

Invited commentary: Medullary thyroid cancer: The importance of *RET* testing

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In this issue of *Surgery*, Bugalho and colleagues from Portugal, present their experience with *RET* testing of patients with sporadic and familial medullary thyroid carcinoma (MTC). Although their targeted approach to *RET* testing for patients in whom the mutation status is unknown (presumed sporadic MTC) is not often practiced in this country (where sequencing of exons 10, 11, and 13 to 16 is commonly performed), their paper serves to emphasize the importance of ordering *RET* gene testing on all patients with MTC. At present, knowledge of the *RET* mutation status and disease extent is required to determine the correct operation for any patient with MTC. Therefore, all surgeons caring for patients with presumed sporadic or inherited MTC need to know how to obtain genetic testing of the *RET* proto-oncogene.

Inherited MTC can occur as part of multiple endocrine neoplasia type 2 (MEN 2) syndrome. MEN 2 is an autosomal dominant inherited cancer syndrome with 3 main subtypes: MEN 2A, MEN 2B, and familial MTC (FMTC). These clinical subtypes differ from each other in the spectrum of endocrine involvement and the biologic behavior of MTC. MEN 2A is clinically defined by the presence of MTC and either pheochromocytoma or hyperparathyroidism (or both) in a single individual, or the presence of 2 or more tumor types in multiple relatives of a single family. MEN 2B is characterized by MTC and pheochromocytoma, without hyper-

parathyroidism, and the presence of visible physical stigmata including mucosal neuromas on the lips and tongue and throughout the digestive track, and a Marfanoid body habitus. FMTC is defined by the isolated finding of MTC in multiple family members. Because FMTC shares a common genetic defect with MEN 2A, it can be difficult to distinguish a family that initially appears to be FMTC from one that may soon prove to become MEN 2A, as the manifestation of pheochromocytoma and/or hyperparathyroidism occurs over time. The diagnosis of FMTC can only be considered when 4 or more family members across a wide range of ages have isolated MTC. Until then, such families with presumed FMTC should be considered “unclassified” and undergo screening for pheochromocytoma—especially prior to exposing such patients to physically stressful events such as general anesthesia.

The *RET* gene is a member of the cadherin superfamily and encodes a single-pass membrane receptor tyrosine kinase. The *RET* gene contains 21 exons, yet up to 95% of families with MEN 2 have germline mutations in 1 of only 6 exons (10, 11, 13, 14, 15, and 16). Within each exon are groupings of 3 nucleotide bases called codons that are translated into 1 of 20 essential amino acids. When a nucleotide is replaced with a different base that results in the new codon encoding a different amino acid, the result is an activating “missense” mutation. Strong genotype–phenotype correlations exist for specific *RET* codon mutations and (1) the clinical subtype of MEN 2, and (2) the age of onset and aggressiveness of MTC.¹

Although some overlap exists between *RET* mutations and the resulting clinical subtype of MEN 2 (Fig), there are some noteworthy consistent relationships between genotype and phenotype. For example, 85% of patients with MEN 2A have a codon 634 mutation in exon 11. Hyperparathyroidism in MEN 2A is most often associated with the

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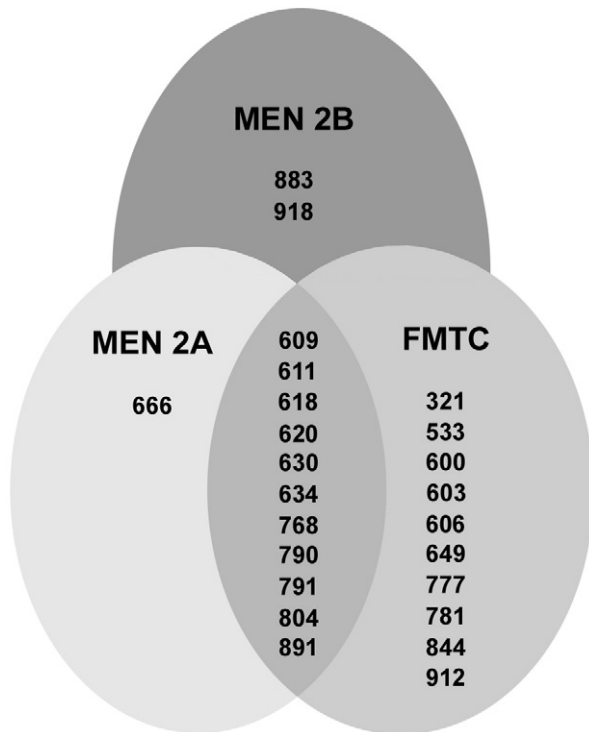


Fig. Correlation of specific *RET* codon mutations with the phenotypic expression of hereditary MTC (MEN 2B, MEN 2A, and FMTC). Additional rare *RET* mutations that are not listed include 532, 534, 635, 636, 638, 639, 778, and 852.

codon 634 mutation (specifically a cysteine-to-arginine amino acid substitution) but much less commonly with mutations in codons 609, 611, 618, 620, 790, and 791. A codon 918 mutation in exon 16 is associated exclusively with MEN 2B, accounts for most patients with this clinical subtype, and is usually produced as a spontaneous new mutation following conception (ie, the de novo mutation is not carried by either parent).

Both the typical age when MTC becomes clinically apparent as a thyroid mass and the biologic behavior of MTC are mutation-dependent. These trends have allowed for the stratification of *RET* mutations into 3 risk levels: level 1, high risk; level 2, higher risk, level 3, highest risk.² Patients with level 3 mutations include those with MEN 2B (codons 883 and 918) and have the highest risk for the early development and aggressive growth of MTC. Patients with level 2 mutations, including codons 611, 618, 620, and 634, have an intermediate risk for the early development and growth of MTC. Patients with level 2 mutations may have evidence of MTC or its precursor C-cell hyperplasia by the age of 5 years or even earlier. In general,

patients with level 1 mutations, including codons 609, 768, 790, 791, 804, and 891, have the most indolent form of MTC.

RATIONALE FOR GENETIC TESTING

When MEN 2 has not yet been established in the family and a patient (the proband) presents with MTC, *RET* testing is indicated to confirm or exclude a hereditary etiology. Approximately 5% to 10% of patients with apparently sporadic MTC (ie, family history is negative) have been found to carry germline *RET* mutations. This is especially likely in patients who are diagnosed with MTC at a young age (<40 years) or in those who are found to have bilateral thyroid nodules (multifocal MTC). Importantly, operative management of the neck (extent of lymphadenectomy and the management of the parathyroid glands) is based on the presence or absence of a *RET* mutation and the specific mutation found (Table). In addition, *RET* testing is appropriate for carrier screening of relatives from an established MEN 2 family to determine the correct timing for prophylactic thyroidectomy, as demonstrated in the manuscript by Bugalho and colleagues. The merits of *RET* testing are now so well described that this genetic test is considered standard of care for all patients with newly diagnosed MTC and for all first-degree relatives of a patient with a known *RET* mutation.

PROCESS OF *RET* TESTING

RET genetic testing is now widely available through many commercially licensed laboratories in the United States and internationally. An updated list of these laboratories including specific mutation testing services, specimen and paperwork requirements, and contact information is available on the GeneTests website (Genetests.org).³ In contrast to the recommendations by Bugalho and colleagues, the most common practice for *RET* analysis in the United States includes DNA sequencing of exons 10, 11, and 13 to 16 for every patient with MTC, regardless of family history or clinical presentation. This approach will diagnose the overwhelming majority of patients with inherited MTC missing only those with the rare mutations in exons 5 (codon 321) and 8 (codon 533). If a patient presents with obvious phenotypic evidence of MEN 2B, it is reasonable to request examination of exons 15 and 16 alone because, to date, all patients with MEN 2B have been shown to have mutations only in these exons. Targeted exon analysis for MEN 2B patients will yield the same sensitivity with reduced cost and faster turnaround time for laboratory processing. Presymptomatic testing

Table. Author's algorithm for the operative management of MTC

<i>Patient classification</i>	<i>Management of neck*</i>	<i>Management of parathyroid glands that are devascularized/removed</i>
Prophylactic thyroidectomy in MEN 2A/FMTC	Performance of central (level VI) neck dissection based on <i>RET</i> mutation, age of the patient, serum calcitonin level, and cervical US findings	-Autograft in neck if <i>RET</i> mutation consistent with FMTC -Cryopreserve/autograft in forearm for <i>RET</i> mutations consistent with MEN 2A
Prophylactic thyroidectomy in MEN 2B	-Level VI dissection -Lateral neck (levels IIA, III, IV, V) dissection based on age of the patient, serum calcitonin level, and cervical US findings	Autograft in the neck as the parathyroid glands are normal in MEN 2B
Therapeutic thyroidectomy in MEN 2A/FMTC; patients with a malignant thyroid nodule(s) and a normal lateral neck by US	-Level 1 <i>RET</i> mutation: Level VI dissection -Level 2 <i>RET</i> mutation: Level VI dissection; ipsilateral/bilateral levels IIA-V dissection based on age, serum calcitonin level -Level 3 <i>RET</i> mutation: level VI and bilateral levels IIA-V neck dissection	-Autograft in neck if <i>RET</i> mutation consistent with FMTC or MEN 2B -Cryopreserve/autograft in forearm for <i>RET</i> mutations consistent with MEN 2A
Therapeutic thyroidectomy in sporadic MTC (no <i>RET</i> mutation) with a malignant thyroid nodule and a normal lateral neck by US	Level VI dissection and ipsilateral levels IIA-V dissection	Autograft in neck

US, Transcutaneous ultrasound.

*It is assumed that if US documents disease in level VI, a level VI dissection would be performed. If disease is discovered in the lateral neck, a functional neck dissection would be performed involving levels IIA, III, IV, and V.

of at-risk relatives from families with a known *RET* mutation also can safely use targeted *RET* testing for the sole exon (or more specifically, the exact codon) known to be affected in the family. When requesting targeted exon or mutation screening for patients suspected to have MEN 2B or for at-risk relatives from families with known mutations, the clinician should clearly state the request for targeted exon (or codon) *RET* testing in the comments or special remarks section of the laboratory requisition form. When a patient with MTC, thought to have a genetic etiology, receives a negative result following standard sequencing of the common 6 *RET* exons, repeat *RET* testing to include sequencing of the entire remaining coding exons should be considered.

Analysis of the entire coding sequence of the *RET* gene (21 exons) is only available at selected commercial laboratories and is an expensive test that should be reserved for patients with evidence strongly suggesting a genetic cause. When requesting full *RET* exon sequencing, the clinician should first verify with the laboratory that this service is still available, obtain preauthorization from the insurance provider for coverage of testing, and obtain authorization from the patient for any out-of-

pocket costs that may not be covered by health insurance.

Prior to ordering a *RET* test, a clinician or preferably a genetic counselor should counsel the patient regarding the potential risks, benefits, and limitations of genetic testing. A genetic counselor specializes in communicating various aspects of the genetic testing process, including legal, ethical, financial, and psychologic implications that make ordering a *RET* genetic test far more complicated than ordering a standard blood test. Unfortunately, many health centers and smaller hospitals lack the benefit of genetic counseling staff. When a genetic counselor is unavailable, it is satisfactory for another healthcare provider (eg, nurse or physician) with formal or informal training to cover these topics in a detailed communication session with the patient. After this communication process, the patient can provide informed consent, which must be documented on a form provided by the laboratory or the ordering institution. When the patient is between the ages of 7 and 17 years, "child assent" by the informed patient may be requested in addition to the "informed consent" by his/her parent or guardian. Copies of these signed documents should be made available to the patient. In some

cases, preauthorization for genetic testing from a patient's health insurance plan may be required.

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