Resource for Individuals Living with Multiple Endocrine Neoplasia

Frontiers of Hormone Research: "Genetic Endocrine Tumor Syndromes"

Multiple Endocrine Neoplasia Type 2

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Short title: MEN 2

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abstract:

Multiple Endocrine Neoplasia type 2 (MEN 2) is an autosomal dominant cancer syndrome characterized by variable penetrance of medullary thyroid carcinoma (MTC), pheochromocytoma (PHEO), and primary hyperparathyroidism (PHPT). MEN 2 consists of two clinical subtypes, MEN 2A and MEN 2B. Familial Medullary Thyroid Cancer (FMTC) is now viewed as a phenotypic variant of MEN 2A with decreased penetrance for PHEO and PHPT rather than a distinct entity. All subtypes are caused by gain-of-function mutations of the RET proto-oncogene. Genotype-phenotype correlations exist that help predict the presence of other associated endocrine neoplasms as well as the timing of thyroid cancer development. Recognition of the clinical entity in individuals and families at risk of harboring a germline *RET* mutation is crucial for the management and prevention of associated malignancies. Recent guidelines released by the American Thyroid Association (ATA) regarding the management of MTC will be summarized in this chapter.

Overview:

MEN 2 occurs in 1:200,000 live births, and is an autosomal dominant syndrome characterized by predisposition to neuroendocrine tumors [1]. The first clinical subtype of MEN 2, MEN 2A is characterized by medullary thyroid cancer (MTC), pheochromocytoma (PHEO), and primary hyperparathyroidism (PHPT). MEN 2A accounts for 70-80% of individuals with MEN 2. MTC is frequently the first neoplastic manifestation in MEN 2A patients, with thyroid cancer occurring as early

as in the first 5 years of life. MTC is a calcitonin-producing tumor that arises from the parafollicular C cells of the thyroid gland, which are derived from the embryonic neural crest. Two rare variants of MEN 2A have been identified, one with Hirschsprung's disease and the other with cutaneous lichen amyloidosis [2, 3]. In families without an established diagnosis of MEN 2A, patients typically present with a neck mass between the ages of 15 and 20 years. The risk of developing MTC in MEN 2A is between 70 and 95%, while approximately 50% of individuals with MEN 2A develop PHEO and 10-30% develop primary PHPT [4, 5].

The second inherited subtype of MEN 2, MEN 2B, accounts for only 5% of MEN 2. MEN 2B is characterized by MTC in all affected individuals, in addition to variable presentation of PHEO, a Marfanoid body habitus, mucosal and eyelid neuromas, and ganglioneuromatosis of the gastrointestinal tract (Figure 1). Patients with MEN 2B do not develop PHPT. Distinct early clinical manifestations in MEN 2B include inability to shed tears and gastrointestinal symptoms including abdominal distension, megacolon, diarrhea, or constipation [6, 7]. MTC in MEN 2B is characterized by a clinically aggressive course; affected individuals who do not undergo prophylactic thyroidectomy in infancy are likely to develop metastatic MTC. Prior to the recommendation for prophylactic thyroidectomy, the mean age of death in MEN 2B was 21 years [1]. Approximately 50% of individuals with MEN 2B develop PHEOs, which are often bilateral.

Familial MTC (FMTC) was previously categorized as a separate, third subtype of MEN 2A. Now, however, FMTC is viewed as a phenotypic variant of MEN 2A with decreased penetrance for PHEO and primary PHPT rather than a distinct entity [8]. FMTC accounts for 10% to 20% of MEN 2 cases; only the thyroid gland is affected in these patients by definition. In general, FMTC patients present later with MTC than those with MEN 2A or MEN 2B, usually between 20 and 40 years of age.

MTC is the most common cause of death in patients with MEN 2A, MEN 2B, and FMTC [9]. Carriers of all variants of inherited MTC have a high penetrance for

developing thyroid cancer: 90% of carriers of such predisposition eventually are diagnosed with MTC and present with a palpable thyroid nodule or elevation of calcitonin levels [10]. The most frequent systemic manifestation of MTC is diarrhea, which is associated with elevated plasma calcitonin and poor prognosis. On histopathology, MTC is a malignant tumor originating from the calcitonin-producing (C cells) of the thyroid gland (Figure 2a&b). C-cell hyperplasia is a precursor to MTC, in which clusters of C-cells extend beyond the basement membrane. Positive immunohistochemical staining with calcitonin antibodies is also found in MTC (Figure 2c).

History

MEN 2 is the first inherited neoplasia syndrome in which a clear genotype-phenotype association was made, notably between the *RET* genotype and the MEN 2 phenotype [5]. MEN 2A was originally described by Sipple, who in 1961 published the case of a man who died of intracranial hemorrhage and autopsy findings were consistent with PHEO, MTC, and parathyroid hyperplasia [11]. The following year, Cushman put forth a link between these endocrine tumors, which led to the classification of MEN 2 as a unique entity [12]. Linkage analysis in families with MEN 2 led to the discovery of the *RET* proto-oncogene in chromosome 10 as the causative gene in this syndrome in 1993 [13-15].

In 1966 the constellation of findings associated with MEN 2B including thick lips, tongue lesions, MTC, and PHEO was described in a patient and his family members by Williams and Pollock [16]. Nearly 30 years later in 1994, distinct activating mutations in the *RET* proto-oncogene were found to cause MEN 2B [17, 18]. Following these discoveries, it became possible to identify relatives of patients with these syndromes who have inherited a mutated *RET* allele and in whom MTC is almost certain to develop. Because MTC is not curable once it metastasizes beyond the thyroid, it is now recommended that all patients with MTC and especially young members of kindreds with MEN 2A and 2B have genetic screening performed to determine if they are carriers of a *RET* mutation [19].

Structure and function of *RET*

The *RET* proto-oncogene is located on chromosome 10q11.2 and includes 21 exons. *RET* (REarranged during Transfection) was first identified by Takahashi *et al*. in 1985 [20]. *RET* encodes a receptor tyrosine kinase with key roles in cell growth, differentiation, and survival. Receptor dimerization leads to autophosphorylation of intracellular tyrosine residues, which subsequently activate downstream pathways of signal transduction [21]. The role of the *RET* oncogene in the development of MTC has been well characterized. Activating *RET* germline mutations have been identified as the primary cause of all the hereditary MTC syndromes: approximately a quarter to a third of all sporadic MTC cases and up to 98% of MEN 2 cases have a germline *RET* mutation leading to constitutive activation of the *RET* receptor; somatic *RET* mutations account for another quarter to half of all sporadic MTCs [5].

The specific site of the particular mutated residue within the RET protein has been correlated to phenotypic differences among patients with inherited MTC. For example, patients with MEN 2A characteristically have missense mutations in exon 10 (codons 609, 610, 611, 618, 620) and exon 11 (codon 634) [19]. These mutations affect one of the six cysteine residues present in the *RET* extracellular domain [22]. Mutations in these cysteine residues lead to receptor homodimerization via the formation of disulfide bonds, rendering the receptor activated regardless of the presence of ligand. The natural history of MTC in MEN 2A is highly variable between different *RET* mutations and even among members of the same family. In the case of MEN 2B, more than 95 percent of patients have a mutation at exon 16 (codon 918), in the tyrosine kinase domain of the protein. This mutation renders the receptor activated in its monomeric state, and leads to increased phosphorylation of intracellular tyrosine residues [23]. Patients with FMTC harbor mutations in exons 10, 11, 13 (codon 768), and 14 (codons 804, 806)[19].

RET gene and practice recommendations

MEN 2 is a well-defined hereditary cancer syndrome in which genetic testing is considered part of the standard management for relatives who are at-risk. Therefore *RET* molecular genetic testing should be offered to at-risk family members. The risk to family members of having a *RET* mutation differs according to the subtype of MEN 2. In MEN 2A, the majority (95%) of individuals have inherited the mutation from a parent, while the remaining 5% have a de-novo germline mutation [24]. In contrast, in MEN 2B, approximately 50% of individuals have an affected parent, while 50% represent de-novo germline mutations. As is the case with autosomal dominant disorders, each child of an individual with MEN 2 has a 50% chance of inheriting the *RET* mutation. Early detection of at-risk children leads to prophylactic thyroidectomy in a timely fashion, potentially averting the risk of metastatic MTC.

Current practice recommends performing prophylactic thyroidectomy prior to the development of MTC in at-risk patients [9]. In fact, total thyroidectomy is indicated in patients with inherited *RET* mutations, regardless of the plasma calcitonin level. Specific genotype-phenotype correlations have been established that associate the clinical aggressiveness of MTC with the mutated codon of the RET protein, as outlined in Table 1 [19]. Therefore, the timing and extent of prophylactic surgery is dictated by germline analysis of mutations. The dilemma arises in terms of balancing the risk of surgical morbidity from performing the thyroidectomy at an early age with doing the surgery prior to the development of metastatic MTC. In 1999, the 7th International Workshop on MEN published a consensus statement that was the first to classify *RET* mutations into risk levels. They correlated patient genotypes with clinical aggressiveness of hereditary MTC, and created guidelines for the timing of prophylactic thyroidectomy: the patients at highest risk include those with MEN 2B and *RET* mutations in codons 883 or 918 [5, 19]. The guidelines call for prophylactic thyroidectomy in these patients within the first year of life. Patients with MEN 2A or FMTC who have mutations in codons 611, 618, 620, and 634 are at high risk and thyroidectomy should be undertaken prior to the age of five years, whereas the timing of surgery of patients with other mutations can be

individualized; such a decision, however, should never be left for later than early childhood [5, 9, 19]. In 2009, the American Thyroid Association (ATA) published updated recommendations for clinical and genetic diagnostic testing and treatment options for MTC, in which the age at which prophylactic thyroidectomy is performed can be guided by the codon position of the *RET* mutation [8]. The risk of aggressive MTC based on genotype are defined in the ATA guidelines, to summarize, level D carries evidence of the highest risk and highest penetrance of developing aggressive MTC. These risk levels are then used to guide the timing of prophylactic thyroidectomy as outlined in Table 2. For those patients identified by screening to have inherited a mutated *RET* allele who go on to have prophylactic thyroidectomy, 5 and 10-year survival rates approach 100% [9, 25, 26]. In patients with sporadic MTC, however, genetic screening has rarely been applied; these patients often present with metastatic disease [25, 26].

For individuals with a *RET* mutation who have not had a thyroidectomy, annual biochemical screening with calcitonin is recommended, followed by thyroidectomy if results are elevated. Calcitonin is secreted by the C-cells of the thyroid and is used as a marker of disease in MTC. Historically, stimulated calcitonin testing was performed by measurement of calcitonin following intravenous administration of the secretagogue pentagastrin, which is not currently available in the United States and many other countries. In addition, current calcitonin assays have superior sensitivities, as low as 1–2 pg/mL. In the present time, most experts believe that there is rarely a need for stimulated calcitonin testing in the diagnosis or follow-up of MTC [8]. Guidelines call for annual serum calcitonin screening to begin for children with MEN 2B at 6 months and at age 3-5 years for children with MEN 2A or FMTC [8].

The molecular genetic basis of MEN 2 are germline mutations in the *RET* proto-oncogene; mutations are listed in Table 1. The algorithm for molecular genetic testing is presented in the most recent ATA MTC practice guidelines [8]. For all patients with isolated MTC, or with MTC along with PHPT or PHEO, initial testing

should include sequencing of exons 10, 11, and 13-16 of *RET*. If this initial testing is negative, potential reflex testing should include full sequencing of the coding region. For individuals with MTC along with features suggestive of MEN 2B, targeted mutation analysis or sequencing of exons 15 and 16 for M918T and A883F is warranted.

Summary of current optimal therapeutic practices

MTC is relatively unresponsive to radiation therapy and to standard chemotherapeutic regimens [25, 27]. Radiation therapy has only a palliative role in the treatment of MTC [28]. Surgery remains the only standard treatment for patients with MTC: total thyroidectomy that is performed before MTC grows or spreads beyond the gland is currently the only curative therapy. It is recommended that surgery be performed by a center with experience in MEN 2. The thyroid surgeon must incorporate knowledge of the patient's genotype into the decision regarding surgical approach. In a prophylactic thyroidectomy, a total thyroidectomy should be performed, yet routine central compartment dissection is not warranted. However, in the setting of a clinically evident tumor, central neck dissection should be performed along with total thyroidectomy [8]. Specific recommendations regarding evaluation and management of metastatic MTC are outlined in the ATA guidelines and are beyond the scope of this chapter.

Once MTC metastasizes, it has a tendency to spread to local and regional lymph nodes (cervical and mediastinal), and more distantly to lung, liver, and bone (Figure 3) [25]. Although MTC is often widely metastatic, it tends to be a slow growing tumor. Patients with metastatic disease continue to have 5 and 10-year survival rates of 80% and 70%, respectively. Decreased survival in MTC can be correlated with the stage of diagnosis. Survival also varies by the extent of local disease, with 10-year survival rates as high as 95% for patients with disease confined to the thyroid gland, and rates as low as 40% for those with distant metastases [29].

Because approximately 50% of individuals with MTC have recurrent disease after total thyroidectomy and neck dissection, surveillance for recurrent MTC is paramount [30]. Annual serum calcitonin is indicated in all individuals with MTC who have undergone thyroidectomy, with more frequent follow-up indicated for those with residual disease [8]. Imaging indicated for localization of metastatic disease in these patients includes neck ultrasound, chest and neck computed tomography (CT), contrast-enhanced magnetic resonance imaging (MRI) of liver, bone MRI of the spine and pelvis, as well as bone scan [8].

Treatment of MTC with Tyrosine Kinase Inhibitors

The only current cure for patients with MTC is total thyroidectomy performed at an early stage, when the disease is confined to the thyroid gland. Standard chemotherapy and radiation have not shown to be effective. Rationally designed small molecular compounds that affect tyrosine kinase-dependent oncogenic pathways, tyrosine kinase inhibitors (TKIs), have emerged recently as treatment for patients with MTC. Vandetanib is an orally available TKI that targets vascular endothelial growth factor-dependent tumor angiogenesis and epidermal growth factor receptor and *RET*-dependent tumor cell proliferation. Like other small molecule TKIs in this class of anticancer agents, vandetanib competes with ATP and blocks autophosphorylation and signal transduction. Vandetanib has been approved by the Federal Drug Administration for use in patients with locally advanced or metastatic medullary thyroid cancer based on results of a randomized, double blind phase III trial [31]. Another tyrosine kinase inhibitors, sorafenib showed suggestion of clinical benefit for patients with sporadic MTC in a phase II clinical trial [32]. Sunitinib has also shown effectiveness in MTC and well-differentiated thyroid carcinoma in a phase II clinical trial [33]. The use of these agents is limited by the development of resistance; multiple drug therapy may hold promise for future studies.

Pheochromocytomas in MEN 2 Syndromes

Pheochromocytomas develop in approximately 50% of patients with MEN 2A or MEN 2B [34]. The timing of the development of PHEO is usually after the presentation of MTC, however it is the presenting disease in 13 - 30% of individuals with MEN 2A [35, 36]. Nodular or diffuse hyperplasia of the adrenal medulla occurs as a precursor to the development of PHEO, and on histopathological examination, bilateral adrenal medullary disease is usually found [34]. Malignant transformation of PHEO occurs in approximately 4% of cases [37].

Evaluation and Treatment of Pheochromocytoma

Recommendations for the management of PHEO in MEN 2A syndromes have evolved from bilateral adrenalectomy, to unilateral adrenalectomy, and most recently to cortical-sparing adrenalectomy as the treatment of choice. Because of the high likelihood of the development of a contralateral PHEO, older surgical recommendations called for bilateral adrenalectomy in MEN 2A and MEN 2B once the diagnosis of PHEO was made, regardless of clear evidence of tumor in both glands. This approach led to the lifelong need for glucocorticoid replacement therapy and the risk of death from adrenal insufficiency. In the 1990s recommendations advocated for the more conservative approach of single gland resection via laparoscopic adrenalectomy for unilateral disease. Lairmore et. Al reported in their series of 23 patients with MEN 2 syndromes who underwent unilateral adrenalectomy, 48% of the patients did not develop a PHEO in the contralateral adrenal gland during at least 10 years of follow up. Even more recently, minimally invasive cortical-sparing adrenalectomy has been very successful in terms of both low risk of local recurrence and reducing the risk of addisonian crisis [38, 39]. Azari et al. reported outcomes in 13 patients with MEN 2A and hereditary PHEO who underwent adrenal sparing surgery; only 5 of the 13 patients (38%) developed recurrence in the contralateral gland [39]. In addition 22% of their patients experienced Addisonian crisis, including a death, after bilateral adrenalectomy. Azari et al. concluded that cortical-sparing adrenalectomy and close monitoring of the remnant may be the treatment of choice for hereditary bilateral PHEO in MEN 2A, as overall recurrence is low [39].

Screening for Pheochromocytoma

The majority of PHEOs in MEN 2A syndromes are intra-adrenal and benign, however the potential for malignancy does exist. As is the case with the development of MTC, there is also a *RET* codon-specific- age-related risk for development of PHEO. The median age of presentation of PHEO in MEN 2B is in the mid-20s [40, 41]. Machens et. al. looked at the timing of development of PHEO in a series of 206 *RET* mutation carriers [42]. Based on their data, annual biochemical screening for PHEO was recommended from age 10 years in carriers of *RET* mutations in codons 918, 634, and 630, and from age 20 years in the remainder [42]. According to the 2009 ATA guidelines for the management of MTC, PHEO preoperative screening should begin by age 8 years for MEN 2B and mutated *RET* codons 634 and 630; otherwise by age 20 years for other *RET* mutations [8].

Current recommendations call for biochemical screening for PHEO with measurement of plasma metanephrines or measurement of 24-h collections for urinary catecholamines or metanephrines [43]. Epinephrine and norepinephrine are the most common metabolites found to be elevated in MEN 2 [44]. If levels are elevated, adrenal imaging with CT or MRI should be performed. [^{18}F]-fluorodopamine positron emission tomography (PET) is a superior imaging modality for PHEO localization and further evaluation, with superior sensitivity as compared to 123I-metaiodobenzylguanidine and 111in-pentetreotide scintigraphy [45]. There is not a consensus on specific recommendations for regular adrenal imaging in the setting of normal metabolic screening for individuals with MEN 2.

Patients with MEN 2 should be screened for PHEO prior to any surgical procedure so that appropriate adrenergic blockade can be administered. Therefore, PHEO must be excluded or treated prior to surgery for MTC. Special care should be taken to ensure that female *RET* mutation carriers are screened for PHEO before or during early pregnancy to avoid the potential of hypertensive crisis activated by pregnancy or delivery [8]. Once identified, patients with phechromocytoma must undergo

proper preoperative management with α- and β-blockade to minimalize adverse events associated with induction of anesthesia and surgery [46].

Primary Hyperparathyroidism

Between 15 and 30% of individuals with MEN 2A will develop primary hyperparathyroidism (PHPT) [19, 26]. Periodic screening with albumin-corrected calcium or ionized calcium is recommended; if serum calcium is elevated along with inappropriately normal or elevated PTH, the diagnosis of PHPT is suspected. PHPT usually presents years after the diagnosis of MTC and most individuals have no symptoms associated with elevated calcium. In one study of 56 patients affected by PHPT among 249 MEN-IIa patients the median age at diagnosis was 37.6 years, and PHPT was asymptomatic in 68% of the patients [47]. Another group looked at 67 patients with MEN 2A and PHPT where the median age at diagnosis of PHPT was 38 years, and PHPT was asymptomatic in 84% of the patients [48]. These studies suggest that MEN-IIa-related PHPT is generally associated with mild, often asymptomatic hypercalcemia, although hypercalcuria and renal calculi may occur. Although some patients had recurrences 5 to 15 years after the first parathyroidectomy, resection of only macroscopically enlarged glands generally appears sufficient. Subtotal or total parathyroidectomy with autotransplantation is associated with a high rate of hypoparathyroidism.

Surveillance with annual serum calcium and PTH levels should begin by age 8 years in carriers of *RET* mutations in codons 630 and 634, and by age 20 years in carriers of other MEN 2A *RET* mutations [8]. Treatment recommendations for PHPT associated with MEN 2A vary according to the particular *RET* mutation. Resection and autotransplantation of parathyroid tissue are performed at the time of thyroidectomy when there is evidence of PHPT. The ATA MTC guidelines recommend that in individuals with a strong family history of PHPT or with *RET* mutations carrying high risk of PHPT, devascularized normal parathyroid glands should be autografted into the forearm, while those individuals with lower risk

conferring mutations may have the parathyroid tissue autografted into either the forearm or the sternoclidomastoid muscle. This will help to preserve the remaining parathyroid tissue in the likely scenario of the patient requiring repeat neck operations for recurrent MTC, thus avoiding hypoparathyroidism. For MEN 2A patients who have had prior thyroidectomy who later get PHPT, these individuals require preoperative localization using sestamibi and CT scans. In patients with persistent or recurrent PHPT, and in those who are suboptimal surgical candidates, medical therapy with calcimimetics may play a role. Calcimimetics reduce PTH secretion by increasing the sensitivity of parathyroid calcium-sensing receptors to extracellular calcium. Cinalcet, an oral calcimimetic, has been studied in a randomized double-blind placebo controlled study and has shown effectiveness in reducing PTH concentrations and PTH levels in patients with PHPT [49]. While cinacalcet may prove to be an attractive alternative to surgery in patients with PHPT, it is unknown if Cinalcet will prove useful in patients with MEN 2A-associated PHPT, and use in these patients is not documented.

Conclusion

MEN 2 is an autosomal dominant hereditary cancer syndrome caused by gain-of-function mutations in the *RET* protooncogene. MEN 2A is defined as the presence of MTC, PHEO, and PHPT. MEN 2B is a multiple tumor syndrome associated with MTC, marfanoid habitus, ganglioneuromatosis of the oral mucosa and GI tract, and PHEO. Early screening of family members who may be affected by MEN 2 is associated with improved prognosis. The timing of prophylactic thyroidectomy is guided by the ATA-risk level of *RET* mutations. Surveillance for PHEO and PHPT, are indicated in select groups of patients with specific *RET* mutations.

Figure Legends:

Figure 1: A) Computed tomography scout film showing dilated bowel due to ganglioneuromatosis of the gastrointestinal tract in MEN 2B. B) Mucosal neuromas on the anterior surface of the tongue in MEN 2B

Figure 2:
A) Low power view of medullary carcinoma of the thyroid comprised of nests of spindle-shaped cells containing amyloid B) high power detail. C) Calcitonin immunohistochemical staining of MTC

Figure 3:
Metastatic MTC to the skull (A), hilar lymph nodes (B) and liver (C)

References

1. **Moline J, Eng C** 2011 Multiple endocrine neoplasia type 2: an overview. Genet Med 13:755-764
2. **Gagel RF, Levy ML, Donovan DT, Alford BR, Wheeler T, Tschen JA** 1989 Multiple endocrine neoplasia type 2a associated with cutaneous lichen amyloidosis. Ann Intern Med 111:802-806
3. **Verdy M, Weber AM, Roy CC, Morin CL, Cadotte M, Brochu P** 1982 Hirschsprung's disease in a family with multiple endocrine neoplasia type 2. J Pediatr Gastroenterol Nutr 1:603-607
4. **Howe JR, Norton JA, Wells SA, Jr.** 1993 Prevalence of pheochromocytoma and hyperparathyroidism in multiple endocrine neoplasia type 2A: results of long-term follow-up. Surgery 114:1070-1077
5. **Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, van Amstel HK, Lips CJ, Nishisho I, Takai SI, Marsh DJ, Robinson BG, Frank-Raue K, Raue F, Xue F, Noll WW, Romei C, Pacini F, Fink M, Niederle B, Zedenius J, Nordenskjold M, Komminoth P, Hendy GN, Mulligan LM, et al.** 1996 The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA 276:1575-1579
6. **Wray CJ, Rich TA, Waguespack SG, Lee JE, Perrier ND, Evans DB** 2008 Failure to recognize multiple endocrine neoplasia 2B: more common than we think? Ann Surg Oncol 15:293-301
7. **Brauckhoff M, Machens A, Hess S, Lorenz K, Gimm O, Brauckhoff K, Sekulla C, Dralle H** 2008 Premonitory symptoms preceding metastatic medullary thyroid cancer in MEN 2B: An exploratory analysis. Surgery 144:1044-1050; discussion 1050-1043
8. **Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JF, Pacini F, Ringel MD, Schlumberger M, Wells SA, Jr.** 2009 Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid 19:565-612
9. **Skinner MA, Moley JA, Dilley WG, Owzar K, Debenedetti MK, Wells SA, Jr.** 2005 Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A. N Engl J Med 353:1105-1113
10. **Ponder BA, Ponder MA, Coffey R, Pembrey ME, Gagel RF, Telenius-Berg M, Semple P, Easton DF** 1988 Risk estimation and screening in families of patients with medullary thyroid carcinoma. Lancet 1:397-401
11. **Sipple JH** 1961 Association of Pheochromocytoma with Carcinoma of Thyroid Gland. Am J Med 31:163-&
12. **Cushman P** 1962 Familial Endocrine Tumors - Report of 2 Unrelated Kindred Affected with Pheochromocytomas, 1 Also with Multiple Thyroid Carcinomas. Am J Med 32:352-&
13. **Mathew CG, Chin KS, Easton DF, Thorpe K, Carter C, Liou GI, Fong SL, Bridges CD, Haak H, Kruseman AC, et al.** 1987 A linked genetic marker for multiple endocrine neoplasia type 2A on chromosome 10. Nature 328:527-528

14. **Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, Moley JF, Goodfellow P, Wells SA, Jr.** 1993 Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet 2:851-856
15. **Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, et al.** 1993 Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 363:458-460
16. **Williams ED, Pollock DJ** 1966 Multiple Mucosal Neuromata with Endocrine Tumours - a Syndrome Allied to Von Recklinghausens Disease. J Pathol Bacteriol 91:71-&
17. **Hofstra RM, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, Pasini B, Hoppener JW, van Amstel HK, Romeo G, et al.** 1994 A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 367:375-376
18. **Carlson KM, Dou S, Chi D, Scavarda N, Toshima K, Jackson CE, Wells SA, Jr., Goodfellow PJ, Donis-Keller H** 1994 Single missense mutation in the tyrosine kinase catalytic domain of the RET protooncogene is associated with multiple endocrine neoplasia type 2B. Proc Natl Acad Sci U S A 91:1579-1583
19. **Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, Gheri RG, Libroia A, Lips CJ, Lombardi G, Mannelli M, Pacini F, Ponder BA, Raue F, Skogseid B, Tamburrano G, Thakker RV, Thompson NW, Tomassetti P, Tonelli F, Wells SA, Jr., Marx SJ** 2001 Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab 86:5658-5671
20. **Takahashi M, Ritz J, Cooper GM** 1985 Activation of a novel human transforming gene, ret, by DNA rearrangement. Cell 42:581-588
21. **Arighi E, Borrello MG, Sariola H** 2005 RET tyrosine kinase signaling in development and cancer. Cytokine Growth Factor Rev 16:441-467
22. **Asai N, Iwashita T, Matsuyama M, Takahashi M** 1995 Mechanism of activation of the ret proto-oncogene by multiple endocrine neoplasia 2A mutations. Mol Cell Biol 15:1613-1619
23. **Santoro M, Carlomagno F, Romano A, Bottaro DP, Dathan NA, Grieco M, Fusco A, Vecchio G, Matoskova B, Kraus MH, et al.** 1995 Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. Science 267:381-383
24. **Schuffenecker I, Ginet N, Goldgar D, Eng C, Chambe B, Boneu A, Houdent C, Pallo D, Schlumberger M, Thivolet C, Lenoir GM** 1997 Prevalence and parental origin of de novo RET mutations in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma. Le Groupe d'Etude des Tumeurs a Calcitonine. Am J Hum Genet 60:233-237
25. **Kebebew E, Ituarte PH, Siperstein AE, Duh QY, Clark OH** 2000 Medullary thyroid carcinoma: clinical characteristics, treatment, prognostic factors, and a comparison of staging systems. Cancer 88:1139-1148
26. **Modigliani E, Cohen R, Campos JM, Conte-Devolx B, Maes B, Boneu A, Schlumberger M, Bigorgne JC, Dumontier P, Leclerc L, Corcuff B,**

Guilhem I 1998 Prognostic factors for survival and for biochemical cure in medullary thyroid carcinoma: results in 899 patients. The GETC Study Group. Groupe d'etude des tumeurs a calcitonine. Clin Endocrinol (Oxf) 48:265-273

27. **Quayle FJ, Moley JF** 2005 Medullary thyroid carcinoma: including MEN 2A and MEN 2B syndromes. J Surg Oncol 89:122-129
28. **Brierley J, Tsang R, Simpson WJ, Gospodarowicz M, Sutcliffe S, Panzarella T** 1996 Medullary thyroid cancer: analyses of survival and prognostic factors and the role of radiation therapy in local control. Thyroid 6:305-310
29. **Roman S, Lin R, Sosa JA** 2006 Prognosis of medullary thyroid carcinoma: demographic, clinical, and pathologic predictors of survival in 1252 cases. Cancer 107:2134-2142
30. **Cohen MS, Moley JF** 2003 Surgical treatment of medullary thyroid carcinoma. J Intern Med 253:616-626
31. **Wells SA, Jr., Robinson BG, Gagel RF, Dralle H, Fagin JA, Santoro M, Baudin E, Elisei R, Jarzab B, Vasselli JR, Read J, Langmuir P, Ryan AJ, Schlumberger MJ** 2012 Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol 30:134-141
32. **Lam ET, Ringel MD, Kloos RT, Prior TW, Knopp MV, Liang J, Sammet S, Hall NC, Wakely PE, Jr., Vasko VV, Saji M, Snyder PJ, Wei L, Arbogast D, Collamore M, Wright JJ, Moley JF, Villalona-Calero MA, Shah MH** 2010 Phase II clinical trial of sorafenib in metastatic medullary thyroid cancer. J Clin Oncol 28:2323-2330
33. **Carr LL, Mankoff DA, Goulart BH, Eaton KD, Capell PT, Kell EM, Bauman JE, Martins RG** 2010 Phase II study of daily sunitinib in FDG-PET-positive, iodine-refractory differentiated thyroid cancer and metastatic medullary carcinoma of the thyroid with functional imaging correlation. Clin Cancer Res 16:5260-5268
34. **Lairmore TC, Ball DW, Baylin SB, Wells SA, Jr.** 1993 Management of pheochromocytomas in patients with multiple endocrine neoplasia type 2 syndromes. Ann Surg 217:595-601; discussion 601-593
35. **Rodriguez JM, Balsalobre M, Ponce JL, Rios A, Torregrosa NM, Tebar J, Parrilla P** 2008 Pheochromocytoma in MEN 2A syndrome. Study of 54 patients. World J Surg 32:2520-2526
36. **Frank-Raue K, Rybicki LA, Erlic Z, Schweizer H, Winter A, Milos I, Toledo SP, Toledo RA, Tavares MR, Alevizaki M, Mian C, Siggelkow H, Hufner M, Wohllk N, Opocher G, Dvorakova S, Bendlova B, Czetwertynska M, Skasko E, Barontini M, Sanso G, Vorlander C, Maia AL, Patocs A, Links TP, de Groot JW, Kerstens MN, Valk GD, Miehle K, Musholt TJ, Biarnes J, Damjanovic S, Muresan M, Wuster C, Fassnacht M, Peczkowska M, Fauth C, Golcher H, Walter MA, Pichl J, Raue F, Eng C, Neumann HP** 2011 Risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germline RET mutations located in exon 10. Hum Mutat 32:51-58
37. **Modigliani E, Vasen HM, Raue K, Dralle H, Frilling A, Gheri RG, Brandi ML, Limbert E, Niederle B, Forgas L, et al.** 1995 Pheochromocytoma in

multiple endocrine neoplasia type 2: European study. The Euromen Study Group. J Intern Med 238:363-367

38. **Alesina PF, Hinrichs J, Meier B, Schmid KW, Neumann HP, Walz MK** 2012 Minimally invasive cortical-sparing surgery for bilateral pheochromocytomas. Langenbecks Arch Surg 397:233-238
39. **Asari R, Scheuba C, Kaczirek K, Niederle B** 2006 Estimated risk of pheochromocytoma recurrence after adrenal-sparing surgery in patients with multiple endocrine neoplasia type 2A. Arch Surg 141:1199-1205; discussion 1205
40. **O'Riordain DS, O'Brien T, Crotty TB, Gharib H, Grant CS, van Heerden JA** 1995 Multiple endocrine neoplasia type 2B: more than an endocrine disorder. Surgery 118:936-942
41. **Leboulleux S, Travagli JP, Caillou B, Laplanche A, Bidart JM, Schlumberger M, Baudin E** 2002 Medullary thyroid carcinoma as part of a multiple endocrine neoplasia type 2B syndrome: influence of the stage on the clinical course. Cancer 94:44-50
42. **Machens A, Brauckhoff M, Holzhausen HJ, Thanh PN, Lehnert H, Dralle H** 2005 Codon-specific development of pheochromocytoma in multiple endocrine neoplasia type 2. J Clin Endocrinol Metab 90:3999-4003
43. **Chen H, Sippel RS, O'Dorisio MS, Vinik AI, Lloyd RV, Pacak K** 2010 The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: pheochromocytoma, paraganglioma, and medullary thyroid cancer. Pancreas 39:775-783
44. **Ilias I, Pacak K** 2009 Diagnosis, localization and treatment of pheochromocytoma in MEN 2 syndrome. Endocr Regul 43:89-93
45. **Ilias I, Chen CC, Carrasquillo JA, Whatley M, Ling A, Lazurova I, Adams KT, Perera S, Pacak K** 2008 Comparison of 6-18F-fluorodopamine PET with 123I-metaiodobenzylguanidine and 111in-pentetreotide scintigraphy in localization of nonmetastatic and metastatic pheochromocytoma. J Nucl Med 49:1613-1619
46. **Pacak K** 2007 Preoperative management of the pheochromocytoma patient. J Clin Endocrinol Metab 92:4069-4079
47. **Kraimps JL, Denizot A, Carnaille B, Henry JF, Proye C, Bacourt F, Sarfati E, Dupond JL, Maes B, Travagli JP, Boneu A, Roger P, Houdent C, Barbier J, Modigliani E** 1996 Primary hyperparathyroidism in multiple endocrine neoplasia type IIa: retrospective French multicentric study. Groupe d'Etude des Tumeurs a Calcitonine (GETC, French Calcitonin Tumors Study Group), French Association of Endocrine Surgeons. World J Surg 20:808-812; discussion 812-803
48. **Raue F, Kraimps JL, Dralle H, Cougard P, Proye C, Frilling A, Limbert E, Llenas LF, Niederle B** 1995 Primary hyperparathyroidism in multiple endocrine neoplasia type 2A. J Intern Med 238:369-373
49. **Peacock M, Bilezikian JP, Klassen PS, Guo MD, Turner SA, Shoback D** 2005 Cinacalcet hydrochloride maintains long-term normocalcemia in patients with primary hyperparathyroidism. J Clin Endocrinol Metab 90:135-141