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Case Report

Medullary Thyroid Carcinoma and Clinical Outcomes in Heterozygous Carriers of the *RET* K666N Germline Pathogenic Variant

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Abstract

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor of the thyroid parafollicular C-cells associated with activating mutations in the rearranged during transfection (*RET*) kinase proto-oncogene. We report the clinical outcomes of a family with a rare germline *RET* K666N pathogenic variant discovered incidentally by genetic testing performed for breast cancer risk stratification in an asymptomatic 24-year-old woman. Subsequent genetic testing identified the same pathogenic variant in her 21-year-old sister, 60-year-old father, and 84-year-old paternal grandmother. The proband and her sister had no biochemical or imaging evidence of MTC. The 60-year-old father had mildly elevated serum calcitonin and multiple thyroid nodules on ultrasound. Fine-needle aspirate thyroid biopsy cytology suggested MTC so he underwent total thyroidectomy. Surgical pathology demonstrated bilateral subcentimeter foci of MTC and C-cell hyperplasia. The 84-year-old grandmother was also found to have multiple thyroid nodules and elevated calcitonin but declined further evaluation. There was no biochemical evidence of other multiple endocrine neoplastic type 2 (MEN2)-associated tumors (ie, parathyroid adenoma, pheochromocytoma) in the family. These data, along with prior rare reports in the literature, suggest that monoallelic germline *RET* K666N pathogenic variants carry a risk of familial MTC that demonstrate age-dependent expressivity but low penetrance of other MEN2 tumors in affected individuals.

Key Words: medullary thyroid carcinoma, MEN2, *RET* K666N, germline, pathogenic variant

Abbreviations: ACR, American College of Radiology; ATA, American Thyroid Association; FNAB, fine-needle aspiration biopsy; GC, Genomic Classifier; IHC, immunohistochemical; MEN2, multiple endocrine neoplastic type 2; MTC, medullary thyroid carcinoma; *RET*, rearranged during transfection; US, ultrasound.

Introduction

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor of the thyroid parafollicular C-cells comprising 5% to 10% of thyroid cancers [1-3]. MTC arises from aberrant growth of thyroid parafollicular cells that normally produce calcitonin. Therefore, patients diagnosed with MTC can present with elevated serum calcitonin and a thyroid mass. The primary treatment for MTC is total thyroidectomy, with the use of local radiation or chemoablation or systemic targeted kinase therapies in some cases for advanced disease [1, 3]. While approximately 75% of MTC cases are sporadic, 25% are associated with multiple endocrine neoplastic type 2A (MEN 2A), MEN 2B, and familial medullary thyroid carcinoma syndromes [1, 2]. MEN2 is an autosomal dominant inherited syndrome caused by activating pathogenic variants in the *RET* proto-oncogene. Patients with MEN2 have an increased incidence of neuroendocrine tumors, such as MTC, pheochromocytoma, and parathyroid tumors [4].

An understanding of inherited *RET* pathogenic variants and associated clinical features is critical for guiding cancer screening [3, 4]. The missense *RET* K666N (c.1998G > T; p.Lys666Asn) germline variant is uncommonly described but has been associated with familial MTC; it has not yet been assigned a risk level for MTC by the American Thyroid Association (ATA) [3]. The largest case series of this germline *RET* K666N pathogenic variant describes 24 carriers from 8 families, including 9 individuals with MTC and 2 with evidence of C-cell disease (C-cell hyperplasia and/or elevated serum calcitonin) [5]. Here, we report the clinical outcomes of 4 related individuals carrying a monoallelic *RET* K666N variant and highlight the finding of isolated MTC in a pattern suggestive of age-dependent expressivity and incomplete penetrance of MEN2.

Case Presentation

A healthy 24-year-old woman sought publicly available genetic testing with the Integrated BRACAnalysis with MyRisk

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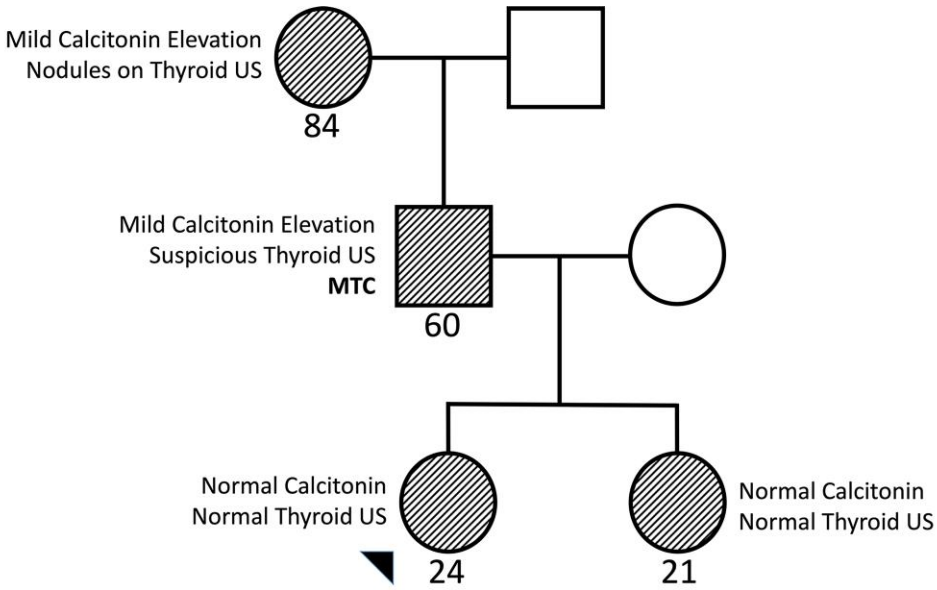

Figure 1. Pedigree showing carrier status of *RET* K666N pathogenic variant and known clinical manifestations in a single family. Arrow indicates proband individual.

Hereditary Cancer Test for breast cancer risk stratification. Results from this commercial assay identified a *RET* c.1998G > T (K666N) germline pathogenic variant. Subsequent clinical genetic testing performed in 3 family members identified that her 21-year-old sister, 60-year-old father, and 84-year-old paternal grandmother were also heterozygous carriers of the same *RET* K666N variant (Fig. 1).

Diagnostic Assessment

Based on the association of *RET* pathogenic variants with the familial MEN2 syndrome, all 4 family members were screened for hyperparathyroidism, pheochromocytoma, and MTC [1]. Serum calcium, parathyroid hormone, and plasma fractionated metanephrine testing results in all individuals were within normal limits. Both 21- and 24-year-old daughters had undetectable serum calcitonin levels and a normal thyroid ultrasound (US). The 84-year-old grandmother had an elevated serum calcitonin of 37 pg/mL (37 ng/L) (0.0-7.5 pg/mL; 0.0-7.5 ng/L) and evidence of several thyroid nodules on US. Because of the grandmother's age and other medical comorbidities, she elected to not pursue further work-up for MTC.

The 60-year-old father had mild elevation of serum calcitonin levels of 13 pg/mL (13 ng/L) (0.0-7.5 pg/mL; 0.0-7.5 ng/L). Thyroid US of the father revealed 3 subcentimeter, intrathyroidal, solid hypoechoic nodules, classified as American College of Radiology (ACR) TI-RADS [6] category 4 in the right lobe (Fig. 2), as well as multiple bilateral cystic/spongiform nodules but no abnormal cervical lymph nodes. Fine-needle aspiration (FNA) of the 5-mm right superior lateral nodule (see Fig. 2, nodule 1) showed benign Bethesda II cytology. The ill-defined 4-mm right central nodule (see Fig. 2, nodule 2) had FNA cytopathology category of Bethesda V, suspicious for malignancy. Cytologic findings were notable atypical cells containing plasmacytoid morphology with mild-to-moderate nuclear atypia, coarse chromatin, and irregular nuclear membranes (Fig. 3A and 3B). Molecular testing of this specimen with ThyroSeq V3 Genomic Classifier (GC), which is reflexively sent for indeterminate cytology at our center, showed that the nodule had strong expression of calcitonin and chromogranin A genes concerning for MTC. Immunohistochemical (IHC) studies confirmed positive staining for synaptophysin (Fig. 3C), a marker for neuroendocrine tumors such as MTC [7]. Thus, preoperative studies of this nodule were consistent with a diagnosis of MTC. Interestingly, ThyroSeq V3 GC identified no pathogenic variants of *RET* or *RAS* genes in this specimen. Finally, cytology from the 6-mm right inferior posterolateral nodule (see Fig. 2, nodule 3) showed atypia of undetermined significance, consistent with Bethesda III, with poorly delineated cells with overlapping nuclei, inconspicuous nucleoli, and delicate cytoplasm with a background sheet of benign thyroid follicular cells, scant colloid, and blood. Reflex ThyroSeq V3 GC molecular testing of nodule 3 FNA reported negative for malignancy with no pathologic gene variants identified.

Treatment

The 60-year-old father elected to undergo total thyroidectomy for a presumed MTC. Preoperative thyroid US showed no evidence of extrathyroidal extension of malignancy and no abnormal cervical lymphadenopathy (levels II-VI). Surgical pathology of the thyroid demonstrated multiple, bilateral microscopic foci of MTC and C-cell hyperplasia (Fig. 4A). The largest focus of MTC was a right-lobe, 0.8-cm intrathyroidal mass; there was no lymphatic invasion, angioinvasion, or extrathyroidal extension, consistent with American Joint Committee on Cancer 8th edition pathologic stage pT1aNxMx. IHC staining for MTC-associated proteins, INSM1 (insulinoma-associated protein 1) and calcitonin, were performed on the surgical specimen and confirmed the diagnosis of MTC (Fig. 4B and 4C). In addition, Ki-67 staining for proliferating cells showed 2% positivity (Fig. 4D).

Outcome and Follow-up

The father's 4-week postoperative calcitonin level was undetectable and remained undetectable 6 months later. His

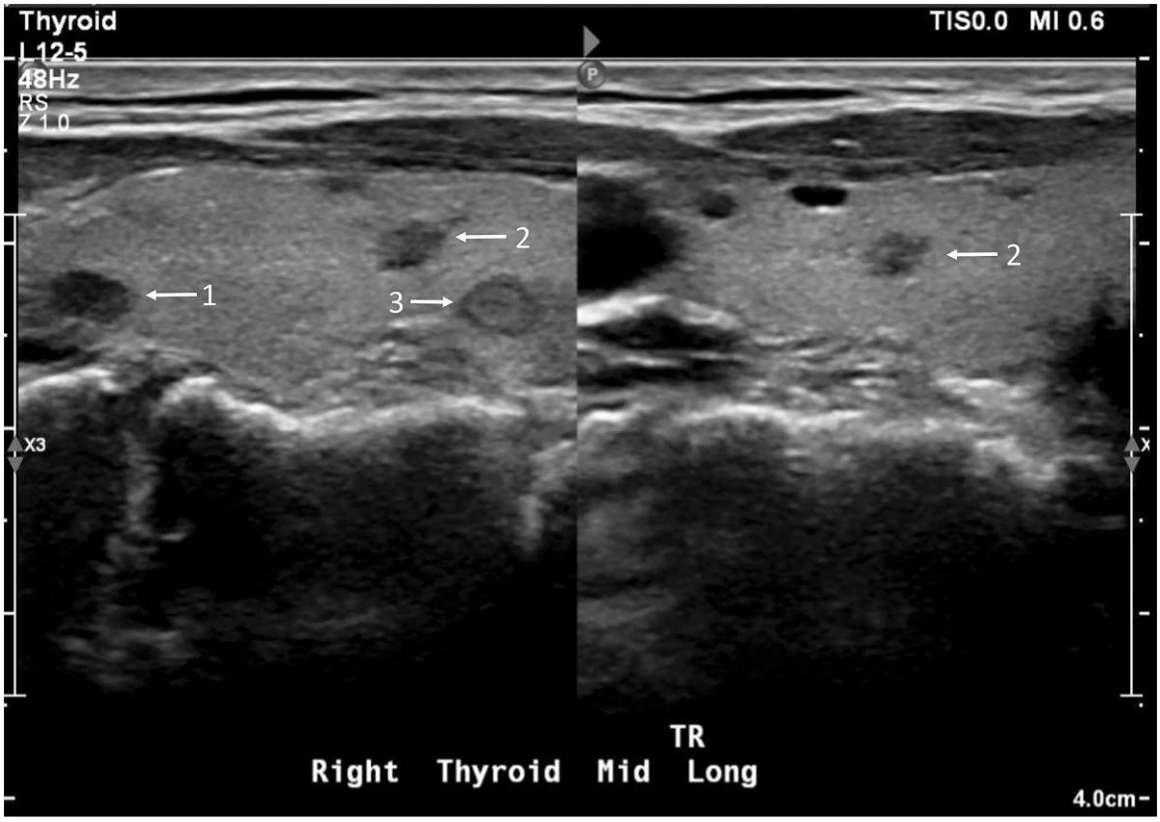

Figure 2. Thyroid nodules identified by screening thyroid ultrasound in a *RET* K666N carrier. Sagittal (left) and transverse (right) views of thyroid nodules in the right thyroid lobe. Thyroid ultrasound imaging in the 60-year-old father, a *RET* K666N carrier, showed multiple solid, hypoechoic thyroid nodules that underwent fine-needle aspirate biopsy. Nodule 2 was positive for medullary thyroid carcinoma.

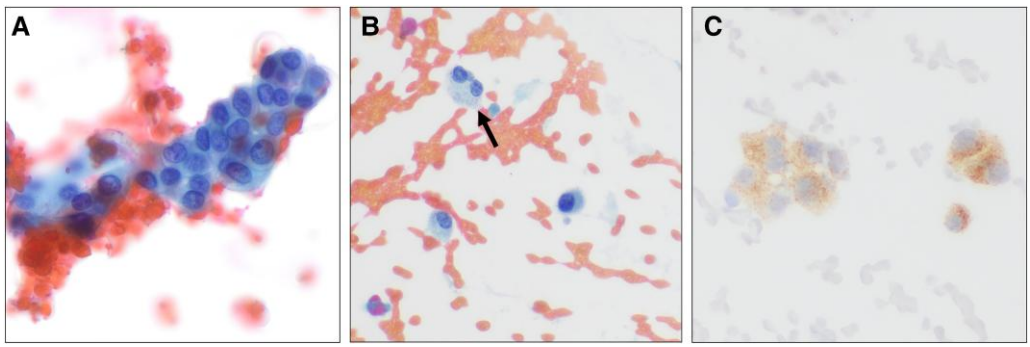

Figure 3. Thyroid fine-needle aspirate cytology from a thyroid nodule in a 60-year-old man with *RET* K666N heterozygous pathogenic variant, ultimately found to be a medullary thyroid carcinoma. A, Small cluster of uniform cells with round to oval nuclei and coarse, stippled chromatin (Papanicolaou stain, ×600). B, Singly dispersed cells with round, eccentric nuclei and abundant granular cytoplasm. Arrow indicates rare binucleated cells (Papanicolaou, ×400). C, Immunocytochemical stain for synaptophysin at ×400 showing diffuse, granular cytoplasmic staining of tumor cells.

postoperative hypothyroidism was treated with levothyroxine thyroid hormone replacement. Annual screening calcitonin testing was recommended to the family members carrying the *RET* K666N pathogenic variant. Genetic counseling was recommended for first-degree relatives of all carriers in the family.

Discussion

We report the occurrence of MTC in a family carrying a monoallelic K666N *RET* proto-oncogene variant. Consistent with prior reports of this rare pathogenic variant [5, 8], MTC presented in mid- and later-life decades in affected heterozygous individuals without additional features of MEN2 syndrome. This disease pattern is suggestive of age-dependent expressivity and perhaps incomplete penetrance. Interestingly, this particular missense mutation affects the intracellular juxtaglomerular domain of the *RET* tyrosine kinase receptor to cause ligand-independent kinase activation of the *RET* protein (Fig. 5) [2, 8]. Indeed, when transfected into NIH3T3 mouse embryonic fibroblast cells, this pathogenic variant was sufficient to drive malignant transformation [8]. In silico protein

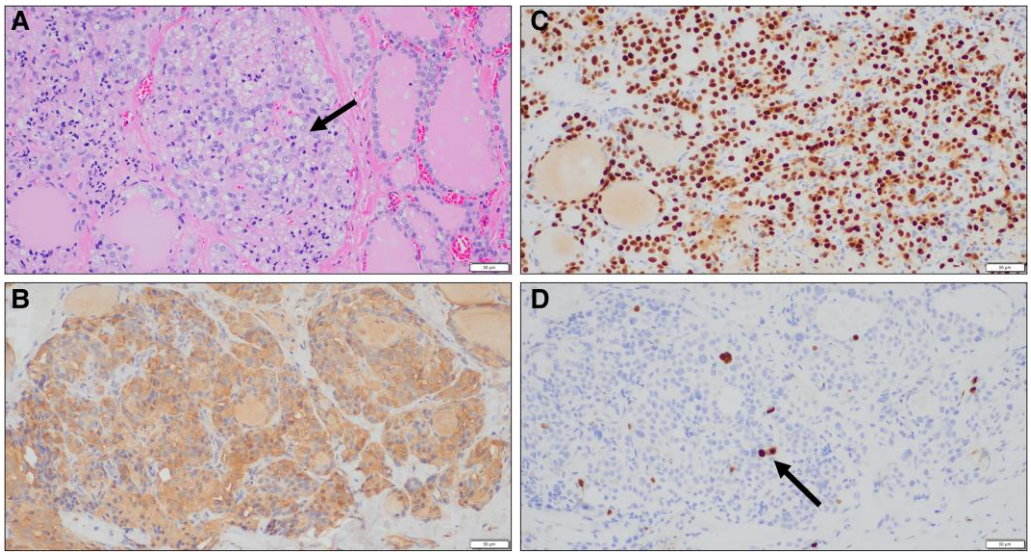

Figure 4. Surgical histopathology of medullary thyroid carcinoma (MTC) in a 60-year-old man with a heterozygous *RET* K666N pathogenic variant. A, Hematoxylin and eosin staining of thyroid nodule. Arrow indicates area of MTC, with normal thyroid follicular tissue seen to the right. Nests of neoplastic cells demonstrating small, round nuclei with finely stippled chromatin and granular to slightly amphophilic cytoplasm. B, Immunohistochemical (IHC) staining for calcitonin showing diffuse cytoplasmic staining of MTC cells. C, IHC staining for INSM1 (insulinoma-associated protein 1) showing strong nuclear staining throughout the MTC, supporting neuroendocrine differentiation. D, IHC staining for proliferation marker Ki-67. Arrow indicates area of positive nuclear stain. (Original magnification ×200 for all images.)

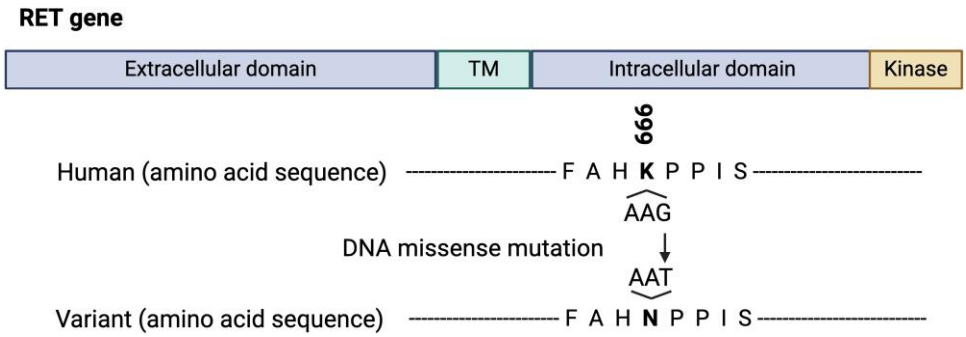

Figure 5. Schematic of the *RET* (rearranged during transfection) gene and K666N missense variant identified in this family. The family described here presented with a pathogenic variant in the intracellular region of the *RET* kinase proto-oncogene at position 1998 G > T. This missense variant results in an amino acid change from lysine (K) to asparagine (N) at position 666 and kinase activation. TM, transmembrane. Created in Biorender.

structure analysis suggests that the K666N pathogenic variant alters the protein transmembrane α-helix to induce an active conformation of the kinase domain. However, because this pathogenic variant does not directly alter the catalytic region of the kinase, the resulting change in kinase activity may be mild. This indirect mechanism of kinase activation may explain its association with more indolent MTC and C-cell disease that presents later in life (decreased expressivity) and the apparent incomplete penetrance for MEN2 compared to other known activating *RET* gene variants.

Four prior reports have identified the *RET* K666N variant in association with MTC. In the largest case series, Xu et al [5] observed that the *RET* K666N variant appears to have an autosomal dominant pattern of inheritance with low MTC penetrance. In this series, 9 individuals presented with isolated MTC across 8 unrelated families (diagnosed at ages 22, 23, 33, 49, 51, 59, 55, 64, and 70 years) and 2 families had 1 individual with elevated calcitonin and C-cell hyperplasia. The other 8 confirmed K666N carriers across the 8 families, ranging in age from 20 to 80 years, had no clinical evidence of MTC and 5 remaining variant carriers, aged 5 to 92 years, did not obtain clinical screening for MTC. Another case of a heterozygous *RET* K666N pathogenic variant was found in a 65-year-old man who presented with isolated MTC, though no additional family information was available [8]. In our series of 4 related heterozygous carriers, MTC was identified at age 60 in the father while younger family members (aged 21 and 24 years) had no current evidence of MTC or MEN2-associated tumors. While we cannot confirm the presence of MTC in the 84-year-old grandmother, the presence of multiple solid thyroid nodules and elevated serum calcitonin is suggestive of C-cell disease. Taken together, these data suggest that the monoallelic *RET* K666N variant is indeed a pathogenic driver of MTC that displays increasing expressivity with age and incomplete penetrance.

While we found no evidence of other MEN2 disease in our case series, data regarding an association of *RET* K666N with pheochromocytoma are mixed. Jaber et al [9] reported the co-occurrence of MTC and bilateral pheochromocytomas in a 59-year-old homozygous carrier of the same *RET* K666N

variant. Both adult children of this individual were found to be heterozygous carriers of the *RET* K666N pathogenic variant and had either isolated MTC or C-cell hyperplasia without extrathyroidal manifestations of MEN2 syndrome. The proband was negative for other pathogenic variants associated with pheochromocytomas. In another case, MTC, unilateral pheochromocytoma, and primary hyperparathyroidism were found in a 40-year-old woman with monoallelic *RET* K666N [10]. Thus, homozygous carriers of *RET* K666N variants or heterozygous carriers who present with MTC at a younger age may be more likely to have other MEN2-associated tumors, suggestive of a possible gene dosage effect and variable expressivity.

Given the incomplete penetrance of this pathogenic variant, it is important to consider the balance of early MTC detection with the burden of unnecessary testing in potential carriers. In our family, serum calcitonin evaluation and baseline thyroid ultrasound were recommended for all carriers and led to the early identification and successful surgical treatment of MTC. However, we note that while thyroid US facilitates early detection of MTC, it may in fact be more likely to identify benign thyroid nodules that nonetheless lead to FNA and additional testing. Therefore, we recommend a patient-centered approach that considers the age-dependent expressivity of this pathogenic variant and uses clinical examination and changes in serum calcitonin levels to guide the need for thyroid US and FNAB. Similarly, while pheochromocytoma and parathyroid adenoma appear to occur rarely in these individuals, baseline biochemical screening for plasma fractionated metanephrines and calcium is reasonable to exclude these diagnoses.

When thyroid US is pursued in *RET* K666N carriers, the increased risk of MTC should be considered in the interpretation of results. Sonographic risk stratifications systems (eg, ACR TI-RADS, ATA) have primarily been validated for malignancy prediction in papillary thyroid cancer, not MTC. Indeed, thyroid ultrasonography in the asymptomatic father showed 3 subcentimeter solid thyroid nodules classified as ACR TI-RADS 4, and below the usual size threshold for FNA biopsy (FNAB). However, the presence of a germline pathogenic variant with increased risk for MTC and mildly elevated calcitonin drove the clinical recommendation to pursue FNAB of these nodules and confirmed a diagnosis of MTC. This is consistent with current recommendations from the ATA [11] that cytologic evaluation of nodules by FNAB may be considered at lower size thresholds in individuals at higher risk of thyroid malignancy, such as those with germline pathogenic variants.

Another evolving area of clinical care is the expanding use of genetic testing. The index patient in our series learned she was a carrier of this pathogenic *RET* variant through a commercially available genetic test. In addition, thyroid nodule evaluation increasingly incorporates molecular testing for preoperative risk of malignancy stratification [12]. While not their intended use, such tests report the presence of thyroid cancer–associated gene mutations, fusions, and copy number alterations, and therefore are increasingly used to inform decisions about cancer prognosis and management. Interestingly, the ThyroSeq V3 test GC [13], which was performed as a reflex test for 2 nodules in the father with MTC, does not evaluate codon 666 of the *RET* gene and therefore did not identify a *RET* pathogenic variant in these specimens. Thus, it is important to note that molecular testing should not supplant more comprehensive genetic testing in patients with MTC to identify germline or somatic *RET* mutations and is not recommended over routine cytology and immunostaining to confirm a diagnosis of MTC.

In conclusion, we describe a family with a monoallelic *RET* K666N germline pathogenic variant associated with isolated MTC and C-cell hyperplasia in later decades of life and with incomplete penetrance. Our case series further highlights the evolving use of commercially available genetic testing and how it may be incorporated into screening for familial genetic cancer syndromes.

Learning Points

- The *RET* K666N missense pathogenic variant is associated with ligand-independent RET kinase activation and increased risk of MTC.
- Heterozygous carriers of the *RET* K666N pathogenic variant present with isolated MTC in the mid to later decades of life, usually without extrathyroidal manifestations of MEN2 syndrome. Data for homozygous carriers are limited, but these individuals may be more likely to present with other MEN2-associated disease, including pheochromocytoma.
- In *RET* K666N carriers, screening with serum calcitonin measurement and thyroid ultrasound can be considered to identify MTC in early-stage disease.
- Multidisciplinary medical and surgical teams should employ shared decision-making with patients carrying the *RET* K666N pathogenic variant that incorporates patient preference, understanding of disease penetrance, and aggressiveness of disease within a family.
- Adjusted size thresholds for thyroid nodule evaluation by FNAB in individuals with germline pathogenic variants associated with thyroid malignancy is recommended and consistent with current ATA guidelines.

Contributors

A.T.Y. analyzed the data and wrote the manuscript. T.H.K. obtained images and provided interpretation of histopathology. E.M.P. assisted with manuscript and figure preparation. M.W.Y. was responsible for the patient's surgery. S.E.J. is an expert consultant in genetics. M.G.L. was involved in the diagnosis and management of the patient, manuscript editing, and overall study oversight. All authors reviewed and approved the final manuscript.

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Disclosures

All authors have no conflicts of interest to declare.

Informed Patient Consent for Publication

Signed informed consent obtained directly from the patients.

Data Availability Statement

Original data generated and analyzed for this case report are included in this published article.

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