The rare intracellular *RET* mutation p.S891A in a Chinese Han family with familial medullary thyroid carcinoma

Xiao-Ping Qi[1,*,†], Rong-Xin Zhang[2,†], Jin-Lin Cao[1,†], Zhen-Guang Chen[1], Hang-Yang Jin[1] and Ren-Rong Yang[1]

[1]*Department of Oncologic and Urologic Surgery, the 117th PLA Hospital, Anhui Medical University, 40 Jichang Road, Hangzhou, Zhejiang Province, 310004, China*

[2]*Department of Neck and Thoracic Oncologic Surgery, Anhui Provincial Cancer Hospital, Anhui Medical University, No. 107 Huan-hu East Road, Hefei 230031, China*

**Corresponding author (Fax, +86-571-87348500; Email, qxplmd@vip.sina.com)*

[†]*These authors contributed equally to this work.*

We report intracellular *RET* mutation in a Han Chinese pedigree with familial medullary thyroid carcinoma (FMTC). Direct sequencing of *RET* proto-oncogene identified a missense c.2671T>G (p.S891A) mutation in 6 of 14 family members. The single nucleotide polymorphisms c. 135A>G (p.A45A), IVS4+48A>G, c. 1296A>G (p.A432A), c. 2071G>A (p.G691S), c. 2307T>G (p.L769L) and a variant c. 833C>A (p.T278N) were also found in 6 carriers. Among 5 of the 6 carriers presented medullary thyroid carcinoma (MTC) as an isolated clinical phenotype, with elevated basal serum calcitonin (Ct). Two underwent non-normative thyroidectomy either two or four times without physician awareness or diagnosis of this disease at initial treatment, but with elevated Ct. One with elevated pre-Ct accepted total thyroidectomy (TT) with modified bilateral neck dissection (MBiND), and whose seventh posterior rib MTC metastases was confirmed 5 months after surgery. Moreover, results of two affected individuals with elevated Ct were reduced to normal after TT with MBiND or prophylactic VI compartmental dissection. However, only another carrier with the variant p.T278N had slightly elevated Ct rejected surgery and was strictly monitored. Given these case results, we suggest that screening of *RET* and pre-surgical Ct levels in the management of MTC patients is essential for earlier diagnosis and more normative initial treatment, that FMTC patients with cervical lymph nodes metastases may be cured by TT with MBiND, and that prophylactic VI compartmental dissection should be avoided when Ct levels are low.

[Qi X-P, Zhang R-X, Cao J-L, Chen Z-G, Jin H-Y and Yang R-R 2014 The rare intracellular *RET* mutation p.S891A in a Chinese Han family with familial medullary thyroid carcinoma. *J. Biosci.* **39** 505–512] **DOI** 10.1007/s12038-014-9428-x

1. Introduction

Medullary thyroid carcinoma (MTC) arising from calcitonin (Ct)-secreting parafollicular C-cells accounts for 5% to10% of thyroid carcinomas (Kloos *et al*. 2009). It develops in the hereditary form in 25% of cases, occurring in almost all patients with multiple endocrine neoplasia type 2 (MEN 2) (Paszko *et al*. 2007). Three distinct forms of MEN 2 are subtyped into MEN 2A, MEN 2B, and familial MTC (FMTC). MEN 2A, the most common form of MEN 2 (80% of all cases), is characterized by the occurrence of two or more specific endocrine tumours, which shows MTC in nearly 95%, pheochromocytoma (PHEO) in 50–57% and hyperparathyroidism (HPT) in about 25% in a single individual or in close relatives. MEN2B, the most rare (5%) and aggressive form of MEN 2, is similar to MEN 2A

Keywords. Familial medullary thyroid carcinoma; MEN 2; polymorphism; *RET* mutation

Supplementary materials pertaining to this article are available on the *Journal of Biosciences* Website at: *http://www.ias.ac.in/jbiosci/jun2014/supp/Qi.pdf*

Published online: 1 May 2014

except that HPT is rare, and characteristic developmental abnormalities, such as mucosal neuroma, marfanoid habitus and ganglioneuromatosis of the gut, are presented. FMTC, as one subtype of MEN 2, is operationally diagnosed in families with four or more cases of MTC in the absence of PHEO or HPT, which occurs in 15% of all MEN2 cases (Eng *et al*. 1996; Brandi *et al*. 2001; Wells *et al*. 2013).

FMTC, MEN2A and MEN2B are regularly correlated with germline point mutations of *RET* gene, which is mapped on chromosome 10q11.2 and contains 21 exons, encoding a receptor tyrosine kinase (RTK) transmembrane protein, and expressed in neural crest-derived cells, adrenal chromaffin cells, and parafollicular C cells of the thyroid gland (Pachnis *et al*. 1993). The protein consists of 3 domains: extracellular (encoded in exons 1–10), intracellular (encoded in exons 12–21) and the connecting transmembrane domain (encoded in exon 11) (Paszko *et al*. 2007). Germline mutations of *RET* are found in MEN2 and Hirschsprung's disease (HD) in an autosomal dominant pattern (Mulligan *et al*. 1993; Romeo *et al*. 1994). HD can occur in patients with MEN2A and FMTC. The survey suggest that MTC-associated *RET* mutations are restricted to exons 10 and 13 affecting 5% of unselected adults with HD (Virtanen *et al*. 2013).

Currently, screening of *RET* and pre-surgical basal serum Ct (pre-Ct) level is an excellent method to ensure earlier diagnosis and more normative initial treatment of MTC (Qi *et al*. 2013). Pre-Ct is nearly always elevated in MTC and patients with Ct levels over 3000 ng/L are likely to have widely metastatic disease, and are unlikely to be cured despite aggressive surgery (Jarzab *et al*. 2013; Schneider and Chen 2013). Moreover, germline mutations in the *RET* proto-oncogene is found in 98% of families with MEN2-related MTC (Elisei *et al*. 2012). More than 90% of mutations are those at the extracellular domain (codons 609, 611, 618, and 620 in exon 10) and the transmembrane domain (codon 634 in exon 11) (Eng *et al*. 1996). However, intracellular p.S891A mutation is rare, accounting for less than 5% of all families with MEN2-related MTC (Schulte *et al*. 2010).

Surgical resection is the most effective treatment and has the best outcomes for patients with early-stage MTC (Vierhapper *et al*. 2005). However, 35% of MTC patients have tumours extending beyond the thyroid into surrounding tissues or regional lymph node metastases, and 13% have MTC metastatic to distant organs, which has a poor prognosis (Roman *et al*. 2006). Even after curative surgery and adjuvant systemic chemotherapy, the 10-year disease-specific survival of MTC is about 75% (Hundahl *et al*. 1998).

To our knowledge, this is the first report of a missense c.2671T>G (p.S891A) mutation of the *RET* proto-oncogene in an Asian pedigree with FMTC. Results of our study may contribute to better diagnosis and treatment of MTC with this rare mutation.

2. Patients and methods

2.1 *Patients*

A four-generation southern Chinese Han pedigree with FMTC from Zhejiang Province, China, was investigated (figure 1). All patients and/or their legal guardians provided written informed consent to participate in the study, as required by the Ethics Committee of the 117th PLA Hospital. The study protocol was also approved by the Ethics Committee.

2.2 *Clinical investigation*

Fourteen members of the subject family all received Doppler ultrasound (US) examination and Ct detection (normal male <8.4 ng/L and female <5.0 ng/L) by FACLIA (fully-automated chemiluminescence immunoassay, Immulite 2000 Immunoassay System, Siemens Ltd.,

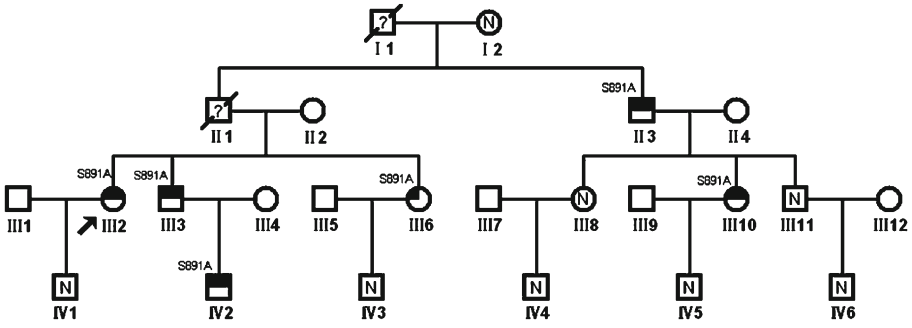

Figure 1. Pedigree of *RET* proto-oncogene mutation p.S891A in familial medullary thyroid carcinoma. ☐ ○ Sample not available; ▣ ◉ No mutation in RET; ◧ ◔ p.S891A carrier; ▬ ◓ MTC patient; ↗ proband; ⊘ Suspected mutation dead carrier

Germany). A medical history and clinical data were obtained from each participant, and a physical examination was performed. Clinical evaluation of FMTC was performed according to the criteria recommended by the Consensus of the International Multiple Endocrine Neoplasia Workshop (Kloos *et al*. 2009). For *RET* gene mutation carriers, further evaluation was performed such as computerized tomography (CT), and if any abnormalities were detected, emission CT (ECT) and nuclear magnetic resonance (MRI) were applied. Biochemical evaluation consisted of carcinoembryonic antigen (CEA) (normal <5.0 ng/L), serum parathyroid hormone (PTH) by electrochemiluminescence, and serum catecholamines (CA) by RIA (Radioimmunoassay), and serum calcium (arsenazo III method). Tumour staging was performed according to the current American Joint Committee on Cancer (7th edition) TNM classification system (Edge and Compton 2010). Lymph-node status was defined according to the criteria of the American Thyroid Association (ATA) recommendations (Kloos *et al*. 2009). All patients who received prophylactic thyroidectomy for treatment of MTC then received follow-up.

2.3 *Molecular genetic analyses*

All individuals were offered molecular genetic testing for germline mutations of the *RET* gene. Genomic DNA was extracted from EDTA anti-coagulated peripheral blood of all family members as previously described (Qi *et al*. 2011, 2012). The entire coding region of *RET* was amplified and sequenced in both sense and antisense directions with the ABI Prism 377 automatic sequencer (Perkin-Elmer, USA). The analyses were performed according to the 2009 ATA recommendations and 2001 European recommendations (Kloos *et al*. 2009; Fugazzola *et al*. 2013).

2.4 *Follow-up management*

All patients were followed-up for evaluation of tumour recurrence and metastasis after surgery. The standard follow-up for MTC consists of determining Ct, plasma calcium (every 6 months), and if the Ct level is abnormal during the postoperative period, an annual detailed imaging examination may be considered. After the first screening of *RET* mutation carriers older than age 20 years, re-examination of biochemical and imaging examinations were performed every year to screen for PHEO and HPT, and lifetime follow-up was also required. Follow-up visits for MTC are conducted according to the criteria of the ATA recommendations and previous reports (Kloos *et al*. 2009; Qi *et al*. 2013).

3. Results

3.1 *Identification of the RET germline mutation*

A heterozygous serine to alanine amino acid substitution within exon 15 of *RET* proto-oncogene, the missense c.2671T>G (p.S891A) mutation, was confirmed in 6 cases of 14 family members (figures 1 and 2). The single nucleotide polymorphisms (SNPs) c.135A>G (p.A45A/exon2), IVS4+48A>G (intron 4), c.1296A>G (p.A432A/exon7), c.2071G>A (p.G691S/exon 11), c. 2307T>G (p.L769L/exon 13) and a variant c. 833C>A (p.T278N/exon 4) were also found in this family (table 1). Of the 6 carriers, only 5 presented MTC as the isolated clinical phenotype. The other carrier with p.T278N was asymptomatic (III-6, 44 years; p.S891A/p.T278N). Meanwhile, 2 unaffected members (IV-1 and IV-3, 26 years and 25 years; respectively) with p.T278N were also identified and had no abnormality including consistently undetectable Ct (figure 3).

3.2 *Clinical features and diagnostic data*

The pedigree of the family is shown in figure 1. The mean age of five MTC patients was 41.2 years (range: 24–62 years), with average elevated Ct of 1995.23 ng/L (range: 12.60–8763.94) and a mean maximum diameter of thyroid nodules of 1.7 cm (range: 0.5–3.3). A total unilateral/

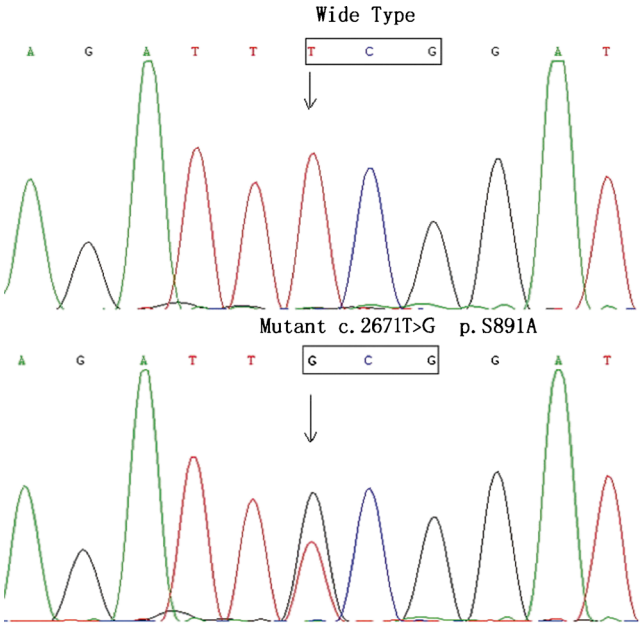

Figure 2. A heterozygous serine to alanine amino acid substitution within exon 15 of *RET* proto-oncogene, the missense c.2671T>G (p.S891A) mutation in proband.

Table 1. Sequence variants/polymorphisms of *RET* found in 14 cases

Individual	Gender	Variant/polymorphism					
		A45A	T278N	IVS4+48A>G	A432A	G691S	L769L
I-2	F				+		+
II-3 (p.S891A)	M				+		+
III-2 (proband)	F			+	+		+
III-3 (p.S891A)	M	+			+		+
III-6 (p.S891A)	F		+		+		+
III-8	F				+		+
III-10 (p.S891A)	F				+		+
III-11	M				+		+
IV-1	M		+		+		
IV-2 (p.S891A)	M	+			+	+	+
IV-3	M		+		+		+
IV-4	M				+		
IV-5	M				+		+
IV-6	M				+		+

F: female, M: male.

bilateral thyroidectomy with unilateral/bilateral neck dissection and/or level VI lymph node dissection was performed. The asymptomatic carrier (III-6) rejected surgery. All six affected individuals had no evidence of other related MEN 2 clinical symptoms such as PHEO, HPT, HD or corneal nerve thickening (CNT).

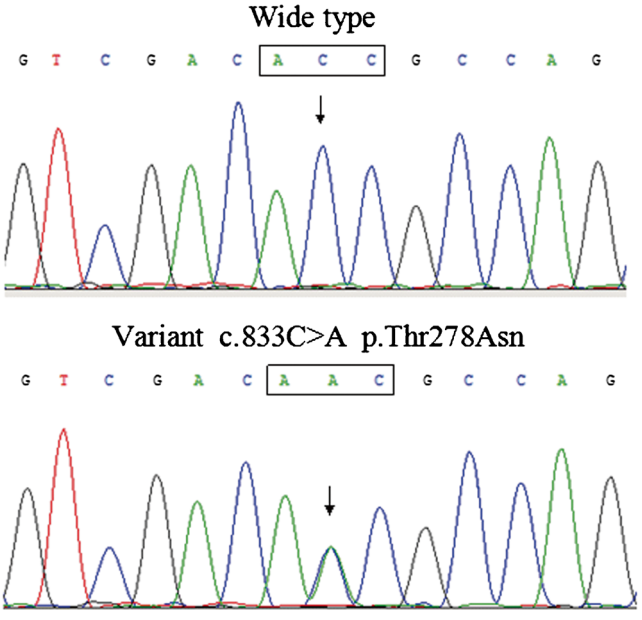

Figure 3. A variant c.833C>A(p.T278N) within exon 4 of *RET* proto-oncogene.

The proband (III-2) was a 40-year-old woman diagnosed with MTC in 1999. This patient underwent surgery successively four times due to lack of awareness of FMTC at initial treatment. The first surgery was in July 1999 when she had a palpable neck mass in the right thyroid. US scanning indicated hypoechoic nodules (3.3 cm × 2.8 cm × 2.2 cm) with calcifications in the right thyroid lobe and right neck mass. Then, a total right thyroidectomy and modified right neck dissection were performed. Pathological examination of both specimens showed MTC ($T_2N_{1b}M_0$; table 2). In 2012, the patient underwent second and third surgeries (modified right neck dissection) based on the presence of multi-right neck masses and elevated biochemical markers. In April 2013, US scanning indicated multi-centric hypoechoic nodules (the maximum size was 1.5 cm × 1.5 cm × 0.7 cm) in the left thyroid lobe and left neck mass, and a pre-Ct level of 688.30 ng/L. Then, total left thyroidectomy and modified left neck dissection were performed. Finally, histopathology revealed bilateral multi-centric MTC with bilateral lymph node metastases ($T_2N_{1b}M_0$; table 2).

In November 2009, the proband's 33-year-old female cousin (III-10) was subjected to a total left thyroidectomy with modified left neck dissection after diagnosis by US and CT of multi-centric hypoechoic nodules (the maximum size was 1.5 cm × 0.8 cm × 0.5 cm) in the left thyroid lobe and left neck mass. Histopathological examination showed left multi-centric MTC with left lymph node metastases ($T_1N_{1b}M_0$). Three years later, US and CT scanning also discovered multi-centric hypoechoic nodules with calcifications in the right thyroid lobe and right neck mass, and biochemical examination

Table 2. Clinical features of p.S891A *RET* mutation carriers

Patient	Gender	Age at Dx(years)	Pre-/ Post-Ct(ng/L)	US Results	Surgery	Histology	LN+/resected	pTNM
II-3	M	62	478.00/2.92	RBL(Multi):0.9 × 0.7 × 0.5 cm; LBL(Multi):0.8 × 0.7 × 0.5 cm; Bi-NLNM	TT+MBiND	Bi-MTC	17/60	$T_1N_{1b}M_0$
III-2 (proband)	F	40	-/-	RL:3.3 × 2.8 × 2.2 cm; R-NLNM	TT(Right)+MRND	R-MTC	NA	$T_2N_{1b}M_0$
		52	-/-	R-NLNM	MRND	R-MTC	NA	$T_2N_{1b}M_0$
		53	982.70/688.30	R-NLNM	MRND	R-MTC	NA	$T_2N_{1b}M_0$
		54	688.30/358.30	LBL(Multi):1.5 × 1.5 × 0.7cm; L-NLNM	TT(Left)+MLND	L-MTC	NA	$T_2N_{1b}M_0$
III-3	M	47	8763.94/1267.20	RBL(Multi):1.8 × 1.5 × 1.0; LBL (Multi):2.3 × 1.7 × 1.5; Bi-NLNM	TT+MBiND	Bi-MTC	25/67	$T_2N_{1b}M_1$
III-6	F	44	5.97–6.72/-	-	Reject	-	-	-
III-10	F	33	-/-	LBL(Multi):1.5 × 0.8 × 0.5 cm	TT(Left)+MLND	Bi-MTC	12/24	$T_1N_{1b}M_0$
		36	33.30/9.20	RBL(Multi):0.6 × 0.5 × 0.5 cm	TT(Right)+MRND	R-MTC	13/39	$T_1N_{1b}M_0$
IV-2	M	25	12.60/<2.00	LL:0.5 × 0.5 × 0.4 cm	TT+L-LND(VI)	Bi-MTC	0/4	$T_1N_0M_0$

F: female, M: male, Dx: diagnosis, Pre-/ Post Ct: pre-/post-surgery basal serum calcitonin, US: ultrasound, RL: right lobe, LL: left lobe, Multi: multi-centric nodules, RBL: right biggest lobe, LBL: left biggest lobe, Bi-NLNM: bilateral neck lymph node metastases, L-NLNM: left neck lymph node metastases, R-NLNM: right neck lymph node metastases, TT: total thyroidectomy, MBiND: modified bilateral neck dissection, MRND: modified right neck dissection, MRND: modified left neck dissection, L-LND(VI): level VI lymph node dissection, Bi-MTC: bilateral MTC, LN+: includes positive lymph nodes proven on histopathology; resected: includes lymph node resected, NA: not available, pTNM: tumour stage.

disclosed elevated Ct (33.30 ng/L). A further total right thyroidectomy with modified right neck dissection was performed. Histopathology revealed multi-centric MTC with lymph node metastases ($T_1N_{1b}M_0$).

In November 2012, based on the systematic family investigation and *RET* gene scanning, eight of the 14 members exhibited normal Ct levels and US images, but the other 6 (II-3, III-2, III-3, III-6, III-10 and IV-2) had the p.S891A mutation of *RET*. Then, the other three *RET* mutations, including the proband's uncle (II-3), brother (III-3) and brother's son (IV-2), had elevated pre-Ct levels (478.00, 8763.94 and 12.60 ng/L, respectively), and all three were subjected to total thyroidectomy and modified bilateral neck dissection (II-3 and III-3) or level VI lymph node dissection (IV-2). Histopathological examination revealed bilateral MTC with lymph node metastases in two patients (II-3 and III-3; $T_1N_{1b}M_0$ and $T_2N_{1b}M_x$, respectively) and only bilateral MTC in one patient (IV-2; $T_1N_0M_0$). In addition, the proband's sister (III-6, 44-year-old) who was a *RET* mutation carrier refused thyroidectomy although she had presented with elevated Ct (range: 5.97–6.72 ng/L) without evidence of clinical symptoms (such as CEA, serum calcium, serum CA, thyroid function tests and serum PTH, as well as neck Doppler US and adrenal CT were all normal) and was strictly monitored.

After surgery, all 5 patients (II-3, III-2, III-3, III-10 and IV-2) received L-T4 substitution therapy and were followed up. Unfortunately, the proband's 47-year-old brother (III-3) exhibited seventh posterior rib MTC metastases confirmed by ECT and MRI examination 5 months after surgery, but Ct was reduced to 1267.20 ng/L and CEA from 186.00 ng/mL to 23.50 ng/mL ($T_2N_{1b}M_1$), and he refused further treatment. In addition, two patients (III-2 and III-10) still presented elevated post-Ct levels (358.20 and 9.20 ng/L, respectively), and biochemical and imaging examinations of the other two (II-3 and IV-2) revealed no recurrence of MTC.

4. Discussion

We identified a missense p.S891A mutation within exon 15 of the *RET* proto-oncogene, which lies in the tyrosine kinase 2 (TK2) region of the RET protein. Its mutation appears to exist as an active monomer as it does not require dimerization to be activated, and ligand binding does not further enhance autophosphorylation or downstream signalling (Plaza Menacho *et al*. 2005; Wagner *et al*. 2012). It belongs to those *RET* mutations with low (p.E768D, p.V804L, p.S891A and p.A919P) rather than high (p.A883F, p.M918T and p.E768D) transforming activity (Iwashita *et al*. 1999). This mutation is the first to be reported in Asian, to our knowledge.

Prior studies have shown that intracellular p.S891A mutation is rare, accounting for less than 5% of all patients with

RET mutations (Schulte *et al*. 2010). It was found in three of 141 German *RET* mutation families (2.1%) and 17 of 356 Continental European *RET* mutation families (4.8%) (Machens and Dralle 2008; Machens *et al*. 2009). Mutation of S891A was initially recognized as an FMTC mutation, but recently has been linked to MEN 2A features (Jimenez *et al*. 2004). Until now, 85 carriers of the intracellular *RET* mutation p.S891A had been summarized (Blom *et al*. 2012): MTC in 72.9% (62/85), PHEO in 3.5% (3/85), HPT in 3.5% (3/85) and CNT in 3.5% (3/85). The mean age of MTC diagnosis in that group was 42.3 years. In Schulte's report (Schulte *et al*. 2010), age-related penetrance of MTC in 36 *RET* mutation S891A patients was 20.0%, 71.4%, 92.3% and 100.0% in those aged 0–20 years, 21–40 years, 41–60 years, and >60 years, respectively. In our pedigree with p.S891A mutation, the mean age of MTC onset in five of six affected individuals, excluding one patient (III-6, age 44 years), was 41.2 years (range: 24–62); 83.3% (5/6) exhibited the typical presentation of MTC as the only clinical symptom with no evidence of PHEO, HPT, CNT or HD. The reason for these differences compared with previous results may be related to the ethnicity, regional distribution and small sample. Additionally, four patients presented with lymph node or bone metastases (80%, 4/5; 2 with $T_1N_{1b}M_0$, 1 with $T_2N_{1b}M_0$, and 1 with $T_2N_{1b}M_1$, respectively), which is consistent with previous clinical data (Schulte *et al*. 2010). Furthermore, we found five SNPs (p.A45A, IVS4+48A>G, p.A432A, p.G691S and p.L769L) and a variant p.T278N. These SNPs have been associated with modification of disease susceptibility and the clinical phenotype of C-cell hyperplasia, sporadic MTC, papillary thyroid carcinoma, HD, MEN 2A and FMTC in some populations (Fitze *et al*. 2003; Sangkhathat *et al*. 2006; Chang *et al*. 2009; Figlioli *et al*. 2013; Lantieri *et al*. 2013). However, no association between these SNPs and phenotypes in this family was found to date. Additionally, the p.T278N did not report to play a role in MEN2-related MTC, to our knowledge (Chang *et al*. 2009; *http://arup.utah.edu/database/MEN2/MEN2_welcome.php*). In our pedigree, the p.S891A/p.T278N mutations carrier (III-6, 44 years) who only presented with elevated Ct (range5.97–6.72 ng/L) without evidence of clinical symptoms, two unaffected members (IV-1 and IV-3, 26 years and 25 years, respectively) with p.T278N had no abnormal consistently, and all other family members presented no p.T278N including III-2 (IV-1'mother), which seems that the p.T278N may play potential inhibitory modified or may benign, or may be a SNP in essential (*http://asia.ensembl.org/Homo_sapiens/Variation/Mappings?db=core;g=ENSG00000165731;r=10:43572475–43625799;source=dbSNP;v=rs35118262;vdb=variation;vf=11442193*). Therefore, further studies are needed involving the tracking of more samples throughout the *RET* gene p.S891A and p.T278N and follow-up studies on FMTC initiation and aggressiveness of this mutation.

Currently, early and normative surgery is the most effective treatment and offers the best results for patients with MTC (Vierhapper *et al*. 2005). Screening of *RET* and pre-Ct level is an excellent method for obtaining an earlier diagnosis of MTC (Qi *et al*. 2013). Thus, surgical resection should be based on the management guidelines of aggressive MTC after a systematic screening for *RET* and pre-Ct detection in at-risk patients and their families. The 2009 ATA stratified mutations at codon p.S891A as ATA-A level with a lower transforming activity of *RET* and a milder form of disease (Kloos *et al*. 2009). However, total thyroidectomy with unilateral/bilateral neck dissection and VI compartmental dissection is needed if clinical lymph node metastases is present, and prophylactic total thyroidectomy at age 5 or earlier is recommended, performed in an experienced tertiary care setting. Unfortunately, no significant clinical symptoms are noted for earlier stages of FMTC at diagnosis. After the disease has developed with lymph node metastasis or elevated Ct, the prognosis remains poor and the disease-free survival of patients has not increased significantly in recent decades (Kebebew *et al*. 2005; Roman *et al*. 2006). In the present series, only one patient (III-2) had a palpable neck mass in the right thyroid, another was (III-10) found by US and CT, and *RET* mutations were confirmed in four patients (II-3, III-3, III-6 and IV-2) after the family investigation and *RET* gene scanning. Regrettably, two patients (III-2 and III-10; $T_2N_{1b}M_0$ and $T_1N_{1b}M_0$, respectively) had undergone multiple non-normative surgery at initial treatment, and post-Ct levels were still elevated. Meanwhile, despite the performance of relative normative surgery in another patient (III-3) with elevated pre-Ct (8763.94 ng/L), distant posterior rib MTC metastasis ($T_2N_{1b}M_1$) still appeared unavoidably. Moreover, recurrence of MTC was not revealed in two affected individuals (II-3 and IV-2; $T_1N_{1b}M_0$ and $T_1N_0M_0$, respectively) with elevated pre-Ct (478.00 and 12.60 ng/L, respectively) by biochemical (post-Ct: 2.92 and <2.00 ng/L, respectively) and imaging examinations after total thyroidectomy and modified bilateral neck dissection (II-3) or prophylactic VI compartmental dissection (IV-2, tumour size: 0.5 cm × 0.5 cm × 0.4 cm). Total thyroidectomy and modified bilateral lymph node excision are curative in FMTC patients with cervical lymph nodes metastases (Wells *et al*. 2013).

However, as with other lower-risk mutations, it is unclear whether these patients should receive prophylactic VI compartmental dissection when no clinical or imaging evidence of lymph nodes metastases is found. The 2009 ATA recommendations for MEN 2A or FMTC patients with thyroid nodules ≥ 5 mm in size at any age, or Ct > 40 ng/L, are that they should undergo prophylactic level VI lymph node dissection. Other studies suggest that surgery can be individualized for *RET* mutation carriers with pre-Ct < 60 ng/L or pre-Ct < 71.4 ng/L and they may forgo lymph node

dissection (Kloos *et al*. 2009; Elisei *et al*. 2012; Qi *et al*. 2013). Thus, treatment of patient IV-2 in the present series, who received prophylactic VI compartmental dissection, may be controversial or there may have been no need to implement prophylactic VI compartmental dissection. Elisei *et al*. (2012) also further substantiated that the timing of prophylactic total thyroidectomy in mutation carriers with negative Ct can be individualized and safely planned when stimulated Ct becomes positive, independent of the type of *RET* mutation and the patient's age. However, in the present study, the proband's sister (III-6), who was a *RET* mutation carrier, refused prophylactic thyroidectomy for lack of clinical symptoms even though Ct was elevated, which highlights the importance of increasing patients' awareness in order to avoid delaying timely treatment. Meanwhile, this suggests that differences in MTC progression exist among individuals, and that genetic modifiers such as *RET* variants/SNPs and marked heterogeneity may influence the transforming activity and the progression of MTC (Sakorafas *et al*. 2008; Qi *et al*. 2012). It is suggested, therefore, that individual treatment of MEN 2-related FMTC should be based on predictive *RET* screening and pre-Ct levels.

In conclusion, based on the results of the present study, screening of *RET* and pre-surgical Ct levels in the management of FMTC patients is essential for earlier diagnosis and more normative initial treatment.

Acknowledgements

The authors thank all the patients and their families who agreed to participate in this study. This work was supported by the Key Scientific Research Project of Nanjing Military Command, China (09Z038 and 10Z036).

References

Blom CB, Ceolin L, Romitti M, Siqueira D and Maia AL 2012 The rare intracellular *RET* mutation p.Ser891Ala in an apparently sporadic medullary thyroid carcinoma: a case report and review of the literature. *Arq. Bras. Endocrinol. Metabol.* **56** 586–591

Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, *et al.* 2001 Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J. Clin. Endocrinol. Metab.* **86** 5658–5671

Chang CF, Yang WS, Su YN, Wu IL and Chang TC 2009 Mutational Spectrum of Multiple EndocrineNeoplasia Type 2 and Sporadic Medullary Thyroid Carcinoma in Taiwan. *J. Formos. Med. Assoc.* **108** 402–408

Edge SB and Compton CC 2010 The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann. Surg. Oncol.* **17** 1471–1474

Elisei R, Romei C, Renzini G, Bottici V, Cosci B, Molinaro E, Agate L, Cappagli V, Miccoli P, *et al.* 2012 The timing of Total Thyroidectomy in *RET* Gene Mutation Carriers Could Be Personalized and Safely Planned on the Basis of Serum Calcitonin: 18 Years Experience at One Single Center. *J. Clin. Endocrinol. Metab.* **97** 426–435

Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, van Amstel HK, Lips CJ, *et al.* 1996 The relationship between specific *RET* protooncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International *RET* mutation consortium analysis. *JAMA* **276** 1575–1579

Figlioli G, Landi S, Romei C, Elisei R and Gemignani F 2013 Medullary thyroid carcinoma (MTC) and RET proto-oncogene: Mutation spectrum in the familial cases and a meta-analysis of studies on the sporadic form. *Mutat. Res*. **752** 36–44

Fitze G, Schierz M, Kuhlisch E, Schreiber M, Ziegler A, Roesner D and Schackert HK 2003 Novel Intronic Polymorphisms in the RET Protooncogene and Their Association With Hirschsprung Disease. *Hum. Mutat.* **22** 177

Fugazzola L, De Leo S and Perrino M 2013 The optimal range of *RET* mutations to be tested: European comments to the guidelines of the American Thyroid Association. *Thyroid Res.* **6** S8

Hundahl SA, Fleming ID, Fremgen AM and Menck HR 1998 A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985–1995 [see comments]. *Cancer* **83** 2638–2648

Iwashita T, Kato M, Murakami H, Asai N, Ishiguro Y, Ito S, Iwata Y, Kawai K, Asai M, Kurokawa K, Kajita H and Takahashi M 1999 Biological and biochemical properties of Ret with kinase domain mutations identified in multiple endocrine neoplasia type 2B and familial medullary thyroid carcinoma. *Oncogene* **18** 3919–3922

Jarzab B, Szpak-Ulczok S, Wloch J, Czarniecka A and Krajewska J 2013 Timing and criteria for prophylactic thyroidectomy in asymptomatic *RET* mutation carriers-the role of Ct serum level. *Thyroid Res*. **6** S9

Jimenez C, Habra MA, Huang SC, El-Naggar A, Shapiro SE, Evans DB, Cote G and Gagel RF 2004 Pheochromocytoma and medullary thyroid carcinoma: a new genotype-phenotype correlation of the *RET* protooncogene 891 germline mutation. *J. Clin. Endocrinol. Metab.* **89** 4142–4145

Kebebew E, Greenspan FS, Clark OH, Woeber KA and Grunwell J 2005 Extent of disease and practice patterns for medullary thyroid cancer. *J. Am. Coll. Surg.* **200** 890–896

Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JF, Pacini F, Ringel MD,Schlumberger M and Wells SA Jr 2009 Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid* **19** 565–612

Lantieri F, Caroli F, Ceccherini I and Griseri P 2013 The involvement of the RET variant G691S in medullary thyroid carcinoma enlightened by a meta-analysis study. *Int. J. Cancer* 132 2808–2819

Machens A and Dralle H 2008 Familial prevalence and age of *RET* germline mutations: Implications for screening. *Clin. Endocrinol.* **69** 81–87

Machens A, Lorenz K and Dralle H 2009 Constitutive *RET* tyrosine kinase activation in hereditary medullary thyroid cancer: clinical opportunities. *J. Intern. Med*. **266** 114–125.

Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, et al. 1993 Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* **363** 458–460

Pachnis V, Mankoo B and Costantini F 1993 Expression of the c-ret proto-oncogene during mouse embryogenesis. *Development* **119** 1005–1017

Paszko Z, Sromek M, Czetwertynska M, Skasko E, Czapczak D, Wisniewska A, Prokurat A, Chrupek M, Jagielska A and Kozlowicz-Gudzinska I 2007 The occurrence and the type of germline mutations in the *RET* Gene in Patients with medullary thyroid carcinoma and their unaffected kindred's from central Poland. *Cancer Invest.* **25** 742–749

Plaza Menacho I, Koster R, van der Sloot AM, Quax WJ, Osinga J, van der Sluis T, Hollema H, Burzynski GM, Gimm O, Buys CH, Eggen BJ and Hofstra RM 2005 R*ET*-familial medullary thyroid carcinoma mutants Y791F and S891A activate a Src/JAK/STAT3 pathway, independent of glial cell line-derived neurotrophic factor. *Cancer Res.* **65** 1729–1737

Qi XP, Chen XL, Ma JM, Du ZF, Fei J, Yang CP, Cheng J, Song QZ, *et al.* 2012 RET proto-oncogene genetic screening of families with multiple endocrine neoplasia type 2 optimizes diagnostic and clinical management in China. *Thyroid* **22** 1257–1265

Qi XP, Ma JM, Du ZF, Ying RB, Fei J, Jin HY, Han JS, Wang JQ, *et al.* 2011 *RET* germline mutations identified by exome sequencing in a Chinese multiple endocrine neoplasia type 2A/Familial medullary thyroid carcinoma Family. *PLoS One* **6** e20353

Qi XP, Zhao JQ, Du ZF, Yang RR, Ma JM, Fei J, Cheng J, Han JS, Jin HY, *et al.* 2013 Prophylactic thyroidectomy for MEN 2-related medullary thyroid carcinoma based on predictive testing for *RET* proto-oncogene mutation and basal serum calcitonin in China. *Eur. J. Surg. Oncol.* **39** 1007–1012

Roman S, Lin R and Sosa JA 2006 Prognosis of medullary thyroid carcinoma: demographic, clinical, and pathologic predictors of survival in 1252 cases. *Cancer* **107** 2134–2142

Romeo G, Ronchetto P, Luo Y, Barone V, Seri M, Ceccherini I, Pasini B, Bocciardi R, *et al.* 1994 Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung's disease. *Nature* **367** 377–378

Sakorafas GH, Friess H and Peros G 2008 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

Sangkhathat S, Kusafuka T, Chengkriwate P, Patrapinyokul S, Sangthong B and Fukuzawa M 2006 Mutations and polymorphisms of Hirschsprung disease candidate genes in Thai patients. *J. Hum. Genet.* **51** 1126–1132

Schneider DF and Chen H 2013 New Developments in the Diagnosis and Treatment of Thyroid Cancer. *CA Cancer J. Clin.* **64** 373–394

Schulte KM, Machens A, Fugazzola L, McGregor A, Diaz-Cano S, Izatt L, Aylwin S, Talat N, Beck-Peccoz P and Dralle H 2010 The clinical spectrum of multiple endocrine neoplasia type 2a caused by the rare intracellular *RET* mutation S891A. *J. Clin. Endocrinol. Metab.* **95** E92 -97

Vierhapper H, Niederle B, Bieglmayer C, Kaserer K and Baumgartner-Parzer S 2005 Early diagnosis and curative therapy of medullary thyroid carcinoma by routine measurement of serum calcitonin in patients with thyroid disorders. *Thyroid* **15** 1267–1272

Virtanen VB Pukkala E, Kivisaari R, Salo PP, Koivusalo A, Arola J, Miettinen PJ, Rintala RJ, Perola M and Pakarinen MP 2013 Thyroid cancer and co-occurring *RET* mutations in Hirschsprung disease. *Endocr. Relat. Cancer* **20** 595–602

Wagner SM, Zhu S, Nicolescu AC and Mulligan LM 2012 Molecular mechanisms of *RET* receptor-mediated oncogenesis in multiple endocrine neoplasia 2. *Clinics (Sao Paulo)* **67** 77–84

Wells SA Jr, Pacini F, Robinson BG and Santoro M 2013 Multiple Endocrine Neoplasia Type 2 and Familial Medullary Thyroid Carcinoma: An Update. *J. Clin. Endocrinol. Metab*. **98** 3149–3164

MS received 31 December 2013; accepted 13 March 2014

Corresponding editor: SARAH H ELSEA