# MULTIPLE ENDOCRINE NEOPLASIA TYPE 2A (including FAMILIAL MEDULLARY THRYOID CARCINOMA), and TYPE 2B

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## ABSTRACT

Multiple Endocrine Neoplasia (MEN) type 2 A and B autosomal dominant syndromes are rare endocrinopathies characterized by tumors of the C cells of the thyroid, adrenal medulla and parathyroid glands.. MEN2A have either C-cell hyperplasia (CCH) or medullary thyroid cancer (MTC), 50% pheochromocytoma (Pheo), and 20-30% hyperparathyroidism (HPT). Patients with MEN2B have a 100% incidence of CCH or MTC, Pheo (30-50%), a Marfanoid habitus and mucosal neuromas, rarely HPT. About 25% of patients with MTC have one of these familial syndromes. FMTC is a variant of MEN2A. Up to 23% of Pheo patients also have either MEN2 or von-Hippel-Lindau disease. Familial forms of these tumors are multifocal, have hyperplasia as a precursor to frank tumors and different clinical behaviors. Ultrasound, FNA cytology of the thyroid combined with immunostains is recommended to make the diagnosis. The MEN2 RET proto-oncogene, on chromosome 10 (10q11-2), is a transmembrane tyrosine kinase with a long extracellular domain, a single transmembrane region, and two cytosplasmic tyrosine kinase domains. Patients with MEN-2B typically have a point mutation in the intracellular kinase catalytic domain. The most important biomarker is calcitonin and provocative testing for elevation of TCT in response to pentagastrin or calcium helpful but the diagnosis is made by genetic testing. Fractionated metanephrines are used for pheochromocytoma and PTH for hyperparathyroidism. Screening should be done early and cure can be achieved with total thyroidectomy for MTC and adrenalectomy for pheochromocytoma. Recurrent MTC requires history, physical examination, neck ultrasound, and measurement of serum calcitonin and carcinoembryonic antigen (CEA) levels every 6 months. F-DOPA PET/CT or conventional FDG PET/CT, are able to detect MTC metastases or disease progression. Treatment can be successful with a TKI such as Sunitinib, Vanetanib and Sorafenib.

# INTRODUCTION

Multiple Endocrine Neoplasia (MEN) type 2 syndromes are rare endocrinopathies characterized by tumors of the thyroid, adrenal medulla and parathyroid glands. They have been described in nearly all ethnics group throughout the world. Based on an analysis of SEER data, MEN2A is by far the more common, with an incidence of about 1 patient in 2 million, compared to MEN2B with an incidence of about 1 patient in 39 million (1). Overall, about 95% of patients with MEN2 are classified as type 2A, and 5% as type 2B (2). Nearly all patients with MEN2A have either C-cell hyperplasia (CCH) or medullary thyroid cancer (MTC), about 50% have pheochromocytoma (Pheo), and 20-30% have hyperparathyroidism (HPT). Patients with MEN2B have a 100% incidence of CCH or MTC, frequently have Pheo (30-50%), and typically have physical characteristics including a Marfanoid habitus and mucosal neuromas. They rarely have HPT. Both syndromes have autosomal dominant transmission patterns. Thus, children of affected individuals have a 50% chance of inheriting the genetic abnormality. The penetrance is virtually 100% by biochemical screening, however only 60-70% develop clinically apparent syndromes (3) (4). About 25% of patients with MTC have one of these familial syndromes. Because MTC is rare, the occurrence of two MTC's in a single family should clearly raise suspicions of a familial syndrome. Indeed, patients who present with MTC and a negative family history need to be screened for MEN2, as up to 3% to 7% will be found to have a hereditary syndrome (2). Up to 23% of Pheo patients also have a familial disorder, mainly either MEN2 or von-Hippel-Lindau disease (5) (6).

MEN2A has been subclassified into 4 variants:

1. Classic MEN2A
2. MEN2A with cutaneous lichen amyloidosis(CLA)
3. MEN2A with Hirschsprung's disease(HD)
4. Familial medullary thyroid cancer(FMTC)

The patient distribution among the subtypes varies with the series and the population studied. A recent series from China showed that of 73 patients with MEN2, 28(38%) patients were from 7 kindreds with classic MEN2A, 14(19%) patients were from 3 kindreds with CLA, 4(6%) patients were from 1 kindred with HD, 26(36%) patients were from 10 kindreds with FTMC, and 1(1%) patient with MEN2B (7).

Previously, patients with familial medullary thyroid carcinoma (FMTC) were classified as a separate entity, because affected families appeared to have medullary thyroid carcinoma alone, lacking other associated endocrine or neural-tissue involvement of MEN2 (8). With longer term follow-up, some members in many of these families developed Pheo or HPT (9). Data such as these suggested FMTC patients might be more appropriately classified as a variant of MEN2A. Indeed, it has been noted that only three families classified as FMTC have not developed other MEN2A associated lesions (1). Furthermore, some of the same genetic abnormalities have been noted in both MEN2A and FMTC, although those with FMTC who develop other

associated lesions do so at a lower prevalence and at an older age. Finally, the diagnosis, screening, management and follow-up of affected families in both syndromes is similar. After much thoughtful discussion, the American Thyroid Association (ATA) as expressed in their most recent guidelines, feels that FMTC should no longer be considered a distinct, freestanding syndrome, but instead should be considered as a variant of MEN2A (1).

Early diagnosis of affected patients and families is critical to obtain the best outcomes. Management of these patients is challenging, and the decision making is often not straightforward, having to balance risks and benefits, particularly in younger patients. Patients and families suspected of harboring or diagnosed with MEN2 should be evaluated by an experienced multidisciplinary team.

# PATHOLOGY

The major difference in patients with familial compared to the sporadic forms of these tumors is the multifocal nature of the lesions, the presence of hyperplastic states as precursors to frank tumors and different clinical behaviors. Indeed, CCH of the thyroid is associated with familial forms of MTC, and adrenal medullary hyperplasia is associated with Pheo in MEN2 (10).

Patients with genetic forms of MTC have diffuse bilateral CCH of the thyroid, occult MTC, or frank MTC. For patients with thyroid nodules on physical examination or ultrasound, FNA cytology combined with immunostains is recommended to make the diagnosis (11). Grossly, MTC appears as firm white, tan or reddish nodules. CCH or occult MTC may not be apparent on routine histologic examination. Thus, detailed immunostaining for calcitonin, CEA, and chromogranin A is essential. Pathologic evaluation should be carried out by an experienced thyroid pathologist, as MTC may have a variable appearance and may be confused with other conditions including papillary or follicular thyroid cancer (1).

Patients with classic MEN-2A and 2B also may have adrenal hyperplasia preceding the development of Pheo. However, the clinical significance and management of adrenal hyperplasia in these patients is controversial. A study of 18 MEN2 patients showed identical molecular aberrations in all 19 adrenal medullary hyperplasias compared to the 13 Pheos, and at similar frequencies (12). Thus, these authors suggest that adrenal medullary hyperplasias should be regarded as micro- Pheos. However, since not all hyperplasias progress to clinically apparent Pheo, this finding, although interesting, is not likely to change patient management.

A question arises as to the significance of CCH found unexpectedly during thyroidectomy for other conditions. Unfortunately, physiologic and neoplastic CCH have been often grouped together, leading to confusion (13). It is important that physiological(reactive) CCH, which is generally felt to have no malignant potential, be distinguished from neoplastic CCH, which is associated with germline mutations in RET and MEN2 (14). This again illustrates the importance

of an experienced thyroid pathologist, as sometimes distinguishing physiologic CCH from neoplastic CCH may not be straightforward (15).

# GENETICS

The gene for the MEN2 syndromes is the RET proto-oncogene, located at the centrometric region of chromosome 10 (10q11-2). The RET proto-oncogene gene is a transmembrane tyrosine kinase with a long extracellular domain, a single transmembrane region, and two cytosplasmic tyrosine kinase domains. The oncogene contains 21 exons spanning more than 60kb of genomic DNA. It is expressed in tissues of neural crest origin, and appears to have important function in cell migration and development. Ligands which bind to RET together with specific co-receptors include glial cell line-derived neurotrophic factor (GDNF), neurturin, artemin, and persephrin (16). All classic MEN2A families studied thus far have germline mutations of the RET proto-oncogene, but a few families with MEN-2B or FMTC do not have such mutations. For example, an ESR2 frameshift mutation has been identified in a FMTC family lacking a RET mutation (17). These investigators showed this mutation resulted in activation of estrogen- responsive elements and increased RET expression through a novel mechanism.

RET germline mutations in MEN-2 typically result in constitutive activation. Different mutations lead to different levels of activation, which may have an effect on the clinical spectrum (18). Somatic mutations in the RET proto-oncogene are noted in as many as 25-45% of patients with sporadic MTC (11), and rarely in patients with sporadic Pheo. Thus, the RET proto-oncogene appears to be associated with growth regulation of neuroendocrine cells. Mutations of the RET proto-oncogene act in a dominant fashion. Both peripheral leukocytes and tumors from patients with these syndromes contain a normal allele of the RET gene. The vast majority of classic MEN-2A or FMTC patients will have a mis-sense mutation in the extracellular domain at a single codon (609, 611, 618, 620, or 634) (19). Eighty-five percent of classic MEN-2A mutations occur at codon 634, whereas only 30% of mutations in FMTC occur at codon 634 (20). More recent data show that the prevalence of mutations in codon 634 in MEN2 has decreased over time to 40-45%, with the remainder distributed to codon 804(15-18%) and other codons (21). Not only are codon 634 mutations the most common mutations found in classic MEN2A, but codon 634 mutations are also noted in nearly all patients with MEN2A and CLA (22) (23) (24), with a single patient reported to harbor a codon 804 mutation (25). HPT is also diagnosed more frequently in those MEN2A patients with codon 634 mutations (26). The prevalence of HPT was noted to be 19.1% in a French study of 188 patients with mutations at codon 634 (27). The specific mutation did not significantly affect the prevalence of HPT, but high interfamily variability of HPT risk was noted.

It is uncertain as to why the same point mutation (e.g. codon 634) can result in different clinical manifestations in the same family or in different families. Although RET is the primary driver in MEN2A associated tumors, other low frequency alterations such as *EIF4G1* may also

contribute, perhaps by regulating RET pathway activity (28). Other genetic abnormalities identified in patients with classic MEN-2A and FMTC include LOH at chromosome 1p, 22q, 17p or 3p. The low frequency of consistent mutations other than RET have led some to suggest that it may be the nature of the second hit, rather than the specific RET germline mutation itself or the age of the patient, that determines the long term clinical behavior (29). Thus, multi-step carcinogenesis may be applicable to patients with MEN-2, with other non-RET genetic changes or modifiers responsible for the phenotypic differences.

Inactivating mutations in the extracellular domain of the RET proto-oncogene have been identified in patients with Hirschsprung's disease (HD), occurring in 50% of familial cases, and in 15%-33% of sporadic cases (30) (31). Patients with MEN2A and HD have point mutations in RET involving codons in exon 10, including codons 620(40-50%), 618(30%), 611(5%), and 609(15%) (32) (1). It seems paradoxical that MEN2A and HD would occur together, since RET mutations with MEN2A result in gain of function, whereas those mutations occurring with HD result in loss of function (1). This has been attributed to the concept of a “Janus” mutation, a mutation which can act simultaneously as both a gain-in-function and loss-of -function mutation (32). Other theories have also been advanced, such as the constitutive RET activation being sufficient to induce neoplastic transformation of C-cells, but insufficient to induce a trophic response in neurons (33). Based on a novel family in Brazil, one group favors the hypothesis that specific haplotypes not associated with RET are the causes of HD (34). Certainly further work will be required to solve this puzzle.

Patients with MEN-2B typically have a point mutation in the intracellular kinase catalytic domain, most commonly a substitution of threonine for methionine at codon 918. Mutations in codon 918 are noted approximately 95% of the time and in codon 883 approximately 5% of the time in patients with MEN2B (35). MTC in MEN2B patients with the codon 883 mutation appears to behave somewhat less aggressively than the codon 918 mutation (36), but this still generally portends a poor prognosis (2). A study of the codon 918 mutation in transgenic mice revealed that it predisposes the mice to MTC (37). Alterations in the TGF-beta pathway, and increased expression of chondromodulin-1 may help explain the earlier onset of malignancy and skeletal abnormalities in MEN2B compared to MEN2A (38).

Data continue to accumulate regarding mutations in various RET codons and genotype-phenotype correlations. Some data suggest MTC aggressiveness is related to the specific mutation present in the codon (39) (40). In recent study of 184 carriers of RET mutations at codon 634, arginine substitutions for cysteine were associated with a higher penetrance of MTC and Pheo, but not with progression and aggressiveness, compared to the reference standard of other substitutions (41). Double mutations in RET are identified very infrequently, but it appears certain double mutations are a more aggressive genotype (42) (1).

Studies have shown that certain mutations are more commonly associated with Pheo. One study showed that 20 of 21 MEN2 patients with Pheo had codon 634 or 918 mutations (39). A recent study of 17 Indian MEN-2 patients with Pheo from 11 kindreds showed the most common mutations were in codon 634, with other mutations in codons 804 and 918 (43). In this

study the mean age of the patients was only 27.7, significantly younger than sporadic patients, and bilateral disease was much more common as well (47.1% vs. 4.5%). An Asian study of 67 patients with codon 634 germline mutations showed that although the C634R mutation showed a higher age-related penetrance of bilateral Pheo, the accumulated risk of bilateral Pheo across all four C634 mutations approached 100% over time (44). Other mutations appear to carry less risk of Pheo. The less common codon 768 and 912 mutations are associated with FMTC, and these patients would generally not be expected to develop Pheo or HPT. However, current data remain insufficient to determine the risk of Pheo or HPT in a given patient or family. Thus, all patients need to be followed for the development of these tumors (16).

Other recently described mutations appear to be moderate to perhaps even "low" risk variants. A French study of 77 patients from 16 families with codon 790 mutations at a mean follow-up of 89 months showed this is a less aggressive variant (ATA moderate risk-class A), with lower penetrance of MTC (< 50%), no deaths due to MTC, only one Pheo, and no cases of HPT (45). The authors suggest that simple surveillance rather than thyroidectomy is appropriate for patients with normal to slightly elevated calcitonin levels. Two new germline mutations in codons 515 and 636 have recently been described, associated with low penetrance of MTC, late onset of MTC (all age 60 or older) and low aggressiveness (46). The authors consequently recommend caution before recommending thyroidectomy in asymptomatic carriers.

There are a number of RET mutations with unclear clinical significance (21). Such genetic abnormalities are often termed variants of unknown significance (VUS). In a patient with Pheo and C-cell hyperplasia without MTC, a VUS was identified in $RET^{E616Q}$, which was also found in his brother with neonatal Hirschsprung's disease, and in their unaffected mother (47). Patients and families that possess this VUS will need to be monitored. A MTC patient was reported to have a missense mutation in codon 666, a minor VUS (48). A study of 8 MTC probands with codon 666 mutations (K666N variant) found 16 additional family members with the variant, but only one additional case of MTC has been noted thus far (49). Given the low penetrance of MTC, this would suggest this is a less aggressive variant, however further study is required before a risk level can be assigned.

The large increase in genetic information and data about genotype-phenotype correlations has led to the development and updating of consensus guidelines by several groups including the European Society of Endocrine Surgeons (ESES) (50), NANETS (11), and the American Thyroid Association (ATA) (51), including the most recent ATA update (1). The ATA initially stratified patients into low risk (A) to high risk (D) levels, based on the specific mutations (51). To help avoid confusion, the most recent ATA guidelines have reclassified level D to highest risk, level C to high risk, and levels B and A to moderate risk (1).

The guidelines of the different groups vary somewhat, but all assign patients to risk groups and management based on the specific mutations. They all assign patients with codon 918 mutations (found in >95% of MEN2B patients) to the highest risk group. As an example of one of the changes that has occurred, codon 883 mutations (also found in MEN2B) appear to be of lower risk than codon 918 mutations (36). Based on data such as these, patients with codon

883 mutations have been moved from the ATA highest risk (level D) category to high risk (level C) (1). Recent compelling data from Germany (41) show that patients with American Thyroid Association (ATA) moderate risk mutations (level A and B) now have a higher percentage of lymph node metastases than high risk mutations (level C). The ongoing accrual of such data will need to be analyzed and guidelines will undoubtedly need to be updated periodically.

# CLINICAL FEATURES

The clinical presentation and behavior differ with each of the syndromes. Virtually 100% of patients with MEN-2A have CCH or MTC, 50% have Pheo, and 20-30% have HPT. Some patients with MEN-2A develop HD disease, which frequently precedes the diagnosis of MEN-2 (52). Patients with MEN-2B also have a 100% incidence of CCH or MTC, and frequently have Pheo (30-50%) (9). They also have a characteristic physical appearance at birth or shortly thereafter with mucosal neuromas of the eyelids, lips, and tongue, marfanoid habitus, and hyperflexible joints (53) (54) (9). They do not have the marfanoid lens or aortic abnormalities. Patients with MEN2B may also have abnormal gastrointestinal motility, due to wide-spread ganglioneuromatosis of the gastrointestinal tract. Thus, even at a young age, patients with MEN-2B may have troubles with diarrhea, constipation, colonic dilatation, or even present with megacolon. A review of the literature shows that diverticulosis, diverticulitis, abscess and other complications have also been noted (55). Patients with MEN-2B seldom have HPT. Biochemically, the tumors that arise in patients with these genetic syndromes are similar to those with the sporadic forms of the tumors. There are no distinct biochemical markers that allow identification of familial versus non-familial forms of the tumors.

MTC in patients with MEN2 tends to occur at a younger age than sporadic MTC. Peak incidence of MTC is in the second and third decades for patients with classic MEN-2A. The cancer usually behaves in a relatively indolent fashion, even though it can metastasize early. Survival for patients with MEN-2A and MTC is somewhat better than for patients with sporadic MTC. A small subset of patients with MEN-2A has MTC which behaves very aggressively. Thus, there is a need for an aggressive surgical approach early on, to prevent widespread metastatic disease. In FMTC, the peak incidence of MTC is in the fourth to fifth decade, and behaves in the least aggressive fashion (8). Although lymph node metastases are frequent, the disease usually follows an indolent course, and virtually never results in death.

About 40% of patients with MEN2 develop Pheo, however it is rarely a presenting feature (56). Although it may be detected at the same time as MTC, Pheo tends to develop at a later age than the thyroid disease (57). Pheo in MEN2 has a peak incidence in the fourth and fifth decades. Occasionally, Pheo is identified in childhood. Pheo in MEN2 is often bilateral and multiple, but only occasionally malignant (58) (59) (60). Classically, the ratio of norepinephrine to epinephrine is lower in the genetic forms of Pheo than in the sporadic form. Common early symptoms in these patients include palpitations, headaches, and anxiety. With aggressive family screening, some patients are identified who have no symptoms from the tumor. In

patients in whom the disorder is not recognized, a hypertensive crisis may be induced by an operation or by childbirth.

Primary hyperparathyroidism (HPT) develops in 20% to 30% of patients with MEN2A (26) and is most often diagnosed concurrently with MTC (2). It characteristically involves multiple parathyroid glands. HPT is typically diagnosed at a median age of 37 years and 68% to 85% of patients are asymptomatic at time of diagnosis (61) (62). HPT rarely occurs in MEN2B patients.

Cutaneous lichen amyloidosis (CLA) is an uncommon disorder characterized by pruritic lichenoid papules, most commonly located in the pretibial regions bilaterally in sporadic cases. CLA is rarely associated with MEN2A, and in these patients CLA is typically located in the interscapular/scapular regions (24). This Brazilian study of 38 patients in 3 kindreds with MEN2A and CLA found that 68% were women, all harbored codon 634 mutations, and overall prevalence of MCT was 94%, CLA 51%, Pheo 30%, and HPT 16%. Overall, it appears that Pheo and HPT occur in similar frequencies to classic MEN2A (23). Combined with a literature review of 214 other individuals, skin lesions or symptoms suggestive of CLA were first noted at a mean age of 20, 11 years prior to the diagnosis of MEN2A (24). Thus patients presenting with CLA in the unusual interscapular/scapular location should be considered for RET testing.

Patients with HD most commonly present shortly after birth. Those with exon 10 RET mutations should be screened for MEN2A (1). Older patients with MEN2A that possess exon 10 RET mutations and have colonic symptoms should be evaluated for HD (1). Patients with MEN2A and HD are much more likely to have long segment (29.3%) and total colonic ganglioneuromatosis (17.3) compared to the general HD population (32).

MTC in MEN-2B occurs in the first and second decades of life. It tends to be more aggressive and lethal (63) (64), and death from metastatic diseases has been reported in young children (65). When MEN-2B is diagnosed, even in infants, surgical treatment for MTC should be performed as early as possible. Despite characteristic physical exam findings such as mucosal neuromas and Marfanoid habitus as noted above, the diagnosis is often delayed beyond childhood (66). In this study of 22 MEN2B patients (18 with M918T mutations), median age at diagnosis of MTC was 13 years (range 6-25 years), and median delay of diagnosis was 26 months (range 0-18 years). This delay comes at a cost, with 55% of patients having persistent disease in the neck after the initial operation (66). A reason given for the delay in diagnosis is that most patients with MEN2B initially present with de novo mutations rather than inherited mutations (67). In this study of 44 MEN2B patients with M918T mutations, the 3 patients with inherited mutations were all diagnosed before the age of 1year, all had CCH, and are apparently cured. Of the 41 patients with de novo mutations, MEN2B was diagnosed in 12 patients after recognition of nonendocrine symptoms including intestinal ganglioneuromatosis, oral symptoms, ocular symptoms (tearless crying), and skeletal stigmata, either alone or in combination. Those diagnosed based on nonendocrine symptoms instead of symptomatic MTC or Pheo were significantly younger (mean of 5.3 vs. 17.6 years) , less often had lymph node metastases (42% vs 100%) or distant metastases (8% vs 79%), and were more often biochemically cured (58% vs 0%) (67).

# DIAGNOSIS AND SCREENING

The diagnosis of MEN2 is made by genetic and biochemical testing. Patients diagnosed with CCH, MTC or Pheo should be studied for the possibility of MEN-2 by performing genetic testing. Those with other clinical syndromes such as HD (particularly those with exon 10 RET mutations), or CLA in unusual locations such as the interscapular/scapular regions should also be tested. Those with physical characteristics suggestive of MEN2B such as mucosal neuromas and Marfanoid habitus also need to be tested. A negative family history is not reliable in excluding patients for genetic testing, since about 40% of MEN-2A gene carriers do not develop clinically apparent disease. Furthermore, 6-10% of patients with perceived sporadic MTC prove to have an occult germline RET mutation (11). In developing countries where genetic testing is not available, the diagnosis of MEN2 may be made with a high degree of accuracy by using biochemical tests and imaging (68).

Genetic screening with mutational analysis can identify gene carriers with extremely high accuracy. Multiple studies have confirmed that using molecular biology techniques, the index case (proband) of a new family, and every gene carrier in a known family of classic MEN-2A, can be identified with 100% accuracy (69-72). The accuracy for identifying patients with MEN-2B and FMTC is not quite as high, because of the small number of families that do not have the common RET proto-oncogene mutations. If initial sequence analysis is negative, and the clinical suspicion of a genetic syndrome remains high based on family history, physical characteristics, young age at diagnosis, or pathologic findings in the thyroid such as extensive CCH or bilateral MTC, then whole gene sequencing should be considered (21). Once a mutation is identified in the proband, other family members should also be tested to see if they are carriers. Newly identified carriers should be screened for both MTC and Pheo. Indeed, it is currently recommended that carriers undergo annual biochemical screening for MTC, Pheo, and HPT, combined with neck ultrasound, starting at an age based on the ATA risk category (1). Members of classic MEN-2A families who are not mutation carriers on genetic screening do not need to undergo biochemical testing.

The importance of screening in patients with sporadic-appearing MTC to deplete the pool of unrecognized RET mutation carriers was confirmed in a recent German 50 year retrospective study (41). In this study of 455 MEN2A carriers at risk, the percentage of index patients among all carriers fell. In non-index carriers, the percentage of MTC and the percentage of node-positive MTC fell, and the percentage of biochemical cure increased. However, improvements were not seen uniformly, particularly in lower ATA levels (previously A and B, currently Moderate risk). The authors point out these findings are not surprising, since index cases in levels A and B are typically diagnosed at an older age, where they consequently have offspring screened later in life. The authors suggest that with continued focus and vigilance, this pool of unrecognized carriers may gradually be depleted. The development of rapid and accurate genetic testing techniques will hopefully facilitate this process (73).

Biochemical testing is similar to patients with sporadic disease. Patients with clinical MTC have elevated serum calcitonin. Reference ranges for calcitonin differ among laboratories, and also differ based on gender. Reference ranges in children are not well defined, especially under the age of 3, and high levels are normally noted in infancy (74). Thus calcitonin levels are of limited help in the initial management very young children and infants. Various cut-off values for calcitonin have been described in the literature, with values >40pg/ml most commonly associated with clinical MTC. A recent study from Korea suggests a value of 19pmol/l (about 65pg/ml) as a useful cutoff point for macroscopic MTC (75). A study from Norway showed that preoperative calcitonin level was predictive of the presence of MTC, and that levels $\geq$68pg/ml were associated with lymph node metastases (76).The degree of calcitonin elevation correlates with tumor volume, and levels of > 100pg/ml are associated with clinical MTC (11).

Patients with CCH or subclinical MTC usually do not have elevated basal serum levels of calcitonin. However, in these patients calcitonin levels usually increase in response to calcium and/or pentagastrin. The combination test described by Wells and colleagues results in less false negatives than tests performed with either of the two agents alone (77). This test consists of a 50 second infusion of calcium gluconate (2mg of elemental calcium/kg) followed by a 10 second bolus of pentagastrin (Peptavalon, Ayerst, 0.5mg/kg). Calcitonin levels are measured at 0, 2, 3.5, and 5 minutes, and generally peak at the 2 or 3.5 minute points. Pentagastrin is however, no longer available and reliance has to be placed on calcium infusion alone. Physicians screening patients for CCH or subclinical MTC must be thoroughly familiar with the particular calcitonin assay being used, as normal ranges vary somewhat. In general, normal females do not have a stimulated calcitonin level greater than 29 pg/ml, and in males the normal limit is 106 pg/ml. False positive results are uncommon. They occur in a small number of patients with normal thyroid glands, including the 5% of normal patients who have CCH (78) (79) (80). Provocative tests become abnormal at an average age of about 8-9 in gene carriers of MEN-2A.

In the past provocative testing was particularly useful in identifying MEN2A family members as they lack the characteristic phenotypic features found in MEN2B patients. However, for the most part provocative testing has been replaced by genetic testing to identify disease carriers. A situation where provocative testing may still be useful is the younger MEN2A child in whom the family declines prophylactic surgery. In this situation, it makes sense to follow the patient with basal and stimulated calcitonin levels, and then strongly recommend surgery when a rise is noted (11).

Screening for Pheo should be performed in all MEN2 patients prior to preventive or therapeutic thyroidectomy, and in female MEN2 patients who are considering pregnancy, to avoid a potential hypertensive crisis. Furthermore, screening for Pheo should be performed in all children in ATA highest and high risk categories (formerly D and C) by age 11, and in moderate risk children (formerly B and A) by age 16 (1). Pheo is identified by elevated urinary epinephrine, norepinephrine, or VMA. Measurements of plasma normetanephrine and metanephrine may be more sensitive at detecting Pheo than urinary testing (81). In a study of

174 patients with MTC screened for Pheo, 5 cases were identified in known MEN-2 families, and another 5 index cases were found, leading to the discovery of 5 new MEN-2 families (82).

Screening for HPT is similar to those with sporadic disease and includes measurement of serum calcium, albumin, ionized calcium, and intact PTH. ATA guidelines recommend that annual screening start at the same time as screening for Pheo, at age 11 for those in the high risk category and at age 16 for those in the moderate risk category (1).

Some authors have suggested that the frequency of biochemical screening for Pheo and HPT should be tailored based on ATA risk group and the patients age, given the variability of penetrance based on age (83). Indeed, these authors suggest that lower intensity screening (every other year) may even be considered in ATA highest risk category (formerly D) patients who are less than 21 years old, and perhaps those more than 40 years old. These authors admit that additional, longer term studies will be required to confirm these findings.

# SURGICAL MANAGEMENT

The advent of genetic screening of first degree family members of those with newly discovered MEN2 has led to earlier diagnosis and treatment. The goal is to do surgery before there are any clinical signs of disease, thus resulting in a high rate of cure. Patients undergoing total thyroidectomy who have CCH or microscopic MTC have greater than a 90% cure rate. Thus, CCH and occult MTC are consistently curable by total thyroidectomy, unlike higher stage, clinically apparent MTC, where cure is infrequent (78) (79) (77) (84). Unfortunately, some patients have distant metastases when first diagnosed, thus preventing any chance for cure (64).

## Adrenalectomy For Pheo

Patients with MEN-2 syndromes must be screened for possible Pheo, before undergoing thyroidectomy. If Pheo is identified, surgery for this should always take precedence over any neck procedure, to avoid a potential hypertensive crisis following induction of anesthesia. Once a diagnosis of Pheo is established biochemically, non-invasive localization and the subsequent surgical approach is carried out as previously described for sporadic Pheo. Pheo in MEN-2 patients almost always occur bilaterally, however they often do not occur synchronously. The contralateral adrenal lesion usually develops within 10years, but can develop many years later. Interestingly, only one third to one half of patients who undergo unilateral surgery need a second surgery for recurrent Pheo in the opposite adrenal gland. Fortunately, Pheos in MEN2 patients are usually benign. Thus, a search for metastatic Pheo is not likely to be helpful, unless the lesion is large, more than 5cm in diameter (85).

Adrenal surgery can be reserved for those patients with demonstrated elevated catecholamine secretion and with a discrete tumor or tumors, or symptoms. Useful imaging tests in these patients include MIBG scans, CT scans, or MRI. Some favor thin-cut CT of the adrenal glands over MRI (85). Imaging studies usually reveal an abnormality when biochemical testing is abnormal (69). Patients who present with bilateral disease traditionally have undergone bilateral adrenalectomy. Patients with unilateral disease have traditionally undergone unilateral adrenalectomy. Cortical-sparing adrenalectomy is an option in patients who require bilateral adrenal resection, resulting in 65% of patients corticosteroid independent, and a low (approximately 10%) recurrence rate (86).The safety and excellent outcomes of cortical-sparing adrenalectomy in MEN2 patients was confirmed in a large international study of 563 patients who underwent adrenalectomy, including 82 with bilateral Pheo who underwent adrenal-sparing surgery (87). Recurrence rates at 6 to 13 years were low in both the standard adrenalectomy and adrenal-sparing groups (2% vs 3%), and 57% of the adrenal sparing group were steroid independent. Indeed, an adrenal sparing approach, if feasible, should be considered even in those with initially unilateral disease, because of the long term risk of developing recurrence in the opposite gland. Patients with residual adrenal tissue need to undergo yearly biochemical testing and be monitored for recurrence.

## Thyroidectomy For MTC

The primary management for genetic forms of MTC is total thyroidectomy with central neck (level 6) lymph node dissection. Ultrasound of the neck is usually performed prior to therapeutic surgery as it may give important information as to the extent of macroscopic thyroid and lymph node disease, not apparent on clinical exam in this era of widespread obesity. Patients with clinically apparent MTC on either clinical exam or neck ultrasound should undergo screening for metastatic disease, with attention to the most common sites such as the lungs, mediastinal lymph nodes, liver, and bones. Patients with markedly elevated serum calcitonin levels (>1000 pg/ml) should also be screened for distant metastatic disease (85). Because of the field defect in the entire thyroid, and the multifocal nature of MTC in the genetic syndromes, total thyroidectomy is essential. Every effort must be made to leave no thyroid tissue or capsule behind. Failure to remove all thyroid tissue inevitably leads to recurrence (62) (88). This point cannot be overemphasized.

There is no controversy regarding the need for central neck (level 6) lymph node dissection for MEN2 patients with clinically apparent MTC. Metastases to central neck lymph nodes are noted in up to 81% of patients with palpable tumors (11). For patients with involved central compartment lymph nodes, ipsilateral or bilateral modified neck dissection should be strongly considered, guided in part by the pre-operative ultrasound and intra-operative findings.

## Surgery For HPT

HPT is present in about 20-30% of patients with MEN-2A, but rarely occurs in MEN-2B patients. It is most often diagnosed concurrently with MTC (2). Thus, surgery for MEN-2A parathyroid disease most often takes place during total thyroidectomy for MTC. In the past, subtotal parathyroidectomy or total parathyroidectomy with autotransplantation into the forearm muscle was performed in MEN2 patients (71) (89).The recent ATA guidelines suggest that these approaches should only be considered in those with enlargement of all 4 glands or in those with a RET mutation associated with a high risk of HPT (1). For those with enlargement of less than 4 glands, only the enlarged glands should be removed. Intraoperative PTH levels are useful to help guide the extent of the surgery (11). Recurrence of HPT rarely occurs, even with long term follow-up. One or two parathyroid glands may be found to be abnormal in normocalcemic MEN-2A patients undergoing thyroidectomy. In patients whose family history reveals little hyperparathyroidism and a RET mutation associated with low risk of HPT, removal of the enlarged parathyroid glands only is reasonable. In the patient who develops HPT after total thyroidectomy, preoperative localization studies to include at a minimum neck ultrasound and Sestimibi scan (85) are essential to allow a focused surgical approach, increase the chances of finding and removing the culprit(s), and minimize the risk of re-operative neck surgery.

## Preventive Thyroidectomy

Patients undergoing preventive thyroidectomy do not need any imaging, but all patients should have Pheo and HPT biochemically excluded prior to surgery (85). In MEN-2 patients diagnosed by genetic testing or provocative testing, there are usually no palpable thyroid abnormalities, and the central lymph nodes are usually free of disease. There is some controversy regarding the need for central lymph node dissection in patients who undergo preventive surgery, particularly in early childhood. A consensus is emerging that a selective approach may be taken in patients undergoing preventative surgery, reserving central lymph node dissection to those patients with elevated basal serum calcitonin levels > 40 pg/ml (90) (91). Of course, those with the unexpected finding of lymph node metastases intraoperatively should undergo at a minimum central neck lymph node dissection to clear all gross disease. For patients undergoing preventive surgery, and have no evidence of HPT, leaving parathyroid glands in-situ appears to be a good option compared to routine total parathyroidectomy with autotransplantation (91). Fewer patients in the in-situ parathyroid group developed permanent hypoparathyroidism compared to the total parthyroidectomy group (1%vs 6%), no patient in either group suffered permanent RLN injury or has developed hyperparathyroidism. This study confirms that HPT rarely occurs after preventive total thyroidectomy, even when the parathyroid glands are left in place.

The timing of preventive thyroidectomy depends on the particular syndrome. As more data have become available, the age at which preventive thyroidectomy is recommended has decreased. Two large European studies were among the first to suggest that the particular codon affected can be used to help determine the timing of thyroidectomy (92) (93). The ATA has published

consensus guidelines for the management of MTC and prophylactic treatment of MEN2 patients based on the specific codon position of the RET mutation (51), and these guidelines were recently updated (1). A risk level is assigned to each codon based on its known clinical aggressiveness highest risk (previously level D), high risk (previously level C), and moderate risk (previously levels B and A) and recommendations made based on the risk level. The ATA highest risk category includes mutations in codon 918 (>95% MEN2B harbor these mutations) and these patients are recommended to undergo prophylactic thyroidectomy before age 1, ideally as soon as the diagnosis is made. Such surgery should not be delayed, even if calcitonin levels are normal (94). Patients with ATA high risk category mutations (codons 634 and 883) should undergo total thyroidectomy before age 5, while patients with ATA moderate risk category mutations may delay total thyroidectomy until after age 5 in select cases. Delay of thyroidectomy is appropriate in those moderate risk patients with serum calcitonin levels below 30 pg/ml (95).

# DIAGNOSIS AND TREATMENT OF RECURRENT OR METASTATIC DISEASE

A fairly standard post-operative follow up regimen to detect recurrent MTC includes history, physical examination, neck ultrasound, and measurement of serum calcitonin and carcinoembryonic antigen (CEA) levels every 6 months (9). CEA is a non-specific marker which may be elevated in patients with MTC. It is not useful for early diagnosis, but has a role for monitoring disease progression and for detecting recurrence after thyroidectomy (1). It is recommended CEA levels be obtained concurrently with calcitonin measurements.

The treatment of recurrent MTC in patients with the genetic syndromes is similar to their sporadic counterparts. A study from the French Calcitonin Study Group involving 226 sporadic and hereditary MTC patients showed that patients with preoperative calcitonin levels <50 pg/ml were more likely to normalize postoperatively (96). If calcitonin levels remain elevated postoperatively, an aggressive surgical approach may be considered in selected patients with disease confined to the neck. Prior to considering neck re-exploration, the evaluation should be thorough to exclude distant metastatic disease.

Patients with MTC may develop metastases to many sites, most commonly the lungs, mediastinal lymph nodes, and liver, less commonly the bones and brain, and rarely cutaneous metastases (1). In addition to CT or MRI of the neck, chest, abdomen and pelvis, and bone scintigraphy, laparoscopy has been advocated by some to exclude occult liver metastases (97). A number of studies support the use of F-DOPA PET/CT or conventional FDG PET/CT , as being better able to detect MTC metastases or disease progression (98) (99). Others have shown that the number of metastatic lesions is often underestimated by F-DOPA PET/CT (85). Since these techniques are expensive, and lack a demonstrable benefit in outcomes, routine use cannot be recommended. Somatostatin receptor scintigraphy is also of little use, attributed to the low density of type 2 receptors in MTC (85). In the uncommon situation where the source

of elevated calcitonin is unclear after an extensive search, selected venous sampling can be performed for calcitonin. Indeed, we have found this technique useful, helping avoid unnecessary re-exploration of the neck. When elevated levels are isolated to one or both sides of the neck, modified unilateral or bilateral neck dissection is then performed. Such surgery can be performed by experienced groups with low morbidity (61).

There is generally little role for external beam radiation therapy (EBRT) to the neck in these patients (100). The recent ATA guidelines suggest that post-operative external beam radiation therapy (EBRT) may be helpful in selected circumstances where the risk of local recurrence is felt to be high (1). We feel that such use should be very limited, given the potential for making subsequent neck re-exploration, if required, more difficult. We agree with NANETS that post-operative radiation therapy is reasonable in those patients in whom there is gross residual disease (11), particularly if there is a concern for potential airway compromise. There is no role for radioactive iodine (RAI) after thyroidectomy for MTC or after a recurrence. C-cells do not take up RAI, so it is not surprising that RAI has not shown a benefit in these patients (101). Treatment of symptomatic bony or brain metastases from MTC is similar to metastases due to other histologic types. Those with painful bony metastases may have a good symptomatic response to EBRT, and bisphosphonate therapy may also be helpful.

There are few data on the use of combination chemotherapy in patients with systemic disease (102-104). A variety of combinations including CVD have had limited success. Better understanding of the molecular aberrations associated with RET mutations has led to exciting therapeutics in MEN2 patients with locally advanced or metastatic MTC. Studies of small molecule tyrosine kinase inhibitors (TKI) targeting the RET tyrosine kinase have shown promising results. Vandetanib is an orally available TKI that competes with ATP and blocks autophosphorylation and signal transduction. It targets RET-dependent tumor cell proliferation as well as vascular endothelial growth factor dependent tumor angiogenesis and the epidermal growth factor receptor. A phase 3 trial with vandetanib at 300 mg/day in advanced MTC showed an improved progression-free survival, an increased objective response rate and a significant biochemical response rate (105). Within the study were 33 patients with hereditary MTC who had a 46% objective response rate, similar to the entire cohort. Those patients with M918T mutations had a higher response rate than those who did not. Similar results have been noted in another study (106). In 15 pediatric patients with M918T mutations and locally advanced or metastatic MTC, the objective response rate was 47%. These responses can be durable, as illustrated by a pediatric patient with a continuing response on Vandetanib for 4 years (107). Sunitinib (37.5 mg/ daily) and sorafenib (400 mg twice daily) have also shown modest efficacy in refractory and metastatic MTC in phase 2 trials (108) (109).

# PROGNOSIS

Early identification of probands and asymptomatic carriers would seem critical if outcomes are to be improved. A perfect example is MEN2B, where despite characteristic physical exam

findings and appearance, there is a significant delay in diagnosis (66). Recognition of the physical characteristics and other nonendocrine manifestations alone or concomitantly results in earlier identification and improved chance of cure (67).

The morbidity and mortality in patients with MEN-2 is due to recurrent medullary thyroid carcinoma. A study of 104 patients with sporadic and hereditary MTC revealed that patient age at presentation and tumor stage were the only independent predictors of survival (110). Another study in 63 consecutive MTC patients with normal postoperative imaging, revealed postoperative basal calcitonin levels and tumor stage as independent prognostic factors (111). Not surprisingly, patients who present with clinical disease before the genetic diagnosis is made do worse, due to more advanced stage at presentation (112). Patients diagnosed with CCH before clinically apparent MTC do uniformly well (113). Thus the importance of screening and follow-up of asymptomatic carriers cannot be overemphasized.

The importance of performing preventive thyroidectomy based on age and level of risk has been supported by a number of studies and guidelines (94) (92) (93) (50) (11) (1). A group of 9 hereditary MTC patients who underwent age appropriate thyroidectomy per NANETS criteria (Group 1) was compared to 19 patients who had thyroidectomy past the recommended age (Group 2) (114). All patients in Group 1 were cured without recurrence whereas 42% of group 2 patients recurred, and the recurrence rate was higher the greater in time past the guidelines the surgery was performed. A meta-analysis supports the value of prophylactic thyroidectomy to prevent MTC in children with MEN2B (115).

Unfortunately, despite recommendations for prophylactic thyroidectomy in childhood for asymptomatic carriers since the 1970s, there are as yet no published data on long term (>40year) outcomes (29). Acquisition of such data would be valuable, and may help families struggling with the decision for their child to undergo such surgery.

The effect of Pheo on the survival of MEN2 patients has been studied (116). Looking at a group of 59 patients with RET codon 634 mutations with Pheo, compared to a group of 48 patients with RET codon 634 mutations without Pheo. Pheo was not associated with more advanced stage of MTC, or with shorter survival (median survival 499 months with Pheo vs. 444 months without).

The amount of data which has accumulated over the last decade has truly been staggering, and has resulted in significant changes in patient management. Further refinements in risk stratification will undoubtedly occur as additional genotype-phenotype data become available. Molecular based therapies now offer hope to those with advanced or metastatic MTC. The increasing molecular knowledge will hopefully lead to new therapies, therapies useful not only for metastatic MTC, but as adjuvant treatment in high risk patients, or perhaps even in prevention.

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