable Ctn and provided no evidence of abnormality after initial thyroidectomy (Table 1).

#### Patients with CLA

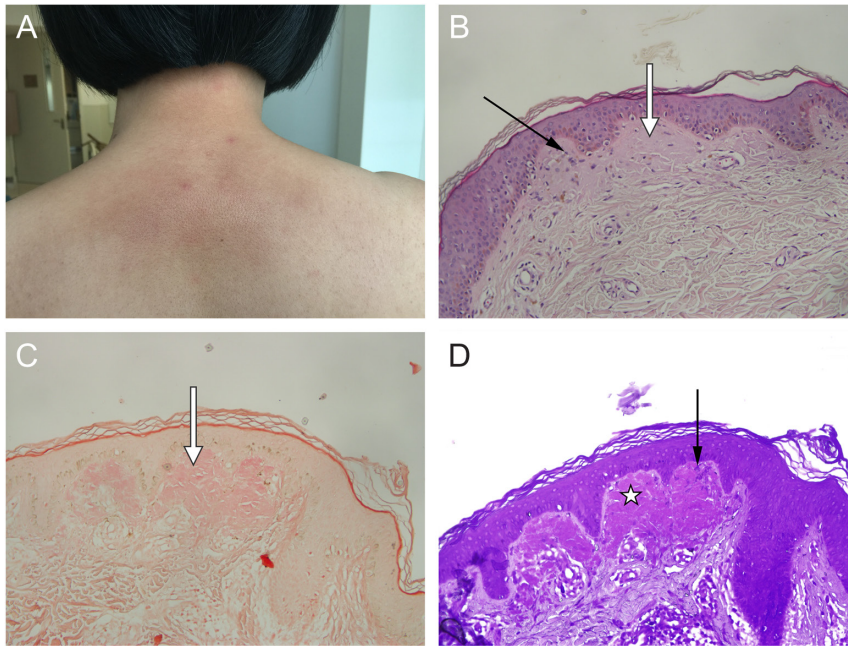
Of all 17 individuals with the *RET* mutation, 1 (5.9%) was diagnosed (III:12) with CLA by a dermatologist, 1 (5.9%) with regional pruritus and scratching without other typical CLA signs (III:14), and none of the other 15 affected relatives presented with pruritus or skin lesions. During the investigation, we stumbled upon an unexpected discovery in 2017. During a routine comprehensive medical history enquiry, two subjects (III:12 and III:14) recounted an intractable but episodic pruritus with the recurrent scratching of the interscapular region, accompanied by a burning sensation occasionally since the age of 22 years or 20s, respectively. Their itch worsened under periods of stress and dry weather. Although glucocorticoid ointment provided incomplete symptom relief of pruritus, it reappeared following the dose reduction. The physical examination revealed that one (III:12) presented with evident cutaneous lesions with brown, hyperpigmented, dry, scaly and thickened papules on the skin with scabby scratches located in the interscapular central region at the T2–T6 level (Fig. 2A). A subsequent skin lesion biopsy and the histological pattern revealed characteristic features of CLA in the skin lesion (Fig. 2B, C and D). However, the other (III:14), who only presented with cutaneous scabby scratches, remained undecided about a skin lesion biopsy (not shown).

**Table 1** Clinical characteristics of the RET C611Y mutation carriers with MEN2A and associated CLA.

Individual	Gender (M/F)	Age (year)	Age at diagnosis (years)			Pre-/Post Ctn (pg/mL)	Pre-/Post CEA (ng/mL)	MTC		Max size (cm)	TNM	PHEO		Follow-up* (months)
			MTC	PHEO	CLA			Surgery	Size (cm)					
II:3	M	74	74	-	-	8.65/1.1	TT + MBiND	1.0 × 0.8 × 0.6	T1aN1aM0	-	-	-	4	
II:6	F	73	73	-	-	4.63/1.87	TT + BiLND(VI)	0.6 × 0.4 × 0.4	T1aN0M0	-	-	-	3	
II:7	M	69	69	69	-	2.05/2.5	TT + BiLND(VI)	0.3 × 0.2 × 0.2	T1aN0M0	ASS (R)	2.6 × 1.6 × 1.0 (R)	-	3	
II:11	M	61	61	-	-	356/9.36	TT + MBiND	1.7 × 1.4 × 0.8	T1bN1bM0	-	-	-	3	
III:1	M	59	30	-	-	NA/4.93	TT(R) + ST(L) <sup>⊙</sup> + TT(L) + MBiND <sup>⊙</sup>	2.8 × 1.7 × 1.3	T2N1bM0	-	-	-	352	
III:6	F	56	56	-	-	1326.3/NA	Reject	2.5 × 1.1 × 1.0	T2NxMx	-	-	-	3	
III:7	M	52	42	-	-	673.5/48.3	TT(R) + ST(L) + MRND <sup>⊙</sup> + TT(L) + MLND <sup>⊙</sup>	3.2 × 2.5 × 1.7	T2N1bM0	-	-	-	126	
III:10	F	45	29	-	-	NA/5.42	TT(R) + ST(L) <sup>⊙</sup> + BiLND(VI) + MRND <sup>⊙</sup>	4.0 × 3.0 × 3.0	T2N1bM0	-	-	-	200	
III:12	F	40	40	37	40	1417.9/<2	TT + MBiND	2.5 × 1.7 × 1.1	T2N1bM0	ASS (R)	4.8 × 4.0 × 3.5 (R)	-	4	
III:14	F	54	35	-	54 <sup>#</sup>	NA/1.0	TT(L) + MLND + ST(R) <sup>⊙</sup> + TT(R) + MBiND <sup>⊙</sup>	3.0 × 3.0 × 2.4	T2N1bM0	-	-	-	236	
III:21	M	42	42 <sup>#</sup>	-	-	9.87/NA	UST	0.5 × 0.3 × 0.2 <sup>#</sup>	T1aN0M0 <sup>#</sup>	-	-	-	3	
III:24	F	39	39 <sup>#</sup>	-	-	1.89/NA	UST	0.4 × 0.2 × 0.2 <sup>#</sup>	T1aN0M0 <sup>#</sup>	-	-	-	3	
IV:1	F	29	29	-	-	41.5/<2	TT + BiLND(VI)	0.7 × 0.5 × 0.3	T1aN0M0	-	-	-	3	
IV:5	F	26	23	-	-	67.12/<2	TT + BiLND(VI)	1.0 × 0.5 × 0.4	T1aN0M0	-	-	-	27	
IV:6	M	20	-	-	-	6.82/NA	WW	-	-	-	-	-	3	
IV:7	M	13	-	-	-	3.01/NA	WW	-	-	-	-	-	3	
IV:15	M	5	-	-	-	3.81/NA	WW	-	-	-	-	-	3	

⊙, initial surgery; <sup>⊙</sup>, second surgery; #, clinical suspected; \*, after the first thyroid surgery or start watchful waiting; -, negative; ASS, adrenal-sparing surgery; BiLND(VI), bilateral level VI lymph node dissection; CLA, cutaneous lichen amyloidosis; F, female; L, Left (thyroid lobe); M, male; Max size, maximum MTC size (all initial); MBiND, modified bilateral neck dissection; MR(L)ND, modified right (left) neck dissection; MTC, medullary thyroid carcinoma; NA, not available; PHEO, pheochromocytoma; pre/post-CEA, pre/post-operative CEA (normal, <5.0 ng/mL); pre/post-Ctn, pre/post-operative basal serum calcitonin (normal males, <8.4 pg/mL; females, <5.0 pg/mL); R, right (thyroid lobe); ST, subtotal thyroidectomy; TNM, tumor-node-metastasis (tumor stage); TT, total thyroidectomy; UST, undergo scheduled thyroidectomy; WW, watchful waiting.



**Figure 2**

Clinical and histological presentation of MEN 2A-related CLA caused by the *RET* C611Y mutation. (A) Clinical characteristics of cutaneous lichen amyloidosis (CLA). Brown hyperpigmentation, scaly, dry and thickened papular skin with scabby scratches on the interscapular region (individual III:12). (B, C and D) Histopathological features of CLA. (B) A skin biopsy of lesional skin reveals overlying epidermal orthokeratotic hyperkeratosis, irregular acanthosis and eosinophilic deposits of amorphous material (white arrow) in the papillary dermis. Numerous scattered melanophages (black arrow) and small amounts of an amorphous material just below the dermoepidermal junction can be observed in the upper dermis (haematoxylin–eosin; original magnification,  $\times 200$ ). (C) The papillary dermal globular deposits of amyloid were positive for Congo red stain (white arrow; original magnification,  $\times 200$ ). (D) The papillary dermal globular deposits of amyloid were positive for crystal violet staining (white asterisk) and overlying pigmentary incontinence (black arrow; original magnification,  $\times 200$ ).

### Other MEN2-associated diseases and surgical outcome

All 17 affected individuals presented no evidence of HPT, Hirschsprung disease, corneal nerve thickening or other endocrine tumors through screening of serum calcium, PTH, slit lamp examination and other relevant imaging examination. Of these, 13 underwent thyroid and/or adrenal gland surgery. After the latest surgery, all 13 attained normal Ctn (biochemical cure), except 5 individuals (4 of thyroid repeat procedure (III:1, III:7, III:10 and III:14); 1 of initial thyroid surgery (II:11)) who exhibited slightly elevated Ctn. In addition, 2 individuals (II:7 and III:12) underwent right adrenal-sparing surgery and have exhibited no recurrence thus far (Table 1). All 13 individuals needed individualized thyroid hormone replacement therapy. Furthermore, one (III:6) still rejected operation and further examination, and the other three individuals (IV:6, IV:7 and IV:15) still selected a watchful waiting approach (Table 1).

### Identification of the *RET* germline mutations

The direct sequencing method confirmed a heterozygous nucleotide substitution in exon 10 of *RET* designated c.1832G>A in the proband (III:1) and other 16 subjects (II:3, II:6, II:7, II:11, III:6, III:7, III:10, III:12, III:14, III:21, III:24, IV:1, IV:5, IV:6, IV:7 and IV:15). This variant substitutes the corresponding amino acid cysteine to tyrosine in codon 611, designated C611Y, which targets

one of five cysteine residues in the extracellular domain of *RET*. Furthermore, we discovered five recurrent exonic polymorphisms of c.135A>G (p.A45A), c.1296A>G (p.A432A), c.2071G>A (p.G691S), c.2307T>G (p.L769L) and c.2712C>G (p.S904S) respectively within exon 2, 7, 11, 13 and 15 (data not shown).

### Discussion

This study demonstrated a recurrent *RET* C611Y mutation in a large south-eastern Chinese family with MEN 2A-related MTC and PHEO. Remarkably, CLA was also present in our patient and first manifested in the *RET* C611Y mutation. Thus, CLA should be one of the clinical manifestations of the *RET* C611Y mutation, which implies a novel genotype–phenotype relationship in MEN 2A.

The genotype–phenotype relationship between CLA and the *RET* mutation has been discussed previously (1, 2, 4, 5, 6, 7, 8, 23, 24, 25, 26). Eng *et al.* (2) reported that CLA was detected in approximately 9% (18/199) of patients with MEN 2A, and all 18 families carried *RET* codon 634 mutations. Verga *et al.* (23) reported that CLA was found in 3 of 10 MEN 2A/FMTC families with codon 634 mutation, and 9 of 25 affected subjects (36%) with presented CLA, other 2 of them (8%) lacked CLA skin lesions but the symptom of neurological pruritus in the upper back. Recently, accumulating evidence suggested that CLA in the scapular region occurred in 45.1% (55/122) of 122 *RET* carriers in 20 MEN2 families with CLA.

The leading mutation at codon 634 in exon 11 (98.2%, 54/55) was a C634Y mutation, followed by C634R/W/G/F and C634. Notably, only one V804M mutation in exon 14 was involved (1.8%, 1/55) (7, 8). The mean age at diagnosis of CLA with the *RET* mutation was 29.5 (range, 5–60) years, and in 51 individuals with CLA, the presence of MTC, PHEO and HPT was approximately 96.1% (49/51), 47.1% (24/51) and 13.7% (7/51), respectively (7). However, to date, no family with MEN2B has been reported to be diagnosed with CLA, as well as families with CLA, but without MEN2 disease features, do not have *RET* mutations (2, 4, 5, 6, 7, 27, 28). In addition, an unexpected finding revealed that the gender-related predominance in the prevalence of CLA was observed as suggested by the male-to-female ratio of approximately 1.0:3.6 (12:43) (7).

In our family, of the 17 *RET* C611Y mutation carriers, only one female patient developed CLA after 18 years of itching and scratching (Table 1). CLA in patients with MEN 2A might be neglected because the skin lesion could not result in a definitive diagnosis (7, 22, 24, 29, 30). Most patients with MEN 2A, including our patient, present with pruritic symptoms of CLA, which occurs before the onset of clinically evident MTC and PHEO (5, 7, 8, 22, 24, 29, 30). Nonetheless, the evolution of CLA might represent independently of the clinical disease course, which is not related to MEN 2A-related endocrine tumors such as MTC, PHEO or HPT (not correlated with increased levels of Ctn, metanephrines or PTH) (7, 8, 22, 23, 30). Notably, not all family members with the *RET* mutation develop CLA. MEN 2A-related CLA in our kindred presented a much lower penetrance (5.9%), although its result revealed in another patient with CLA-suspected clinical symptoms (5.9%) in our study. Individuals with same or different *RET* mutation typically presented with a variable clinical manifestation of CLA, especially a diverse evident in the scapular region of the upper back, exhibiting on the side, midline or bilateral extending across the midline, followed by hyperpigmentation and then papules developed in the same area after many years of itching and scratching (6, 7, 8, 23). Conversely, previously reported cutaneous biphasic amyloidosis encompassing the upper back, shoulders, arms and legs harbored the *RET* S891A mutation and *OSMR* variant G513D in a Chinese FMTC family (9), whereas MEN 2A-related CLA was strictly located in the scapular region and had the *RET* C634F/G mutation without *OSMR* mutation in four Chinese patients (7). Only a small proportion of kindred develop this manifestation, and despite a sensory neuropathy, secondary scratching or other modifying factors involved have been suggested, but there is a lack of *RET* polymorphisms (5, 6, 7, 23).

However, the underlying molecular mechanism remains elusive. Diagnostically, the histology-based definition of MEN 2A-related CLA should result in an apparent delineation of skin lesions for localized amyloid. However, it would be prudent to treat these cases conservatively, and the treatment of CLA in MEN 2A remains disappointing to date (4, 5, 6, 7, 31). Nonetheless, CLA in MEN 2A could be a relatively early clinical marker, as a characteristic of the clinical phenotype in 'MEN 2A with CLA', one of four variants of MEN 2A (5). Furthermore, CLA located in the scapular region is highly suggestive of MEN 2A, which should be investigated clinically, followed by germline *RET* screening (4, 5). Thus, CLA and MEN 2A in this individual should be attributed to the germline *RET* C611Y mutation rather than a coincidental situation, similar to a V804M mutation with CLA and MTC (5, 8).

The *RET* C611Y mutation comprising c.1832G>A and c.1832\_1833delinsAT exhibited the typical presentation of classical MEN 2A or FMTC, and occur at a 'moderate risk' (ATA-MOD) form of disease (4, 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19). In our kindred, all 17 affected patients (except 3 carriers (IV-6, IV-7 and IV-15)) presented with MTC or elevated serum calcitonin levels (82.4%). The mean age of patients was 45.9 (range: 23–73) years; two of them (11.8%) had unilateral PHEO (mean age, 53 (range: 37–73) years; Table 1). The clinical data in this family might imply the clinical pattern of a later diagnosis/onset of MTC and a lower penetrance of PHEO. Nonetheless, the genetic *RET* screening can instructively classify the pathology of thyroid tumors, and individuals with C611Y should be managed using a personalized approach and be related to psychological support (5, 32, 33, 34, 35, 36, 37, 38, 39, 40).

In summary, this study highlights a novel genotype-phenotype relationship between MEN 2A-related CLA and C611Y mutation of *RET*. CLA located in the interscapular region might represent earlier and 'premonitory' symptom should facilitate the early recognition of individuals at risk of MEN 2A-specific tumors. Furthermore, the timing of total thyroidectomy in carriers with the *RET* C611Y mutation should be considered to use a personalized approach based on the Ctn level.

#### Declaration 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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#### Author contribution statement

All authors were involved in the study. X-P Qi conceived and designed the experiments. X-P Qi, J-Z Peng, X-W Yang, Z-L Cao, X-H Yu, X-D Fang and J-Q Zhao performed the experiments; X-P Qi, J-Z Peng, D-H Zhang and J-Q Zhao analyzed the data; X-P Qi, X-W Yang, Z-L Cao and J-Q Zhao contributed reagents/materials/analysis tools. The manuscript was written by X-P Qi.

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