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RET Proto-oncogene Gene Mutation Is Related to Cervical Lymph Node Metastasis in Medullary Thyroid Carcinoma

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Abstract

RET proto-oncogene (*RET*) mutations were proved to be related to the development of medullary thyroid carcinoma (MTC). We aimed to analyze the role of *RET* mutations in cervical lymph node metastasis in patients with MTC. Forty-nine patients with preoperatively diagnosed MTC by fine-needle aspiration cytology (FNAC) who underwent bilateral total thyroidectomy with cervical lymphadenectomy were included. Postoperative *RET* gene test and pathological analysis were performed with the surgical specimens; serum calcitonin (Ctn) and carcinoembryonic antigen (CEA) levels were tested pre- and postoperatively, to evaluate the association between *RET* mutations and cervical lymph node metastasis in MTC. In these 49 patients, the *RET* mutation rates of Exon 11, Exon 10, Exon 11&13, Exon 13, and Exon 16 were 20.4%, 4.1%, 38.8%, 22.4%, and 0%, respectively. The lymph node metastasis rates of patients with *RET* mutation in the central and lateral compartments were 71.4% and 64.3%, respectively, versus 28.6% and 14.3% of patients without *RET* mutation. The preoperative basal serum levels of Ctn (234.8 ± 188.4 vs. 44.4 ± 27.5, $p < 0.01$) and postoperative Ctn (49.8 ± 86.4 vs. 3.7 ± 2.2, $p = 0.001$) in MTC patients with *RET* mutations were significantly higher than those in MTC patients without *RET* mutation. In addition, the preoperative (50.2 ± 76.7 vs. 7.4 ± 6.8, $p = 0.001$) and postoperative serum levels of CEA (13.2 ± 19.5 vs. 1.3 ± 1.6, $p < 0.01$) in MTC patients with *RET* mutations were significantly higher than those in MTC patients without *RET* mutation ($p < 0.05$). *RET* mutation was related to cervical lymph node metastasis in patients with MTC, especially the mutation in Exon 11&13. Patients with *RET* mutation in Exon 11&13 might be regarded as the predictor for prophylactic ipsilateral total cervical lymphadenectomy even without clear evidence of lateral cervical lymph node metastasis.

Keywords Medullary thyroid carcinoma · *RET* proto-oncogene mutation · Cervical lymph node metastasis · Prognostic factors · Surgical treatment

Abbreviations

C cells	Thyroid follicular cells
CEA	Carcinoembryonic antigen
Ctn	Calcitonin
FNAC	Fine-needle aspiration cytology
MTC	Medullary thyroid carcinoma
OR	The odds ratio
RET	*RET* proto-oncogene
SMTC	Sporadic MTC
TG	Thyroglobulin

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Introduction

Medullary thyroid carcinoma (MTC) is one of the malignant neuroendocrine tumors that is derived from thyroid follicular cells (C cells). Although MTC accounts for only 5–10% of all thyroid cancers, about 13.4% of all thyroid cancer-related mortalities are caused by MTC [1, 2]. Similar to other kinds of thyroid cancer, surgery is the effective treatment for MTC.

Total thyroidectomy and dissection of cervical lymph node compartments, depending on serum calcitonin (Ctn) levels and ultrasound (US) findings, are the standard treatment for patients with sporadic or hereditary MTC [3]. It is recommended that patients with MTC who have no evidence of neck lymph node metastases by preoperative US examination and no evidence of distant metastases should have a total thyroidectomy and lymphadenectomy of the central compartment (level VI) [3], but the dissection of lymph nodes of the lateral compartments (levels II–V) is controversial, as lateral

compartment lymphadenectomy may result in a relatively high incidence of surgical trauma and complications.

Studies have shown that the ratio of the number of lymph node metastases to the total number of dissected lymph nodes is associated with prognosis in this disease [4], especially for sporadic MTC (SMTC) where approximately 32–67% was bilateral and multifocal [5]. Moreover, cervical lymph node metastasis is relatively common and presents at the early stage of the disease [6], with metastasis rate reaching 50–80% [7]. Preoperative imaging tests, including the US, were routinely used to detect lymph node metastasis, but the detection of small and hidden lesions can be difficult. Some researchers believe that occult metastasis is very common in MTC, and extensive regional neck dissection can effectively remove these occult lesions. Also, researchers believe that bilateral lymphadenectomy in both central and lateral compartments can be performed regardless of unilateral or bilateral lesions and with or without cervical lymph node metastasis [8].

In recent years, the relationship between *RET* proto-oncogene and the development of MTC caused lots of attention [9]. Approximately 95% of hereditary MTC (HMTC) incidences and more than 80% of SMTC cases were related to *RET* mutations [10, 11]; the most common mutation sites occur in Exon 10, Exon 11, Exon 13, and Exon 16 [12, 13]. We conducted this study to analyze the relationship between the *RET* mutation sites (Exon 10, Exon 11, Exon 13, and Exon 16) and cervical lymph node metastasis in MTC, to study the feasibility of prophylactic ipsilateral lateral compartment cervical lymph node dissection in patients with fine-needle aspiration cytology (FNAC)–diagnosed MTC which were absence in preoperative detection of cervical lymph node metastasis.

Methods

Research Subjects

Patients who were newly diagnosed with MTC by FNAC and treated in our hospital from January 2010 to December 2016 were included in this study. This study was approved by the local Ethics Committee. All patients provided written informed consent before participating in the study.

Inclusion Criteria

Inclusion criteria for this study were as follows: (1) patients who were newly diagnosed with MTC by FNAC; (2) sporadic form of MTC which was defined by the absence of a familial history for MTC as well as a lack of *RET* germline mutations and other MEN2A-related tumors; (3) surgical resection which was performed; (4) MTC which was confirmed by postoperative pathology; (5) patients with completed medical data; and (6) whether preoperative computed tomography (CT) and ultrasound detect cervical lymph node metastasis or not.

Exclusion Criteria

Exclusion criteria for this study were as follows: (1) patients who had a history of other primary malignancies; (2) patients who presented with additional types of thyroid cancer; (3) patients who had a history of thyroid diseases and thyroid-associated medications; (4) patients regarded as germline form of MTC.

RET Gene Testing

In this study, *RET* gene testing and relevant technical support were provided by Mayo Clinic (Beijing, China) and validated by the Laboratory of Kindstar Global Co., Ltd. (Beijing, China). RET testing was performed using the surgical specimens after the operation. PCR and gene sequencing were used to detect *RET* gene mutations in Exon 10, Exon 11, Exon 13, Exon 16, and nearby splice site(s) of the mutant transcript, covering the expected range of point mutations, insertions, and deletions. A total of six PCR and 12 gene sequencing reactions were performed.

Surgical Approaches

All surgeries were performed by the same group of surgeons. All patients underwent bilateral thyroidectomy + lymphadenectomy in the central compartment. Ipsilateral lateral compartment lymphadenectomy was performed in patients without suspect of cervical lymph node metastasis; bilateral lateral compartment lymphadenectomy was performed if cervical lymph mode metastasis was suspected before the operation. Routine pathological examinations were performed postoperatively.

Results Analysis

All patients were grouped according to the presence or absence of *RET* mutations, postoperative pathological examination data about lymph node metastasis (in both central and lateral cervical compartment), serum Ctn and CEA level pre- and postoperative were collected and compared between RET mutation-positive and negative groups.

Despite grouping the patients according to the presence of the *RET* mutations, we also grouped all patients according to different *RET* mutation sites and evaluated cervical lymph node metastasis in these patients to analyze the relationship between the *RET* mutation sites and cervical lymph node metastasis.

Table 1 Demographic profiles of patients

	Ret mutation group $N = 42$	Non-mutated group $N = 7$	p
Age (years old)	50.7 ± 10.8	51.6 ± 10.0	0.674
Sex (male/female)	20/22	3/4	0.815
BMI (kg/m^2)	29.2 ± 2.9	28.1 ± 3.3	0.762

Age and BMI are presented as the mean ± SD with the range

MTC, medullary thyroid carcinoma

Statistical Analysis

SPSS19.0 (SPSS Inc., Chicago, IL) and STATA14.0 (StataCorp LLC., College Station, TX) software were used for statistical analysis and figure preparations. Count data were analyzed using the chi-square test or Fisher's exact probability test (*F*-test), mean *T* test, and ANOVA analysis.

Results

In total, 51 patients were suspected to be MTC by preoperative FNAC, but two patients were eventually diagnosed with papillary thyroid carcinoma and excluded from our study. Thus, in total, 49 patients were included in our study. Table 1 shows the demographic profiles of these patients. Age was ranging from 28 to 77 years (mean age of 50.8 ± 10.6 years), and 23 cases were males. All the patients underwent bilateral thyroidectomy + lymphadenectomy in the central compartment, and ipsilateral lateral compartment lymphadenectomy was performed in patients without suspect of cervical lymph node metastasis, or bilateral lateral compartment lymphadenectomy if cervical lymph node metastasis was suspected, with postoperative pathological examinations performed to confirm the diagnosis of MTC and cervical lymph node metastasis.

The results of *RET* gene testing showed 42 MTC patients with *RET* mutation and seven patients without *RET* mutation. Among the 42 MTC patients with *RET* mutations, 10 cases had a mutation in Exon 11 (23.8%), 2 cases in Exon 10 (4.8%), 19 cases in Exon 11&13 (45.2%), and 11 cases in Exon 13 (26.2%), and 0 cases had a mutation in Exon 16 (0%). This study showed that most of the patients analyzed exhibited *RET* mutations in Exon 11, Exon 13, and Exon 11&13 (Figs. 1 and 2).

Correlation analysis between the presence of *RET* mutation and the rate of cervical lymph node metastasis in MTC patients showed that *RET* mutation was associated with cervical lymph node metastasis in different regions. Among the MTC patients with *RET* mutations, 71.4% of them had lymph node metastasis in the central neck area, and 64.3% of them had lateral cervical lymph node metastasis. Among the MTC patients without *RET* mutation, only 28.6% of them had lymph node metastasis in the central compartment, and 14.3% of them had lateral cervical lymph node metastasis. The odds ratios (OR) for lymph node metastasis in the central compartment of the patients with *RET* mutation was 6.25, and the OR value of lateral cervical lymph node metastasis of these patients was 10.8. The OR of overall metastasis was 8.0 (Fig. 3).

Correlation analysis between different *RET* mutation sites and lymph node metastasis in different regions in the MTC patients was carried out by regrouping all patients based on their *RET* mutation sites. In this study, the number of cases was too small in Exon 10 mutation, and no case had mutation in Exon 16, so we only observed the correlation between Exon 11, 13, and 11&13 mutations and lymph node metastasis in the central neck area, with the ORs for Exon 11&13, Exon 11, and Exon 13 of 3.4, 0.93, and 0.48, respectively. Also, the

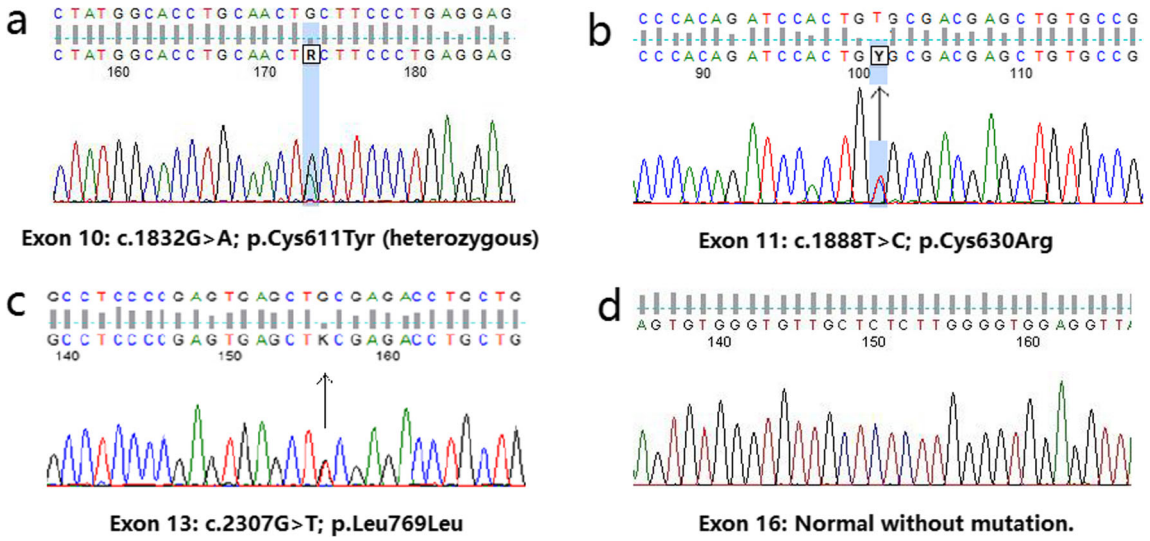

Fig. 1 Different *RET* mutation sites. **a** Exon 10: c.1832G>A; p.Cys611Tyr (heterozygous). **b** Exon 11: c.1888T>C; p.Cys630Arg. **c** Exon 13: c.2307G>T; p.Leu769Leu. **d** Exon 16: normal without the mutation

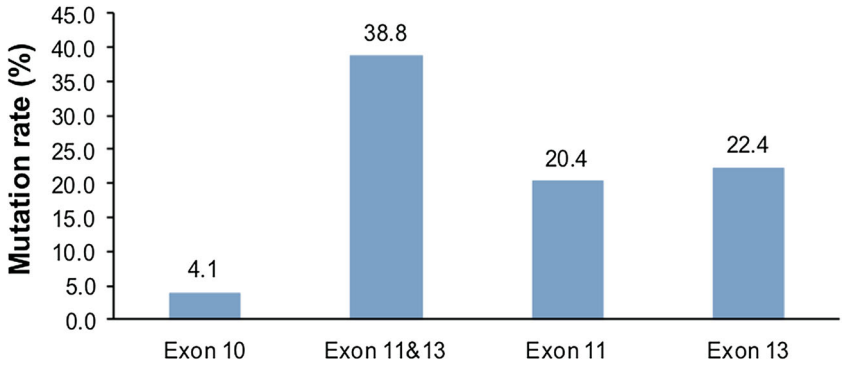

Fig. 2 Mutation rates of different *RET* mutation sites

correlation between Exon 11&13 mutation and lateral cervical lymph node metastasis was the highest among the *RET* mutation sites, with the ORs of 2.96, 0.83, and 0.46 for Exon 11&13, Exon 11, and Exon 13, respectively. The ORs of different *RET* mutation sites associated with lymph node metastasis in region VI and lateral cervical lymph nodes were similar (Fig. 4).

Besides, the study found that the basal serum levels of Ctn (234.8 ± 188.4 vs. 44.4 ± 27.5, $p < 0.05$) and CEA (50.2 ± 76.7 vs. 7.4 ± 6.7, $p = 0.001$) of the patients with *RET* mutation were significantly higher than that those of patients without *RET* mutation (Table 2). Furthermore, though patients in both groups had a significant reduction of postoperative Ctn and CEA, patients with *RET* mutations showed a higher level of postoperative serum Ctn (49.8 ± 86.4 vs. 3.7 ± 2.2, $p = 0.001$) and CEA (13.2 ± 19.5 vs. 1.3 ± 1.6, $p < 0.05$) compared with patients without *RET* mutations (Table 3). Subgroup analysis between *RET* mutation sites and the serum levels of Ctn and CEA both pre- and postoperatively revealed that patients with Exon 11&13 mutations had the highest level of serum Ctn and CEA both pre- and postoperative, while patients with Exon 11 mutations had the lowest level of serum Ctn and CEA both pre- and postoperative (Tables 4 and 5).

Discussion

Although the incidence of MTC is relatively low, the risk of regional lymph node metastasis and distant metastasis is high and occurs early. Besides, thyroid stimulating hormone inhibition therapy and iodine-131 irradiation therapy had a minimal effect on MTC. Therefore, surgical treatment remains the most critical treatment regimen for patients with MTC. Total surgical resection and tumor staging are independent risk factors for the prognosis of MTC [14]. A previous study revealed that cervical lymph node metastasis is an important prognostic factor for MTC [6]. Patients with unilateral intrathyroidal tumors had lymph node metastases in 81% of central compartment dissections, 81% of ipsilateral lateral compartment dissections, and 44% of contralateral lateral compartment dissections. While in patients with bilateral intrathyroidal tumors,

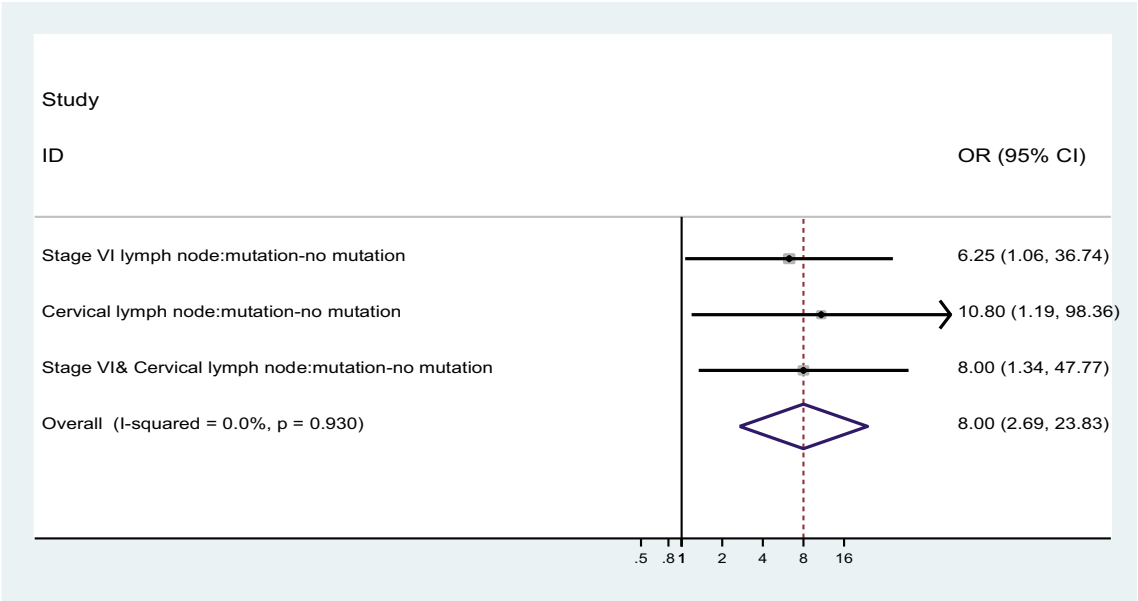

Fig. 3 Analysis of the risk of *RET* mutation in MTC patients with lymph node metastasis in the VI region and lateral cervical lymph nodes. MTC, medullary thyroid carcinoma

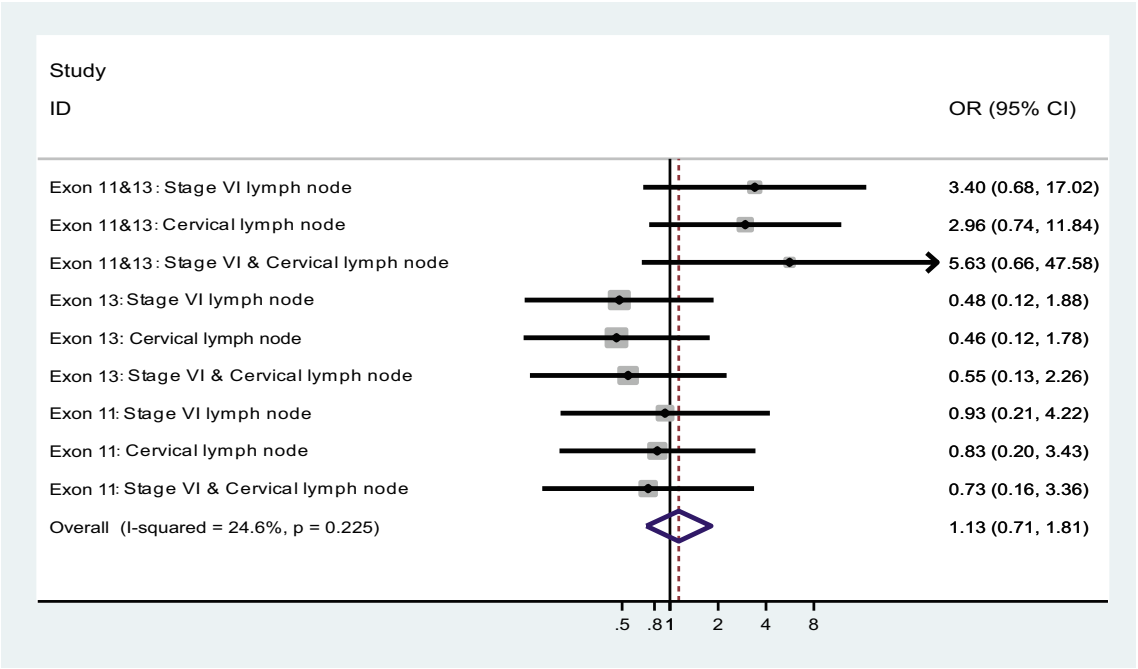

Fig. 4 Analysis of the risk of different *RET* mutation sites in the MTC patients with lymph node metastasis in the VI region and lateral cervical lymph nodes. MTC, medullary thyroid carcinoma

nodal metastases were present in 78% of central compartment dissections, 71% of lateral compartment dissections ipsilateral to the most massive intrathyroidal tumor, and 49% of lateral compartment dissections contralateral to the largest thyroid tumor. Machens et al. [15] reported that approximately 98% of MTC patients exhibit lateral cervical lymph node metastasis when the number of lymph nodes metastases at the central compartment area is ≥4, suggesting a correlation between cervical lateral lymph node metastasis and the level of lymph node metastasis in the central neck area in MTC patients.

Although the values of preoperative serum CEA and Ctn, ultrasound, and CT scan have significantly increased in preoperative diagnosis of cervical lymph node metastasis, the preoperative decision of lateral compartment lymph node dissections can be challenging to be made in MTC patients if cervical lymph node metastasis was not diagnosed before the operation. Diagnostic testing of the genotypes of RET before MTC treatment has aroused extensive attention. The current study attempted to evaluate the correlation between RET mutations, RET mutation sites, and lymph node metastasis in MTC to determine if any of these factors affect the prognosis of MTC patients, which can provide evidence for individualized, precise, radical, and accurate staging of surgical treatment for MTC patients.

RET proto-oncogene is located on autosome 10q11.2 and encodes a membrane-penetrating tyrosine kinase receptor that is an essential cellular surface molecule for signal transduction during cell proliferation and differentiation. *RET* mutation types are associated with the invasiveness of MTC. Mutation of different codons on the Ret protein determines different MTC phenotypes. Indeed, the degree of MTC invasion and malignancy can be determined by elucidating the different codons affected by *RET* mutation [10]. Studies about *RET* mutations and related codons are becoming more and more popular in this area. Existing research in MTC has predominantly focused on *RET* mutations in Exon 10, Exon 11, and Exon 13 [12, 16–18]. With more and more related studies, the possible value of preoperative genetic screening in predicting the resection range of prophylactic lateral compartment lymphadenectomy in patients with MTC might be detected.

Table 2 Comparison of the preoperative basal Ctn and CEA levels between the *Ret* mutation and non-mutated groups

	Ret mutation group $N = 42$	Non-mutated group $N = 7$	*t* value	*p*
Ctn (pg/mL)	234.8 ± 188.4	44.4 ± 27.5	6.167	0.000
CEA (ng/mL)	50.2 ± 76.7	7.4 ± 6.8	3.571	0.001

Ctn, calcitonin; *CEA*, carcinoembryonic antigen

Table 3 Comparison of the postoperative Ctn and CEA levels between the *Ret* mutation and non-mutated groups

	Ret mutation group $N = 42$	Non-mutated group $N = 7$	*t* value	*p*
Ctn (pg/mL)	49.8 ± 86.4	3.7 ± 2.2	3.450	0.001
CEA (ng/mL)	13.2 ± 19.5	1.3 ± 1.6	3.881	0.000

Ctn, calcitonin; *CEA*, carcinoembryonic antigen

Table 4 Comparison of the basal calcitonin and CEA levels at different *Ret* mutation sites

	Exon 11&13 $N = 19$	Exon 11 $N = 10$	Exon 13 $N = 11$	p_1	p_2	p_3
Ctn (pg/mL)	372.8 ± 176.2	66.6 ± 60.0	168.8 ± 117.1	0.001	0.049	0.023
CEA (ng/mL)	96.9 ± 95.4	8.6 ± 6.7	15.0 ± 9.5	0.000	0.003	0.065

Ctn, calcitonin; *CEA*, carcinoembryonic antigen

p_1: data from Exon 11 compared with data from Exon 11&13; p_2: data from Exon 13 compared with data from Exon 11&13; p_3: data from Exon 13 compared with data from Exon 11

The present study showed that *RET* mutation and mutation location are associated with cervical lymph node metastasis in MTC. MTC patients with *RET* mutation had a significantly higher rate of lymph node metastasis than those without *RET* mutation in both central and lateral compartments. Among all the *RET* mutations observed, Exon 11&13 mutations showed the most significant correlation with lymph node metastasis, and the association with the OR value was significantly higher than that in other mutation sites or single point mutations.

RET mutation sites have been used to evaluate the invasion risk and prognosis of MTC and classify the disease into four stages [10]: (1) low risk—the least invasiveness caused by *RET* mutation, involving the *RET* mutation at codons 768, 790, 791, 804, and 891; (2) moderate risk—relatively low invasiveness caused by *RET* mutation, involving *RET* mutation at codons 609, 611, 618, 620, and 630; (3) high risk—relatively high invasiveness caused by *RET* mutation involving mutations at codon 634; and (4) extremely high risk—the highest invasiveness caused by *RET* mutation, involving mutations at codons 883 and 918. The higher the stage is, the higher the risk of lymph node metastasis and distant metastasis can occur.

In this study, *RET* mutations in Exon 10, Exon 11, and Exon 13 were associated with MTC invasion mostly. *RET* mutations in the cysteine-rich region encoded by Exon 11 impede the formation of intermolecular disulfide bonds, which allows free cysteine residues to form intermolecular bonds that result in the generation of aberrant homodimers. The resultant homodimers spontaneously activate the phosphorylation of intracellular tyrosine residues, thereby activating downstream signaling pathways and promoting the activation of kinases as well as the transformation of proto-oncogenes [19]. The most common mutation associated with these phenomena was codon 634 in Exon 11 [20], suggesting that this mutation is a high-risk invasive factor. Indeed, codon 634 in Exon 11 was also found to be a simple *RET* mutation site in MTC patients, suggesting that associated mutations are essential in hereditary MTC cases [21]. A relatively high prevalence of related mutations at the codon 634 in Exon 11 has also been found in European patients with MTC [22].

RET mutations in Exon 13 have been shown to affect catalytic properties and activate substrate phosphorylation of RET kinase. These mutations have also been shown to facilitate inappropriate substrate phosphorylation by affecting the normal signal transduction pathway of RET. The latter phenomenon can result in disease occurrence [19], with the major mutation sites associated with this exon being located at codons 768, 790, and 791 [17, 18].

RET mutations are predominantly located at codons 609, 611, 618, and 620 in Exon 10; these codons encode for the extracellular domain. These mutations result in changes to cysteine residues, thereby leading to ligand-independent activation of the RET receptor protein following auto-phosphorylation and cross-phosphorylation and simultaneous initiation of the activation state [23]. Several studies have revealed a relatively high prevalence of Exon 10 mutations in MTC patients in India [24, 25]. However, in this study, the *RET* mutation rate in Exon 10 was relatively low, which might be due to the sample size and ethnic diversity, as *RET* mutations were shown to exhibit radial differences in the population analyzed.

Absence of exon 16 mutations is an unusual finding as the latter is common in sporadic MTCs [12, 13]. We have checked the results and confirmed that no mistakes were made in our study. This may be resulted from limited case number or may due to the different race we had analyzed, as we only included Han people of China. Further study should be done about this question.

Table 5 Comparison of the postoperative calcitonin and CEA levels at different *Ret* mutation sites

	Exon 11&13 $N = 19$	Exon 11 $N = 10$	Exon 13 $N = 11$	p_1	p_2	p_3
Ctn (pg/mL)	116.4 ± 26.7	3.6 ± 1.2	30.5 ± 9.2	0.000	0.021	0.009
CEA (ng/mL)	24.7 ± 24.3	2.1 ± 2.0	5.6 ± 4.6	0.000	0.011	0.035

Ctn, calcitonin; *CEA*, carcinoembryonic antigen

p_1: data from Exon 11 compared with data from Exon 11&13; p_2: data from Exon 13 compared with data from Exon 11&13; p_3: data from Exon 13 compared with data from Exon 11

Besides, the results of this study showed that the basal serum levels of Ctn and CEA in MTC patients with *RET* mutations were significantly higher than those in MTC patients without *RET* mutations. Also, MTC patients with *RET* mutations in Exon 11&13 had significantly higher basal and postoperative serum levels of calcitonin and CEA than MTC patients with *RET* mutations at other exons. Serum Ctn and CEA levels show significant value to MTC screening, preoperative diagnosis, prediction of prognosis, follow-up monitoring, and evaluation of therapeutic efficacies. Moreover, many studies have shown that the basal serum levels of Ctn are positively associated with tumor size and staging of MTC [26, 27]. Previous studies have also shown that patients with higher basal serum level of Ctn also exhibit a higher rate of lymph node metastasis in MTC, so basal serum level of Ctn was one predictor for prophylactic lateral cervical lymphadenectomy [3]. Detection of postoperative serum level of Ctn during follow-up also helps to the monitoring of potential of recurrence and metastasis. Saltiki et al. [27] reported a postoperative serum level of calcitonin higher than 4.65 ng/L as a predictor of lesion recrudescence. The elevation of postoperative CEA levels might also help in the detection of lesion residues, recurrence, and metastasis [28]. In this study, *RET* mutations, especially in Exon 11&13, were related to higher Ctn and CEA levels pre- and postoperatively, which suggested the potential presence of hidden lesions, recurrence, and metastasis, and indicated poor prognosis for the MTC patients.

Based on the results from the current study and previous reports, we think that *RET* mutations, especially in Exon 11&13, are associated with lymph node metastasis in cervical lymph nodes and invasiveness of MTC. Particular attention should be paid to patients with *RET* mutations in Exon 11&13 detected preoperatively, which might be a predictor of prophylactic lateral cervical lymphadenectomy. While in patients without RET mutation, preoperative "doubling times" for calcitonin and CEA was still considered to be the predictor for prophylactic lateral cervical lymphadenectomy, as recommended in Revised American Thyroid Association Guidelines [3].

However, one limitation of our study was that we did not test RAS variants in our cases, as a large percentage of RET-negative tumors are positive for HRAS and KRAS mutations, which could be the target of targeted therapy. RAS mutation can count for about 48% of benign follicular adenomas, 57% of follicular thyroid cancer, and 21% of papillary thyroid cancer, as mentioned in a previous study [29]. Shortage of research funding prevented us from testing for RAS variants in this study. Further study is needed to include RAS mutation as an arm of this research.

Conclusions

Our results revealed that MTC patients with *RET* mutations, especially in Exon 11&13, had a high risk of cervical lymph node metastasis. Besides, *RET* mutations in Exon 11&13 was related to a higher level of serum Ctn and CEA both pre- and postoperatively, suggesting mutation in Exon 11&13 was associated with a poor prognosis in patients with MTC. Based on those above, MTC patients with *RET* mutations at Exon 11&13 may be considered to receive prophylactic ipsilateral cervical lymphadenectomy.

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Compliance with Ethical Standards

This study was approved by the local Ethics Committee. All patients provided written informed consent before participating in the study.

Conflict of Interest The authors declare that they have no conflicts of interest.

References

1. Dequanter D, et al. Medullary thyroid cancer: surgical results and prognostic factors. Rev Med Liege 2010, 65:450-452.
2. Dackiw AP, et al. The surgical management of medullary thyroid cancer. Otolaryngol Clin North Am 2010, 43:365-374.
3. Wells, SA, et al. Revised american thyroid association guidelines for the management of medullary thyroid carcinoma. Thyroid 2015, *25*(6):567-610.
4. Leggett MD, et al. Prognostic value of lymph node yield and metastatic lymph node ratio in medullary thyroid carcinoma. Ann Surg Oncol 2008, 15:2493-2499.
5. Pinto AE, et al. Familial *vs* sporadic papillary thyroid carcinoma: a matched-case comparative study showing similar clinical/prognostic behavior. Eur J Endocrinol 2014, 170:321-327.
6. Alevizaki M, et al. Medullary thyroid carcinoma: the influence of policy changing in clinical characteristics and disease progression. Eur J Endocrinol 2012, 167:799-808.
7. Milan SA, et al. Current management of medullary thyroid cancer. Minerva Chir 2010, 65:27-37.
8. Moley JF, et al. Patterns of nodal metastases in palpable Medullary thyroid carcinoma: recommendations for extent of nodedi section. AnnSurg 1999, 229:880-887.
9. Mulligan LM. RET revisited: expanding the oncogenic portfolio. Nat Rev Cancer 2014, 14:173-186.
10. Kloos RT, et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid 2009, 19:565-612.
11. Morrison PJ, et al. Genetic aspects of familial thyroid cancer. Oncologist 2009, 14:571-577.
12. Kitamura Y, et al. Novel germ line RET proto-oncogene mutations associated with medullary thyroid carcinoma (MTC): mutation analysis in Japanese patients with MTC. Oncogene 1997, 14: 3103-3106.
13. Koch CA, et al. Allelic imbalance of the mutant and wild-type RET allele in MEN 2A-associated medullary thyroid carcinoma. Oncogene 2001, 20:7809-7811.
14. Roman S, et al. Prognosis of medullary thyroid carcinoma. Cancer 2006, 107:2134-2142.
15. Machens A, et al. Prediction of lateral lymph node metastases in medullary thyroid cancer. Br J Surgery 2008, 95:586-591.

16. Sanso GE, et al. Very early detection of RET proto-oncogene mutation is crucial for preventive thyroidectomy in multiple endocrine neoplasia type 2 children: presence of C-cell malignant disease in asymptomatic carriers. Cancer 2002, 94:323-330.
17. Lombardo F, et al. Familial medullary thyroid carcinoma: clinical variability and low aggressiveness associated with RET mutation at codon 804. J Clin Endocrinol Metab 2002, 87:1674-1680.
18. Berndt I, et al. A new hot spot for mutations in the ret protooncogene causing familial medullary thyroid carcinoma and multiple endocrine neoplasia type 2A. J Clin Endocrinol Metab 1998, 83:770-774.
19. Raue F, et al. Genotype-phenotype relationship in multiple endocrine neoplasia type 2. Implications for clinical management. Hormones (Athens) 2009, 8:23-28.
20. Heshmati HM, et al. Genetic testing in medullary thyroid carcinoma syndromes: mutation types and clinical significance. Mayo Clin Proc 1997, 72:430-436.
21. Sarika HL, et al. Genetic screening of patients with medullary thyroid cancer in a referral center in Greece during the past two decades. Eur J Endocrinol 2015, 172:501-509.
22. Machens A, et al. Molecular epidemiology of multiple endocrine neoplasia 2: implications for RET screening in the new millennium. Eur J Endocrinol 2013, 168:307-314.
23. Machens A, et al. Constitutive RET tyrosine kinase activation in hereditary medullary thyroid cancer: clinical opportunities. J Intern Med 2009, 266:114-125.
24. Menon M, et al. RET mutation status in medullary thyroid cancer (MTC) patients and the significance of genetic screening for mutations in their immediate relatives–a preliminary report. Indian J Pathol Microbiol 2005, 48:161-165.
25. Sharma BP, et al. RET gene mutations and polymorphisms in medullary thyroid carcinomas in Indian patients. J Biosci 2011, 36:603-611.
26. Bao-Dong G, et al. Experimental study on the mutation rate of exon 16 codon 918 from RET proto-oncogene in different human races with sporadic medullary thyroid carcinoma. Chinese Journal of Experimental Surgery 2006, 23(3):319-321.
27. Saltiki K, et al. Small medullary thyroid carcinoma: post-operative calcitonin rather than tumour size predicts disease persistence and progression. Eur J Endocrinol 2014, 171:117-126.
28. Faggiano A, et al. A decrease of calcitonin serum concentrations less than 50 percent 30 minutes after thyroid surgery suggests incomplete C-cell tumor tissue removal. J Clin Endocrinol Metab 2010, 95: E32-E36.