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# Medullary Thyroid Carcinoma Associated with Germline *RET*$^{K666N}$ Mutation

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***Background:*** Multiple endocrine neoplasia type 2 is an autosomal dominant inherited syndrome caused by activating mutations in the *RET* proto-oncogene. The *RET*$^{K666N}$ DNA variant was previously reported in two isolated medullary thyroid carcinoma (MTC) cases, but no family studies are available, and its oncogenic significance remains unknown.
***Methods:*** The clinical features, genetic data, and family information of eight index MTC patients with a germline *RET*$^{K666N}$ variant were assessed.
***Results:*** Four probands presented with MTC and extensive nodal metastasis, one with biopsy-confirmed distant metastasis. Two additional probands presented with localized disease. However, nodal status was not available. Of the final two probands, one had an incidental 1.5 mm MTC and C-cell hyperplasia uncovered after surgery for papillary thyroid carcinoma, and one had two foci of MTC (largest dimension 2.3 cm) detected after surgery for dysphagia. Genetic screening identified 16 additional family members carrying the K666N variant (aged 5–90 years), 11 of whom have documented evaluation for MTC. Of these, only two were found to have elevated basal serum calcitonin upon screening, and the remaining patients had calcitonin levels within the reference range. One patient who elected to have a thyroidectomy at 70 years of age was confirmed to have MTC. The other subject, 57 years old, elected surveillance. Four prophylactic thyroidectomies were performed, with one case of C-cell hyperplasia at 20 years and three cases that revealed normal pathology at ages 21, 30, and 30 years. None of the K666N DNA variant carriers had evidence of primary hyperparathyroidism or pheochromocytoma.
***Conclusions:*** From this case series, the largest such experience to date, it is concluded that the *RET*$^{K666N}$ variant is likely pathogenic and associated with low penetrance of MTC. However, the findings are insufficient to define its pathogenicity clearly and make firm recommendations for screening and treatment. Given the potential benefit associated with early detection of aberrant C-cell growth, and the noninvasive nature of genetic testing, ''at risk'' individuals should be screened, and if the K666N variant is identified, they should be managed using a personalized screening approach for detection of MTC.

**Keywords:** medullary thyroid cancer, multiple endocrine neoplasia type 2A, *RET* proto-oncogene, K666N mutation

## Introduction

Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant cancer syndrome comprised of two primary subtypes: MEN2A and MEN2B. Since the initial identification of activating mutations in the *RET* proto-oncogene as the cause of MEN2, a total of 155 variants have been described as potentially disease associated, with 79 of uncertain significance (1). The association of *RET* genotype with MEN2 phenotype has clearly established medullary thyroid carcinoma (MTC) risk for the most common *RET* mutations (2). As a result, risk levels associated with specific *RET* mutations help to guide the management of presymptomatic *RET* mutation carriers, including the timing for

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early thyroidectomy (3). For the infrequently observed *RET* mutations or variants of uncertain significance (VUS), making a clinical decision can be challenging (4). These are variants for which there is insufficient evidence to determine whether they are benign or pathogenic, and therefore appropriate screening and treatment for MTC is unclear (5).

The missense *RET*$^{K666N}$ variant (c.1998G>T; p.Lys666Asn) has been reported in two cases with relatively indolent MTC (6,7). However, the absence of large family studies confirming segregation of disease with this DNA variant and the lack of published cases of K666N carriers with the classical MEN2A phenotype raises questions about its oncogenic significance. Support for a possible oncogenic role is provided by the demonstration that K666N has transforming activity in NIH3T3 cells (6), and four additional codon 666 mutations (K666E, K666M, K666delinsNS, and K666R) have been associated with MTC (8–13). This study reports eight isolated kindreds harboring the *RET*$^{K666N}$ DNA variant.

## Materials and Methods

### *Patients*

Index patients from eight families were registrants of the Genetics of Endocrine Neoplasia Registry (GENR), and all data were retrospectively reviewed under approved Institutional Review Board protocols. The index patients from families 1 and 3–6 were evaluated for persistent or metastatic disease at the University of Texas MD Anderson Cancer Center (MDACC) after primary surgery was performed elsewhere. The index patient from family 2 was initially diagnosed and treated at MDACC. The index patient for family 7 was initially treated at Duke University Medical Center (DUMC). The index patient from family 8 was treated at an outside facility. All index patients were seen by a genetic counselor, who assessed family history and confirmed genetic testing information. Two family members with the K666N variant underwent prophylactic thyroidectomy, one each at MDACC (family 1) and DUMC (family 7). The remainder of the pedigrees were generated utilizing information obtained from index case interviews and affected family members participation in the GENR. Clinical evidence of C-cell disease was defined by either elevated basal serum calcitonin (Ctn) measurement or C-cell hyperplasia (CCH) or MTC on final pathology. Where noted, screening for primary hyperparathyroidism (PHPT) and pheochromocytoma (PHEO) was performed by measuring serum total calcium, intact PTH levels and plasma free fractionated metanephrines. Cancer staging was performed utilizing the American Joint Committee on Cancer (AJCC) version 7 (14).

### *Screening for* RET *variants*

All index cases had germline *RET* mutation screening performed on DNA extracted from leukocytes in a CLIA-certified laboratory. For the index case in family 3, all *RET* exons and intron/exon boundaries were screened. For the other index cases, bidirectional sequencing analysis was performed on exons 10, 11, 13, 14, 15, and 16 and intron/exon boundaries. At-risk family members where noted (Fig. 1) were tested specifically for the K666N variant.

## Results

Information is presented on 24 individuals from eight apparently unrelated families carrying a germline *RET*$^{K666N}$ mutation (Fig. 1). Nine individuals had MTC, including all eight index cases and one first-degree relative of an index case. Of the 15 remaining gene carriers, only two had evidence of C-cell disease: one with CCH, and one with an elevated serum Ctn level. There was no clinical evidence of MTC in eight patients, and four gene carriers and one obligate carrier had not been evaluated for MTC (Fig. 1).

### *Family 1*

The index case is a 55-year-old woman (II-2) diagnosed with MTC at the age of 22 years who was treated by total thyroidectomy and lymph node dissection. The medical record reported bilateral positive lymph nodes, but her initial pathology report was not available for confirmation of compartmental location of the metastatic nodes (AJCC Stage TXN1M0). She subsequently received external beam radiotherapy to the neck region at another facility. During more than 30 years of follow-up, Ctn has remained mildly elevated (12.2–25.2 pg/mL), but she has had no radiographic evidence of structural disease. Initial *RET* testing performed in 2004 was negative, but more comprehensive testing performed in 2011 revealed a germline *RET*$^{K666N}$ variant. Her daughter (III-1), a K666N carrier, had an undetectable Ctn but elected to undergo a prophylactic total thyroidectomy at 20 years of age. Pathology revealed a single 0.5 mm focus of CCH. Both the proband and her daughter have had negative screening tests for PHPT and PHEO.

### *Family 2*

The index case is a 34-year-old woman (III-2) with a 10-year history of enlarged right neck lymph nodes. A fine-needle biopsy eventually confirmed to be metastatic MTC. Her preoperative Ctn was 1988 pg/mL; her serum carcinoembryonic antigen (CEA) was 35.8 ng/ml. A lytic lesion of her sternum was biopsied and found to be metastatic MTC. Radiologic staging identified no other sites of metastasis. Preoperative genetic testing identified a germline *RET*$^{K666N}$ variant. She had no biochemical evidence of PHPT, no clinical evidence of PHEO, and no adrenal lesions on imaging. At the age of 33 years, she underwent total thyroidectomy, central neck dissection, and bilateral lateral selective neck dissection to include levels IIA, IIB, III, IV, and V, with pathology demonstrating a unifocal MTC (0.6 cm) with confirmed metastasis to 30/39 nodes with extracapsular extension present (AJCC Stage T1N1bM1). No CCH was identified. The sternal metastasis doubled in size over seven months and was treated with external beam radiotherapy. She currently has stable disease, with a Ctn of 300 pg/mL and a CEA of 4.3 ng/mL 20 months after her initial surgery. The patient's 57-year-old mother (II-3), a K666N carrier, was found to have a Ctn of 26 pg/mL (normal <5 pg/mL) and a 3 mm calcification within the right thyroid lobe. She has declined surgery and chosen to be followed with observation. She has no clinical evidence of PHPT or PHEO. The patient's 80-year-old maternal grandmother (I-2) was reported to be positive for the K666N DNA variant. She had a mildly elevated Ctn of 7 pg/mL, which was considered to be within the

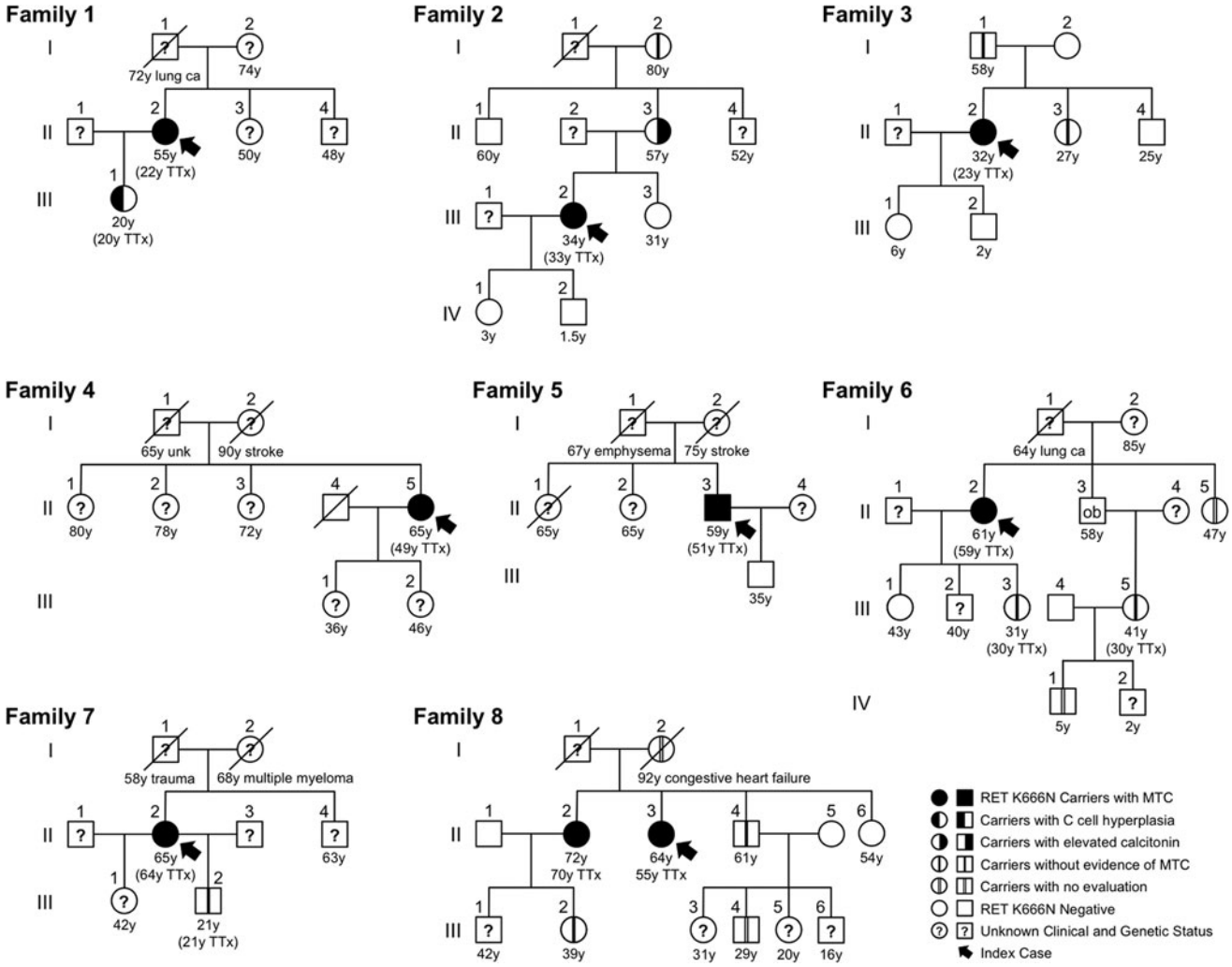


**FIG. 1.** Pedigrees with genotypes and clinical presentations for eight families with a germline *RET*$^{K666N}$.

reference range because of her chronic kidney disease (15). Four additional family members who tested negative for the presence of K666N mutation were found to have normal Ctn levels.

### *Family 3*

The index case is a 32-year-old (II-2) woman diagnosed with MTC at the age of 23 years, when she was noted to have a palpable neck mass. Her preoperative Ctn was 28,000 pg/mL; her CEA was 51 ng/mL. As per the operative note, she underwent total thyroidectomy, central neck dissection, and bilateral modified lateral neck dissections. Pathology revealed a left unifocal 1.2 cm MTC with extrathyroidal extension, and 17/37 lymph nodes were positive for metastasis, with the largest focus of 5.1 cm with extracapsular extension (AJCC Stage T3N1bMX). Her postoperative Ctn decreased to 1000 pg/mL. Follow-up was not routinely performed. Six years after initial diagnosis, her Ctn was 12,541 pg/mL, and her CEA was 12.7 ng/mL, without clear radiographic evidence of disease based on positron emission tomography imaging. *RET* genetic testing with sequencing of all the exons and intron/exon boundaries identified the presence of the K666N variant. At presentation at MDACC, nine years after initial surgery, her Ctn was 14,450 pg/mL, with a CEA of 27.2 ng/mL, without evidence of neck disease. However, multiple small liver and spine metastases were identified on appropriate cross-sectional images. Her distant disease and tumor markers have remained stable to date (11 years since her initial diagnosis). The index patient had negative screening tests for PHPT and PHEO. The patient's 58-year-old father (I-1), a K666N carrier, was reported to have a normal Ctn and normal neck ultrasound. Her 27-year-old sister (II-3), also a K666N carrier, was reported to have a normal Ctn and a benign appearing thyroid nodule, and is currently being observed. The patient's mother, younger brother, and her two children were all reported to be negative for the K666N variant and have no clinical symptoms of MTC.

### *Family 4*

The index case is a 65-year-old woman (II-5) who had MTC first treated at the age of 49 years. Pathology reported bilateral multifocal MTC with C-cell hyperplasia. The

dominant tumor was located in the left lobe and measured 3 cm. No lymph nodes were noted in the pathology report, and her AJCC Stage was T2NXM0. She required a left lateral modified neck dissection for recurrent disease at the age of 64 years, from which 2/13 lymph nodes were positive for metastasis with no extracapsular extension. Her Ctn has remained in the 16–22 pg/mL range since this surgery. The patient was ultimately found to have a germline *RET*$^{K666N}$ variant. She had negative screening tests for PHPT and PHEO. Her sister (II-3) was reported to have PHPT in her 50s and underwent parathyroidectomy. Her father died of unknown disease at the age of 65 years, but prior to that, he had persistent diarrhea. Her mother died of a stroke. Genetic testing was not available for any of her family members.

### *Family 5*

The index case is a 59-year-old man (II-3) who underwent a total thyroidectomy, central neck dissection, and bilateral lateral neck dissection for papillary thyroid cancer (stage IVa) at the age of 51 years. Pathology reviewed at MDACC identified a previously unrecognized 1.5 mm MTC and C-cell hyperplasia in the background thyroid. No lymph nodes contained metastatic MTC for an AJCC stage of T1N0M0, and his Ctn has remained negative more than 10 years after initial MTC diagnosis. The patient's genetic testing was positive for the germline *RET*$^{K666N}$ variant. The index patient had negative evaluation for PHPT and PHEO. His son (III-1) is healthy and negative for the K666N variant. The proband's father died from emphysema at 67 years of age, and his mother died at the age of 75 years with a history of stroke. The proband has two sisters: one with a history of papillary thyroid carcinoma, and one deceased from kidney failure. None underwent germline *RET* testing.

### *Family 6*

The index case is a 61-year-old female (II-2) who underwent, per the operative report, a total thyroidectomy and right modified radical neck dissection at the age of 59 years after MTC was identified during surgery for dysphagia. Two foci of MTC were found within the right lobe, the largest with a dimension of 2.3 cm in diameter with extrathyroidal extension. There was no evidence of CCH, and 1/23 lymph nodes was positive for metastatic disease with extracapsular extension (AJCC Stage T3N1bM0). She presented to MDACC two years after surgery for evaluation of an elevated Ctn of 67.4 pg/mL. No structural disease was identified with imaging of the neck, chest, and abdomen. She was found to carry the germline *RET*$^{K666N}$ variant and had negative evaluations for PHPT and PHEO. The patient's daughter and niece were both identified to have the germline K666N variant and elected to have prophylactic thyroidectomies at the age of 30 years, despite normal Ctn levels. Pathology reported the absence of MTC. However, no information was available regarding CCH. Three other K666N carriers, including a brother who is an obligate carrier (II-3), a 47-year-old sister (II-5), and a five-year-old child (IV-1), did not have a clinical evaluation for MTC.

### *Family 7*

The index case is a 65-year-old female (II-2) who underwent a total thyroidectomy, bilateral central neck dissection, and right lateral selective neck dissection to include levels IIA, IIB, III, IV, and V at the age of 64 years after an asymptomatic right thyroid nodule was incidentally discovered on a cervical computed tomography scan performed after the patient sustained a fall. At presentation, her Ctn was 973 pg/mL, and her CEA was 22.3 ng/mL. There was no family history of endocrinopathy, but routine *RET* testing revealed the K666N variant. She had negative evaluations for PHPT and PHEO. Surgical pathology revealed a 2.2 cm unencapsulated unifocal MTC with 17 central compartment and 26 lateral compartment lymph nodes negative for carcinoma (staged T2N0M0). Postoperatively, she experienced normalization of her serum tumor markers, with an undetectable Ctn. She has two adult children. A son tested positive for the K666N variant and underwent prophylactic thyroidectomy at the age of 20 years. Biochemical testing preoperatively was unremarkable for PHPT and PHEO, and Ctn and CEA levels were within normal limits. Surgical pathology revealed no evidence for MTC or C-cell hyperplasia. A 63-year old brother and 42-year old daughter of the index case have not yet been tested.

### *Family 8*

The index case is a 64-year-old female (II-3) who initially underwent a right thyroid lobectomy at the age of 55 years after an asymptomatic thyroid nodule was identified on carotid artery screening and was biopsied as an indeterminate lesion. Pathology showed a 1.1 cm MTC; no lymph nodes were removed (AJCC Stage T1NXM0). This surgery was followed by a completion thyroidectomy within six months; lymph node dissection was not performed. No evidence of MTC or CCH was found within this lobe on final pathology. She underwent genetic testing at her request a year later and was found to have a germline *RET*$^{K666N}$ variant. She continues to have an undetectable Ctn on yearly follow-up and no evidence of PHPT or PHEO. Her mother underwent genetic testing at the age of 90 years and was found to carry the variant. She did not undergo screening for the presence of MTC, PHEO, or PHPT, and died at the age of 92 years of congestive heart failure. The index case's eldest sister (II-2) also had the *RET*$^{K666N}$ variant, had a screening Ctn of 9 pg/mL, and underwent thyroidectomy at 70 years of age. The final pathology showed a 0.4 cm MTC in the left thyroid lobe, as well as bilateral C-cell hyperplasia and benign lymphoid tissue. She had no clinical evidence of PHEO or PHPT on screening and an undetectable serum Ctn value two years following thyroidectomy. Patient II-2 has two adult children: a son who has not been tested, and a 39-year-old daughter who possesses the variant and on initial screening had an undetectable Ctn value. The index case also has a younger brother (II-4), aged 61 years old, who carries the *RET*$^{K666N}$ variant. His initial screening identified a small thyroid nodule on ultrasound and an undetectable serum Ctn level. He has four children, one of whom underwent genetic testing at 29 years of age and was found to carry the variant; he has not undergone further evaluation.

### RET *variant analysis*

The index cases in families 2 and 3 presented with distant metastasis at relatively young ages, and both had other *RET* variants reported in addition to K666N. For index case 2, genetic testing revealed the benign sequence variant, intron 14 c.2608-24G>A, which has been reported to occur more frequently in patients with elevated Ctn levels or sporadic MTC (16). *RET* genetic testing in index case 3, which included sequencing of all *RET* exons, also identified a second variant V412M in exon 6. The relevance of this amino acid change is unclear. It was found reported in a single case of Hirschsprung disease (17), and not in >120,000 sampled chromosomes (rs746979700) nor in MEN2A (18). No additional polymorphisms were reported for the remaining index cases. Because of the use of confirmation testing on family members, no information is available beyond the presence or absence of the K666N variant. A CLIA-certified, institutionally generated somatic tumor standardized hotspot mutation analysis of 50 genes (appendix 1A of Meric-Bernstam *et al.*) (19) that included *RET* and *RAS* found no mutations for index cases from families 2 and 3.

## Discussion

The $RET^{K666N}$ variant has been reported in two cases of MTC in the literature (6,7), and conflicting interpretations exist regarding its pathogenicity (20). One reported case was a patient diagnosed with MTC at the age of 65 years. His stage was T2N0M0, and he was in remission after surgery (6). The other case, presented in abstract form, was diagnosed with a T2N0M0 MTC at the age of 54 years and PHPT reported to be due to left inferior parathyroid hyperplasia (7). Neither case had family members available for genetic testing. The present study described eight additional probands diagnosed with a $RET^{K666N}$ germline variant and MTC, representing the largest case series reported in the literature to date. Additionally, it has been possible to provide genotype–phenotype data on 10 first-degree relatives and six more distantly related family members with the $RET^{K666N}$ variant in an effort to determine better the penetrance of the MTC phenotype associated with this DNA change. In one family (family 8), two individuals were identified with MTC, and in two others (families 1 and 2), individuals were identified with elevated Ctn and C-cell hyperplasia, known precursors of hereditary MTC. Of note, eight confirmed K666N carriers, ranging in age from 20 to 80 years, had no clinical evidence of MTC. The five remaining variant carriers (age range 5–92 years) were reported as asymptomatic for MTC, although they had not undergone clinical evaluation for the disease. Despite the presence of multiple carriers without evidence of MTC, the occurrence of MTC and its precursor lesions in close relatives seen in three families is unlikely to occur by chance alone. Collectively, these findings are most consistent with an autosomal dominant pattern of inheritance with a low disease penetrance.

The pattern of phenotypic variability (relatively broad age range of MTC diagnosis of 22–70 years) and the breadth of clinical presentation (seen in Table 1) is consistent with patterns observed with other uncommon *RET* germline mutations associated with MEN2, such as V804M and A883F (21–23). Further support for a hereditary role is that some of these eight cases exhibited MTC multifocality and/or CCH within their pathology specimen; such characteristics are often associated with hereditary MTC. However, this is by no means an exclusive relationship, as sporadic MTC specimens also have been found to harbor these features, though to a much lesser extent.

Several factors have led to supporting a pathogenic role for the $RET^{K666N}$ variant. First, codon 666 of the *RET* gene encodes an evolutionarily conserved residue in the intracellular juxta-membrane domain of *RET*, and PolyPhen computational analysis predicts the change to be damaging (24). Second, in addition to the missense variant K666N, other variants at this codon have been reported to be associated with MTC (8–13). They include a missense mutation K666E in four families, a complex mutation leading to K666N followed by a serine insertion (K666delinNS) in two families: a K666M in one family coexisting with the *MEN1* mutation IVS4 + 1G>T, and finally a K666R in one isolated case. Table 2 summarizes the clinical features of the family members carrying DNA variants at codon 666, including the current series. Finally, previous *in vitro* studies have demonstrated that K666N, K666E, and K666delinsNS have increased oncogenic potential compared with wild-type *RET*, and that K666E activity is further enhanced by the presence of the exon 11 polymorphism G691S (6). Nevertheless, only K666E has been well recognized as a mutation with moderate risk of aggressive MTC (3).

However, support also exists for a nonpathogenic role of K666N. First, of five established methods for analyzing

Table 1. Characterstics of Patients with Germline $RET^{K666N}$ Variants with Medullary Thyroid Carcinoma

| *Family* | *Individual* | *Age at MTC diagnosis* | *Stage of MTC at diagnosis* | *Multifocality on pathology* | *CCH on pathology* | *Developed regional recurrence* | *Developed distant metastasis* | *Length of follow-up (months)* |
|---|---|---|---|---|---|---|---|---|
| 1 | II-2 | 22 | TXN1M0 | NR | NR | No | No | 397 |
| 2 | III-2 | 33 | T1N1bM1 | No | No | No | No | 23 |
| 3 | II-2 | 23 | T3N1bMX | No | NR | No | Yes | 118 |
| 4 | II-5 | 49 | T2NXM0 | Yes | Yes | Yes | No | 202 |
| 5 | II-3 | 51 | T1N0M0 | No | Yes | No | No | 132 |
| 6 | II-2 | 59 | T3N1bM0 | Yes | No | No | No | 26 |
| 7 | II-2 | 64 | T2N0M0 | No | No | No | No | 12 |
| 8 | II-3 | 55 | T2NXM0 | No | No | No | No | 108 |
| 8 | II-2 | 70 | T1N0M0 | No | Yes | No | No | 24 |

MTC, medullary thyroid carcinoma; NR, not reported; CCH, C-cell hyperplasia.

Table 2. Clinical Characteristics of Families with a *RET* Variant at Codon 666

| *RET variant* | *Family no.* | *Carrier no.* | *No. of carriers with MTC* | *Youngest age at diagnosis of MTC (years)* | *No. of carriers with C-cell hyperplasia*[b] | *No of carriers with elevated Ctn only* | *No. of carriers with PHPT* | *No. of carriers with PHEO* | *No. of carriers with NOD* |
|---|---|---|---|---|---|---|---|---|---|
| K666N[a,6,7] | 10 | 26 | 11 | 22 | 1 | 1 | 1 | 0 | 13 |
| K666E[8,9] | 4 | 15 | 5 | 35 | 2 | 2 | 0 | 1 | 6 |
| K666delinNS[10,11] | 2 | 6 | 3 | 12 | 1 | 1 | 0 | 0 | 1 |
| K666M[12] | 1 | 8 | 2[c] | 45 | 3 | 0 | 3[c] | 0 | 2 |
| K666R[13] | 1 | 1 | 1 | 38 | 0 | 0 | 0 | 0 | 0 |

Reference numbers indicated in superscripts.
[a]Current series and two reported cases.
[b]In the absence of MTC.
[c]Carriers with this clinical manifestation concurrently carried a *MEN1* mutation IVS4 + 1G>T.
PHPT, primary hyperparathyroidism; PHEO, pheochromocytoma; NOD, no evidence of disease.

mutation severity, including those measuring a *RET* gene-specific Bayes probability classification, amino acid substitution penalties, structural disruption, sequence homology, and neural nets, only one classified K666N as pathogenic (24). Second, it is important to consider the example of the *RET*$^{Y791F}$ variant that was identified in MTC patients during extended gene analysis that included exon 13. Initially reported as a potential pathogenic mutation, subsequent studies have demonstrated that *RET*$^{Y791F}$ alone does not increase the risk of MTC (25). Database analysis revealed that Y791F is a rare genetic polymorphism instead of a disease-causing mutation (25). In the North American cohort (GO-ESP), dbSNP currently reports K666N minor allele frequency as 0.0005 (ss342296546), which is the highest among *RET* codon 666 variants, K666E is 0.00008 (ss342296545) with other MTC-associated codon 666 variants not found in the GO-ESP cohort (26). Additionally, unlike the *RET*$^{K666E}$ variant, which has been associated with PHEO (9), none of the germline *RET*$^{K666N}$ carriers had evidence of PHPT or PHEO, though again this does not rule out hereditary disease. Further, the role of somatic mutations in tumor oncogenicity should be considered. Unfortunately, tumor screening for the most common mutations associated with sporadic MTC, *RET*$^{M918T}$, and H-, K-*RAS*, was only performed on 2/8 patients, and it was negative. Finally, it cannot be overlooked that none of the parents of our index cases, with ages ranging from 58 to 92 years, had clinically apparent MTC.

Reporting variants of unknown or suspected pathogenicity is a complicated decision in which the clinician is balancing the creation of baseless anxiety with failing to provide potentially important information to patients and their families (27). Particularly when the variant is found in the same location as pathogenic mutations, as is the case with many *RET* variants, the clinician's inclination to disclose this information to the patient is heightened. Frustration occurs with the concordant necessary disclosure that the clinical meaning of the variant is uncertain. Such dilemmas demand the continued search to determine genotype–phenotype relationships, a task that is often difficult in rare diseases such as MEN2A and with evolving methods associated with mutation detection. In these settings, it becomes essential to use registries to accumulate data on germline mutations, along with the associated clinical presentations and relevant family data, in order to establish genotype–phenotype correlations firmly. Without the use of a registry in the current study, it would not have been possible to amass such a relatively large number of individuals who harbor this variant. However, the registry has its limitations. It is voluntary, and enrollment focuses on those patients who either visit two tertiary care institutions or are motivated to contact the registry after learning about it through public announcements, potentiating selection bias. Within the confines of the registry, the purpose of this study was to explore the phenotypic expression of *RET*$^{K666N}$. It is not possible to comment upon a founder effect beyond stating the families are not related in the multiple generations that were studied, though most appeared to be of European descent. A goal of a larger international registry could be to comment on the potential of a founder effect in, as well as frequency of, this VUS.

The data here are most consistent with the *RET*$^{K666N}$ variant being associated with an autosomal dominant pattern of inheritance and a low MTC penetrance. However, definitive association with MTC pathogenicity is lacking. Furthermore, no evidence was found for *RET*$^{K666N}$ variant association with the other MEN2A pathogenic features of PHPT and PHEO. However, given the variable penetrance of tumorigenesis associated with other well-established pathogenic *RET* mutations, it is felt that disclosing to K666N variant carriers the possibility that this alteration may be pathogenic is appropriate. The creation of an American Thyroid Association risk level category to capture *RET* variants of unknown or suspected pathogenic significance should be considered for the next guidelines. This would allow treatment recommendations to be created to provide assistance for clinicians encountering such mutations while further correlative data are gathered.

Currently, as individuals with MTC and a germline *RET*$^{K666N}$ variant are encountered, it is recommended that they be biochemically evaluated for PHPT and PHEO. Additionally, under the guidance of a genetic counselor, germline K666N testing within the family is recommended. Family members harboring the variant should then undergo initial evaluation for MEN2-associated diseases through screening, which is straightforward and relatively noninvasive. Given the potential benefit associated with early detection of aberrant C-cell growth, a personalized approach to follow-up screening is supported, which involves assessment of disease aggressiveness in the family, incorporation of patient preferences, and a

vigilant survey for the most recent information on *RET*$^{K666N}$ pathogenicity.


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