Long-term Clinicopathological Features of a Family with Multiple Endocrine Neoplasia Type 2A Caused by C634R *RET* Gene Mutation

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Abstract

Type 2 multiple endocrine neoplasia (MEN2A) is a variant of hereditary medullary thyroid carcinoma (MTC). MEN2A is characterized by the presence of the following: MTC, hyperparathyroidism, and pheochromocytoma (PHEO). The pathogenesis includes *RET* proto-oncogene mutation; the most frequently observed mutation is in exon 11 codon 634. We report pedigree of a large Indian family involving three generations including 21 members with MEN2A, in whom *RET* mutation status was determined. We then analyzed their clinical follow-up details, with a median duration of follow-up of 60 months (range: 9–276 months). Calcitonin (Ctn) levels were routinely checked during the follow-up. The index case was found to carry p.C634R mutation involving exon 11 of the *RET* gene. *RET* mutation was positive in 12 members in the family (12/21, i.e., 57%), was negative in 7 patients, and was not tested in 2 patients, as they were not available for the genetic test. Thirteen were clinically affected with MTC and 10 members had PHEO. At the last follow-up, the median Ctn level was 14.3 pg/mL (range: 2–12655 pg/mL). Four patients developed lymph nodal recurrence during follow-up, for which they underwent re-operations with median duration to recurrence being 48 months (range: 9–156 months). We highlight in this article that early diagnosis, adequate surgery, and appropriate genetic counseling with genetic screening are essential to improve the outcome of persons with MTC. Every case of MTC should be seen as familial or index case of hereditary MTC unless otherwise *RET* mutation excludes it.

Keywords: *C634R, medullary thyroid carcinoma, RET gene mutation, type 2 multiple endocrine neoplasia*

Introduction

Medullary thyroid carcinoma (MTC) is a tumor arising from the parafollicular (C) cells of the thyroid gland. It accounts for 5%–10% of thyroid cancers. MTC tumors secrete calcitonin (Ctn).[1] MTC occurs in a hereditary form (HMTC, 25%) or in a sporadic form (SMTC, 75%). HMTC occurs as part of type 2 multiple endocrine neoplasia (MEN2A) syndromes, MEN2A and MEN2B. MEN2A is characterized by the presence of the following components: MTC (100%), hyperparathyroidism (HPTH, 25%), and pheochromocytoma (PHEO, 50%). It has four variants: (1) classical MEN2A (represented by the uniform presence of MTC and the less frequent occurrence of PHEO or HPTH or both); (2) MEN2A with cutaneous lichen amyloidosis (CLA); (3) MEN2A with Hirschsprung's disease; and (4) FMTC (FMTC is characterized by the presence of a *RET* germline mutation in families with MTC [or single individuals with MTC and no family history of MTC] who develop neither PHEOs nor HPTH). MEN2B is characterized by the presence of MTC, PHEO, mucosal neuromas, and marfanoid body habitus.[1] The *RET* (Rearranged during Transfection) proto-oncogene is located on chromosome 10q11.2, which encodes a single-pass transmembrane receptor of the tyrosine kinase family. SMTC and HMTC variants (MEN2A and MEN2B) are defined according to the absence/presence of germline *RET* mutations, coexistent PHEO, and/or other associated features or first-degree relatives affected by MTC.[1] On the basis of *RET* codon mutations, current American Thyroid Association (ATA) risk categories for HMTC are subdivided as follows:[1] (i) highest risk: patients with MEN2B and *RET* codon M918T mutation; (ii) high risk (H): patients with *RET* codon C634 and A883F mutations; and (iii) moderate risk:

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patients with HMTC and *RET* codon mutations other than M918T, C634, and A883F. In this study, we report pedigree involving three generations including 21 members of a large Indian family with MEN2A, in whom *RET* mutations were analyzed. Twelve of them were mutation positive in codon p.C634R involving exon 11 of the *RET* gene. Of the RET-positive mutation members, 11 were clinically affected with MTC and underwent total thyroidectomy (TT) and 9 of them had PHEO and underwent adrenalectomy. Seven were *RET* mutation negative. We want to highlight in this article, the importance of detailed early evaluation, genetic counseling and long-term follow-up of MTC patients, extensive familial screening, *RET* gene mutation analysis, and prophylactic thyroidectomies before the clinical disease sets in.

Patients Details

Index case (A) was a 47-year-old female, who presented with multinodular goiter for 12 months. Her fine-needle aspiration cytology results were positive for MTC, Bethesda 6 category. She underwent TT and central compartment clearance (TT + CCC) in 1994. In the 12th year of initial diagnosis of MTC, she had signs and symptoms of PHEO. She underwent left adrenalectomy for PHEO along with splenectomy in 2006. Furthermore, the patient developed lymph node recurrence a year later and underwent lymph node dissection in 2007. In her long follow-up of 23 years, her Ctn progressed from 4863 pg/mL in 2007 to 12655 pg/mL in 2018. The patient also underwent 68Ga-DOTANOC positron-emission tomography/computed tomography (PET/CT) in 2011 where it showed Somatostatin Receptor (SSTR) expressing lesions in the right supraclavicular lymph node and a lytic lesion in C4 vertebra with no uptake. Unfortunately, on follow-up 68Ga-DOTANOC PET/CT in 2017, the patient developed the progressive disease with the development of liver and multiple new skeletal site metastases. The patient received thyroxine replacement and also completed 6 cycles of 177 Lu-PRRT. She was the eldest of her siblings, with 5 younger sisters and 1 younger brother. She was married for 19 years and had two sons. On further evaluation, the patient revealed her family history that her deceased mother, four of her sisters, and her younger brother had thyroid surgeries for MTC [Figure 1 shows family pedigree of three generations showing 13 affected members [red] and 8 unaffected members [blue]. Index case [A] is indicated by black arrow]. Here, we inferred that the index patient's mother (C) with MTC would be *RET* gene mutation positive, as the father (B) did not have MTC. Her two sons were screened for MTC. Her elder son (J) was diagnosed with MTC and PHEO (presented with raised Ctn levels and 24-h urinary metanephrines) and underwent TT as well as left adrenalectomy in 2013 and was followed up for 5 years and was disease-free with the last Ctn value of 14.3 pg/mL. Her younger son (K) also was diagnosed with MTC during screening and subsequently underwent TT + CCC in 2013. Although he was initially diagnosed with Stage 1 disease, he eventually had to undergo twice redo lymph node dissections in 2014 and 2016. At the last follow-up after 5 years, he was disease-free with Ctn level of 2.38 pg/mL. All three were positive for *RET* gene mutation p.C634R.

Immediate younger sister (D), 45 years, was also operated for MTC and PHEO. However, we do not have details about this patient to analyze her clinical outcome. Her three children, first child, 20 years female (L), during the screening was detected to have raised catecholamines and was diagnosed with left sided PHEO. On further evaluation, patient was also diagnosed with MTC. She underwent TT + CCC and left adrenalectomy in 2013. She had lymph node recurrence after 17 months, for which she was re-operated. At the last follow-up at 5 years, she had persistent disease with raised Ctn of 471 pg/mL. The second child, 18 years female (M), was also diagnosed with MTC and PHEO during screening. She was operated for both in 2017. However, she had a follow-up for only

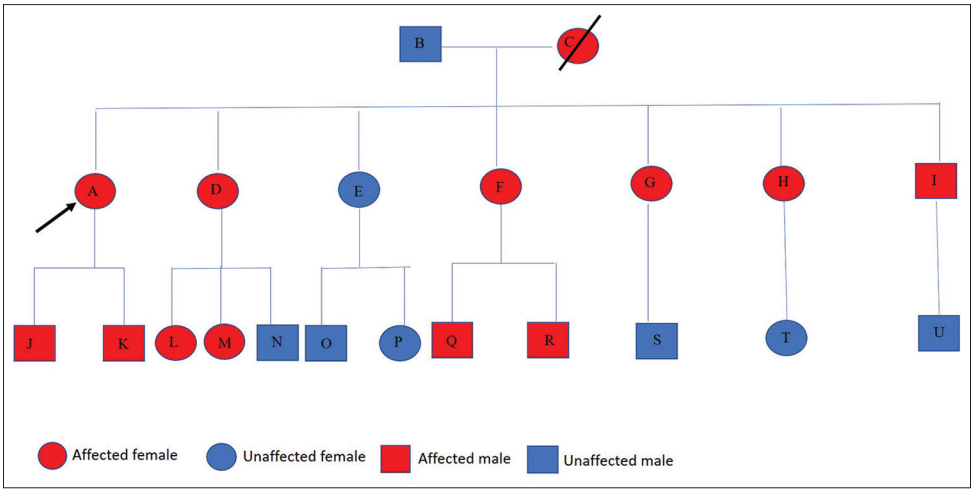

Figure 1: Family pedigree of three generations showing 13 affected members (red) and 8 unaffected members (blue). Index case (A) is indicated by black arrow

9 months, and her last Ctn value was 602 pg/mL. The youngest son, 12 years (N), was *RET* negative.

Index case's second younger sister, 42 years female €, and her son (O, 22 years male) and daughter (P, 20 years female) underwent genetic testing and were both *RET* negative.

The third younger sister (F) aged 38 years presented with hypertension and was diagnosed with left-sided PHEO. Her Ctn values were elevated (>220 pg/mL) and metanephrines (MA) and normetanephrines (NMA) were also elevated (NMA: 680; MA: 1756 (normal <800). She underwent left adrenalectomy in July 2013 and subsequently TT + CCC and right lymph node dissection in September 2013. Histopathology revealed MTC, 1 cm tumor with none of the eight lymph nodes dissected had MTC, hence Stage 1 disease. On her 5-year follow–up, she was disease-free and maintained a Ctn level <2 pg/mL. She had two sons, who were later screened because of a strong family history. They were diagnosed with MTC at the age of 18 years and 17 years. Both her sons underwent TT and LND in 2013 and 2017. While one was diagnosed with Stage 1 and the other was Stage 4A with positive lateral lymph nodes. Elder son (Q) was followed up for 4 years and is doing fine with normal Ctn levels of <2 pg/mL. Younger son [R] had a follow-up of 14 months and is also doing fine with the last Ctn level of <2 pg/mL. All three were positive for *RET* gene mutation C634R.

The fourth younger sister, a 36-year-old female (G), underwent screening in 2013, was found to have high Ctn levels of 131 pg/mL, and underwent left adrenalectomy followed by TT in 2013. Her Ctn levels were normalized postsurgery. She also received 1 cycle of 131I-MIBG therapy. During her last follow–up, she was disease-free with the last Ctn level of 6.3 pg/mL. She was positive for similar *RET* gene mutation (C634R). However, her only son [S], 17-year-old male, was screened negative for MTC, and *RET* gene mutation was also negative.

The fifth younger sister, a 35-year-oldfemale (H), was diagnosed with MTC in 2007, underwent TT + CCC, and then in 2013, underwent left adrenalectomy. At the last follow-up after 10 years, her Ctn value was 88 pg/mL; however, there was no evidence of disease on 18FDG PET/CT. Hence, she had a biochemical persistent disease with no structural disease. Her only daughter (T), aged 13 years, was *RET* gene mutation negative.

The only younger brother, the youngest of all siblings aged 33 years (I), was diagnosed with left-sided PHEO and MTC in 2013. He underwent left adrenalectomy and TT + Modified radical neck dissection (MRND) subsequently for the same. The patient had lymph node recurrence after 3 years, for which he was operated. At the last follow-up after 5 years, he had a high Ctn value of 2703 pg/mL with recent 68Ga-DOTANOC PET/CT showing SSTR expressing lesions in right thyroid bed, bilateral cervical lymph nodes, and right upper lobe lung nodule – suggesting of progressive disease. His only son [U], aged 7 years, however, was *RET* gene mutation negative.

Clinicopathological characteristics of all the family members are described in Table 1.

Screening of hotspot mutations in *RET* gene

Peripheral blood (5–10 ml) in ethylenediaminetetraacetic acid vial was collected from the index patient and her relatives for identification of *RET* proto-oncogene mutation. This procedure was performed in the Department of Nuclear Medicine, AIIMS, New Delhi. The DNA

Table 1: Clinicopathological characteristics of all the family members

Family members	Age (years)	Gender	*RET*	MTC	Pheochromocytoma	Surgery	Recurrence	Last Ctn (pg/ml)	Persistent disease	Alive/dead
A	48	Female	+	+	+	+	+	12,655	+	+
B	72	Male	NA	−	−	−	−	−	−	−
C	68	Female	+*	+	+	NA	NA	NA	NA	−
D	45	Female	+	+	+	NA	NA	NA	NA	+
F	38	Female	+	+	+	+	−	2	−	+
G	36	Female	+	+	+	+	−	6.3	−	+
H	35	Female	+	+	+	+	−	88.1	BCD	+
I	33	Male	+	+	+	+	+	2783	+	+
J	30	Male	+	+	+	+	−	14.3	−	+
K	25	Male	+	+	−	+	+	2.38	−	+
L	20	Female	+	+	+	+	+	471	+	+
M	18	Female	+	+	+	+	−	602	+	+
Q	18	Male	+	+	−	+	−	2	−	+
R	17	Male	+	+	−	+	−	2	−	+
E, N, O, P, S, T, U			−	−	−	−	−	−	−	+

*Assumed *RET* positive, though not tested. Ctn: Calcitonin, MTC: Medullary thyroid carcinoma, NA: Not available, BCD: Biochemical disease

was isolated using the modified salting-out method.[2] The quality check and quantification of the DNA was performed using NanoQuant spectrophotometer. On the basis of reported mutation in online database (http://www.hgmd.cf.ac.uk/ac/index.php), we have designed the primers flanking to the hotspot region, which covers essentially exons 10, 11, 13, 14, 15, and 16 [Table 2]. The initial denaturation temperature was 94°C for all the primer sets followed by 35 cycles of 94°C for 30 s – T_a-30 s – 72°C for 45 s [Table 1 for T_a]. The final extension was set as 72°C for 10 min. The sequencing reaction was done using either a forward primer or a reverse primer, and amplicons were run in the "Applied Biosystems™ 3500 Genetic Analyzer". The data were analyzed using the ABI sequencing analysis software version 5.1. Electropherogram shows wild type and mutation involving codon 634 (p.C634R) on exon 11 in Figure 2.

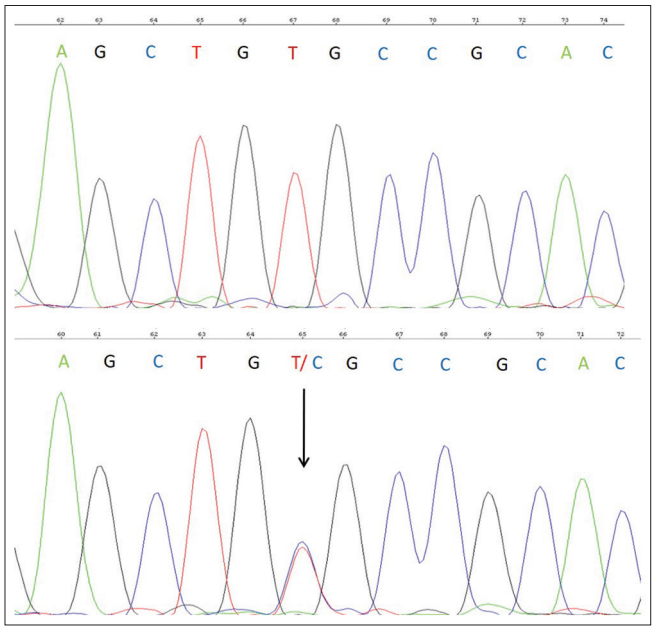

Figure 2: Electropherogram showing wild-type and mutation involving codon 634 (p.C634R) on exon 11 in type 2 multiple endocrine neoplasia patient

Results

In our study, the median age was 22 years (range: 4–68 years), with 10 males and 11 females (17 adults and 4 children). Most of the family members presented with a neck swelling, few were identified during screening, and few presented with symptoms of PHEO. *RET* mutation was detected in 12 members in the family, *RET* mutation was negative in 7 patients, and was not tested in 2 patients. Heterozygous missense variation c. 1900T >C (p.C634R) in exon 11 of the *RET* gene (chr10) was detected in all positive patients in the family. Thirteen patients developed MTC (61.9%) and 10 patients had PHEO (47.6%). Interestingly, none had developed parathyroid adenoma/CLA in this mega family. Ten patients who developed PHEO had concurrent MTC and were operated for both. Regarding surgery details, 10 patients of the family underwent TT and some form of lymph node dissections, 2 patients underwent TT alone, 7 patients were not operated, and details about surgery were not available for 2 patients. Stage of the disease was known in 11 patients: 3 patients each belonged to Stage1 and Stage 3, 2 patients belonged to Stage2, and 3 patients to stage 4A; none of the patients belonged to Stage 4B or C. Postoperatively, all our patients were on replacement doses of levothyroxine. Eight patients at the end of reporting had undergone adrenalectomy (unilateral or bilateral) for PHEO. Our study had a median duration of follow-up of 60 months (range: 9–276 months). Ctn levels were checked during the follow-up. At the last follow-up, the median Ctn level was 14.3 pg/mL (range: 2–12655 pg/mL). Four members developed lymph nodal recurrence during follow-up, for which they underwent reoperations. The median duration for recurrence was 48 months/4 years (range: 9–156 months). At the end of the study, only 2 members were dead, presumably due to age-related issues; 14 members were disease-free, 4 members had persistent disease, one had biochemical persistent disease – Biochemical disease (BCD), (defined as Ctn level >20 pg/mL – twice the higher normal range of 10 pg/mL), and details were not available for 2 members.

Table 2: Polymerase chain reaction primers and conditions for amplification of *RET* gene exons

Exon	Primers	Amplicon Size (bp)	Annealing temperature (°C) (T_a)
10	*RET*_10F: ACACTGCCCTGGAAATATGG	255	55.3
	*RET*_10R: TGCTGTTGAGACCTCTGTGG		
11	*RET*_11F: CAGAGCATACGCAGCCTGTA	372	53.8
	*RET*_11R: GGAGGGCAGGGGATCTTC		
13	*RET*_13F: CTGGTATGGTCATGGAAGGG	246	55.3
	*RET*_13R: GGAGAACAGGGCTGTATGGA		
14	*RET*_14F: AAGACCCAAGCTGCCTGAC	328	55.3
	*RET*_14R: GTGGTGGGTCAGGGTGTG		
15	*RET*_15F: CTGGTCACACCAGGCTGAG	346	56.7
	*RET*_15R: GGGTCAGAAAGATTTGGGGT		
16	*RET*_16F: TCTCCTTTACCCCTCCTTCC	177	55.3
	*RET*_16 R: CTGTAACCTCCACCCCAAGA		

Discussion

RET germline mutations in MEN2A can occur in codons 609, 611, 618, or 620 of exon 10 or codon 634 of exon 11.[3] *RET* codon 634 mutations are associated with a high penetrance of PHEO, which in one study increased with age, being 25% by age 30 years, 52% by age 50 years, and 88% by age 77 years.[4] There is a much lower penetrance of PHEO in patients with exon 10 *RET* codon mutations (609 [4%–26%], 611 [10%–25%], 618 [12%–23%], and 620 [13%–24%]).[5] The HPTH in patients with classical MEN2A is usually mild and associated with few if any symptoms. A *RET* codon 634 mutation is associated with a moderate penetrance of HPTH (up to 30%), and *RET* mutations in codons 609, 611, 618, and 620 are associated with a penetrance between 2% and 12% (62, 65). However, none of the patients in this family developed HPTH.[1] Ctn is a sensitive tumor marker and postoperative Ctn levels correlate well with the tumor size and stage of the disease.[6] Ctn levels of 150–400 pg/mL are seen in those with distant metastasis and extrathyroid extension.[7] *RET* gene mutations and polymorphisms in MTC in Indian patients were evaluated by Sharma and Saranath, in 140 samples, comprising 51 clinically diagnosed MTC patients, 39 family members of patients, and 50 normal individuals. The most frequently observed mutation was exon 11 codon 634 (60% patients), followed by mutations in exon 10 (20%), exon 16 (13.3%), and exon 14 (6.6%).[8] Their data as well as a similar report from the International *RET* Mutation Consortium state that codon 634 is commonly mutated in 85% MEN2A patients, with specific mutations TGC → CGC in 52% of patients, TGC → TAC in 26% of patients, and TGC → AGC in 18% of patients,[9,10] and therefore, indicates codon 634 as the "hot-spot codon" in MEN 2A patients. Rajan *et al*., in their cohort of MEN2-associated PHEO from the Indian population, concluded mutations in codon 634 as the most common cause.[11] Mahesh *et al*. reported p.C634S mutation involving exon 11 of the *RET* gene in six members of an Indian family spread over three generations.[12] Patients detected by screening have improved survival as compared to the index patients and patients with sporadic disease.[13] This underlines the importance of screening for *RET* mutations and initiating treatment at an early stage to improve outcomes and survival. Identification of mutations in asymptomatic carriers adds value to the existing data from India, especially since there is a paucity of information in this area. Relatives with *RET* mutations should undergo prophylactic total thyroidectomy. In one of the reports, authors recommended patients with genetic predisposition to have an early prophylactic thyroidectomy to prevent and cure MTC. The authors recommended a multidisciplinary team (endocrinology, clinical genetics, and pediatric surgery) to study, manage, and follow-up patients with MEN2A and their families.[14] Agarwal *et al*. in their case report first reported in India in 2012 where siblings underwent codon-oriented prophylactic total thyroidectomy based solely on genetic analysis for MEN2A syndrome.[15] For couples carrying this mutation, we would suggest that they screen their children for *RET* mutations as early as possible.[16] In this article, we stress the importance of better evaluation regarding MEN2A syndrome. Subsequently, when physicians examine a proven case of MTC, we urge them to look closely for PHEO and HPTH. Furthermore, we want to highlight the importance of familial screening in MTC patients which is lacking while evaluating the index cases and to compulsorily get the *RET* gene mutation analysis. Also, the need to address prophylactic thyroidectomy, as it plays an important part of management in these cases.

Conclusion

Early diagnosis, adequate surgery, and appropriate genetic counseling with genetic screening are essential to improve the outcome of persons with MTC. Every case of MTC should be seen as familial or index case of HMTC unless otherwise *RET* mutation excludes it.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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