Medullary thyroid carcinoma (MTC) arises from thyroid C cells that secrete calcitonin (CT). It accounts for only about 5% of thyroid carcinomas in the United States (Hundahl 1998), but has aroused considerable interest because of its distinctive biochemical, genetic and clinical features. Although this is usually a sporadic tumor, some are familial tumors that occur as a result of autosomal dominant genetic mutations in the RET protooncogene that produce unique clinical syndromes (Dunn 1993). The explication of the genetic basis of MTC has revolutionized management of the familial form of this tumor and has provided insight into its pathogenesis and clinical behavior. In this chapter we review the important clinical characteristics, hereditary and sporadic forms of the disease, and its biochemical and molecular diagnosis, treatment and follow-up. Several recent publications summarize the major advances in this field, (Eng 1996; Machens 2003b; Massoll 2004) and the Seventh International Workshop on Multiple Endocrine Neoplasia held in Gubbio, Italy in 1999 provides some consensus on the diagnosis and therapy of familial MTC (Brandi 2001), although major questions remain concerning the timing of thyroidectomy in certain gene carriers.

PATHOLOGY

C-cell Hyperplasia and MTC

RET germ-line mutations in humans affect four major types of tissues that originate from neural crest cells: thyroid C cells, parathyroid cells, chromaffin cells of the adrenal medulla, and enteric autonomic plexus (Eng 1996). MTC, which arises from thyroid
C cells, is mainly found in the upper third of the thyroid lobes. In familial disease, this is the site of its first identifiable manifestation: C cell hyperplasia (CCH), which is a precursor of familial MTC that progresses to microscopic MTC (Modigliani 1998). The progression of CCH to MTC occurs at different rates depending on the RET mutation (Machens 2003b). Hereditary MTC is thus bilateral and multicentric, whereas sporadic MTC is generally manifest as a single thyroid tumor (Beressi 1998; Bachelot 2002).

A wide spectrum of histologic patterns may be seen with MTC. Although lymph node metastases are rarely present when MTC is diagnosed early by genetic screening (Wells Jr 1994), they are almost always present when the tumor is palpable, whether it is sporadic or familial MTC. The tumor typically metastasizes to lymph nodes in the central and lateral cervical compartments, to mediastinal lymph nodes, or to the lung, liver or bone.

In fine-needle aspiration (FNA) cytology samples, MTC cells may appear cuboidal, spindled or plasmacytoid. MTC tends to be over-diagnosed by cytology because it may mimic a variety of benign and malignant entities and should therefore be confirmed by immunohistochemical staining for CT.

CCH is usually diagnosed when more than 6 C-cells are seen per thyroid follicle and/or more than 50 intrafollicular CT-positive cells are seen in at least one low-power (100x) field. CCH can be confirmed by a immunohistochemical reaction for CT and can range in appearance from mild to diffuse CCH, which can develop into nodules that replace preexisting follicular epithelium (Hinze 1998). The transition from benign CCH to invasive MTC is marked by disruption of the follicular basement membrane by C-cells. Familial tumors undergo a transition from a RET mutation that leads to early clonal C-cell expansion, which then proceeds to transformation from neoplastic CCH to MTC, and eventually to lymph node and distant metastases, all proceeding at strikingly different rates with different RET mutations (Machens 2003a, 2003b).

HORMONAL ACTIVITY OF MTC

MTC secretes several proteins in addition to CT, including ACTH, CEA, histamines and vasoactive peptides, but clinically the most important is CT, which serves as the major clinical marker for the tumor. In fact, plasma CT levels correlate closely with MTC size (Engelbach 2000), especially in familial cases, and preoperative CT levels <50 pg/mL predict postoperative normalization of CT (Cohen 2000).

SURVIVAL RATE OF PATIENTS WITH MTC

The 10-year survival rate of patients with MTC ranges from about 50% to 80%, and averaged 75% in over 2,000 cases of MTC in a national cancer database with 53,856 cases of thyroid carcinoma treated in the US between 1985–1995 (Hundahl 1998). Survival rates are tightly linked to early diagnosis and tumor stage, and vary significantly among patients with sporadic and familial MTC (Cohen 2000; Brandi 2001). Early thyroidectomy has lowered the mortality rate of hereditary MTC to less than 5%, well
below that in sporadic cases; however, the longest follow-up period of survival with MTC, done well before current screening methods, is less than 25 years (Gagel 1988).

SPORADIC MTC

Clinical Presentation

Sporadic MTC tends to be unifocal without prominent CCH, and is not associated with other endocrine tumors. Dense tumor calcifications may be apparent on ultrasound or other imaging studies (Tokuue 1990; Yokozawa 1996). Sporadic MTC usually presents at about 55 years of age, and at a more advanced stage than familial MTC (Beressi 1998). The tumor is usually palpable in sporadic MTC, and up to 80% is metastatic to cervical lymph nodes and in 20% of the cases to distant sites (Moley 1999). Advanced stage tumors may be rapidly growing and associated with hoarseness, dysphagia or other symptoms of invasion or may present with systemic symptoms of diarrhea, flushing and bone pain (Kebebew 2000; Dolan 2000). Most patients, however, do not have these symptoms, but simply present with a long-standing multinodular goiter or an asymptomatic thyroid nodule.

Diagnosis of CCH and MTC

Sporadic MTC is usually diagnosed by FNA of a palpable thyroid nodule or lymph node; whereas CCH is usually apparent only on histochemical staining of the permanent surgical pathology sections (Aulicino 1998). In some situations accurate FNA diagnosis requires a more objective method than cytological examination alone. RET somatic mutations at codon 918, which occur only in the tumor and are not present in peripheral blood cells, can sometimes be detected in the tumor cells rinsed from the needle after preparing slides from an FNA of a malignant nodule (Russo 1997), establishing a diagnosis of MTC before surgery. Plasma CT or CEA mRNA can also be used for this purpose (Takano 1999). Routine preoperative plasma CT measurements may be the only clue to a diagnosis of MTC in a multinodular goiter (Elisei 2004), although normal CT levels do not rule out MTC (Redding 2000). European endocrinologists widely advocate routinely measuring plasma CT in all patients who undergo FNA for multinodular goiter (Henry 1996; Ozgen 1999; Bonnema 2000; Elisei 2004) but most American endocrinologists do not do this for a variety of reasons (Hodak 2004), but mainly because patients without MTC often have high plasma CT levels and the diagnosis often must be confirmed by pentagastrin injection, which stimulates plasma CT to rise only in CCH and MTC, but the drug is not available in the U.S.A.. Still, the index case of an MTC kindred may be identified by measuring plasma CT levels in a patient with an isolated thyroid nodule in which MTC is not suspected (Mayr 1999). This is a major conundrum (Hodak 2004).

Factors Affecting Mortality Rates with Sporadic MTC

In some cases survival with MTC is prolonged, even with distant metastases, whereas others die within a few years of diagnosis. A French study (Cohen 1996) of 119 deceased
patients with MTC showed that the tumor was usually the cause of death (87%). Prog-
nosis depends on the clinical form of the disease (sporadic or familial), the patient’s age at the time of diagnosis, the tumor stage at the time of surgery, including size, the presence of local tumor invasion, lymph node and distant metastases, and the extent of surgery (Hyer 2000). Although 10-year mortality rates average about 75%, when sporadic MTC presents with systemic symptoms of diarrhea, bone pain, or flushing and is widely metastatic, 33% die within 5 years (Kebebew 2000).

**Initial Management of Sporadic MTC**

Surgery is the only completely effective form of therapy. Total thyroidectomy and central neck dissection is the minimum surgical procedure that should be performed (Wells Jr 1994; Kebebew 2000; Hyer 2000). Preoperative staging and the extent of previous surgery determine the need for further neck dissection, and if metastases are identified in the lateral neck, compartment-oriented lymphadenectomy is advised (Kebebew 2000; Weber 2001; Franc 2001). Modified radical neck dissection provides the best outcomes in patients with lateral lymph nodes metastases (Kebebew 2000; Hyer 2000; Weber 2001). Preoperative plasma CT levels predict tumor size and postoperative normalization of CT (Pentagastrin-stimulated CT < 10 pg/mL) (Cohen 2000) but this does not always predict freedom from recurrence (Franc 2001), and long-term follow-up is always necessary.

External beam radiotherapy (EBRT), which is effective in eradicating foci of residual tumor, is indicated when surgical excision is incomplete (Rougier 1983; Sarrazin 1984). It significantly reduces local relapse in those with ipsilateral lymph node metastases (Rougier 1983; Hyer 2000). Chemotherapy has little effect on MTC. Although some have advocated I-131 therapy, there are neither data nor enthusiasm to support its use (3).

**FAMILIAL MTC: MULTIPLE ENDOCRINE NEOPLASIA TYPE2 (MEN2) SYNDROMES**

The MEN2 syndromes comprise a clinical framework to conceptualize the manifesta-
tions of the familial RET protooncogene mutations, thus providing clinicians the basis for an approach to patients with these disorders.

**Classification of MEN2 Syndromes (Table 1)**

Nearly 40% of MTC cases are inherited as one of several autosomal dominant syndromes, which affect about 1,000 kindreds around the world (Eng 1996; Brandi 2001). The familial MTC syndromes occur either as part of multiple endocrine neoplasia (MEN) type 2A or 2B syndromes or as familial MTC without other endocrine tumors (FMTC, Table 1). Different RET germ-line mutations are responsible for each these syndromes, but the specific mutations and clinical features of the syndromes differ substantially among affected families (Phay 2000).

MTC is the main tumor manifested in these syndromes, but its expression is variable. MTC appears at different times during life and displays different growth rates according to the specific RET mutation. Still, the penetrance of MTC is high enough that 90% of the carriers eventually develop a palpable tumor or blood CT abnormality if the trait is
### Table 1. Clinical Syndromes Associated with MTC and the Exons with Mutations Involved.

<table>
<thead>
<tr>
<th></th>
<th>MEN2A</th>
<th>FMTC</th>
<th>MEN2B</th>
<th>Sporadic MTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTC Incidence</td>
<td>100%</td>
<td></td>
<td>0–20</td>
<td>100%</td>
</tr>
<tr>
<td>Multicentricity</td>
<td>100%</td>
<td></td>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td>Bilaterality</td>
<td>100%</td>
<td></td>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td>C-Cell Hyperplasia</td>
<td></td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of Onset (years)</td>
<td>20–30</td>
<td>40–50</td>
<td>0–20</td>
<td>40</td>
</tr>
<tr>
<td>Clinical Features</td>
<td>MTC Pheochromocytoma (50%)</td>
<td>MTC</td>
<td>MTC Pheochromocytoma (50%)</td>
<td>MTC</td>
</tr>
<tr>
<td></td>
<td>Parathyroid hyperplasia (20%–30%)</td>
<td></td>
<td>Mucosal Neuromas (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Marfanoid habitus (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ganglioneuromas</td>
<td></td>
</tr>
<tr>
<td>Inheritance</td>
<td>Autosomal Dominant</td>
<td>Autosomal Dominant</td>
<td>Autosomal Dominant</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Affected gene</td>
<td>RET</td>
<td>RET</td>
<td>RET</td>
<td>RET</td>
</tr>
<tr>
<td>Exons</td>
<td>G* 10,11</td>
<td>G* 13,14,15</td>
<td>G* 16,15</td>
<td>S† 10,11,13,14,15,16</td>
</tr>
<tr>
<td>Codons</td>
<td>609‡, 611‡, 618‡, 620‡, 630‡, 634, 635, 637, 790‡, 791 and 804‡, 891‡</td>
<td>532‡, 609‡, 611‡, 618‡, 620‡, 630‡, 790‡, 768‡, 791†, val804met, 844§, A891S</td>
<td>918, 833</td>
<td>918 (25%) 664, or A833F (rare)</td>
</tr>
</tbody>
</table>

* G = Germline mutations.
† S = Somatic mutations.
‡ Mutations that may be found in families with either MEN2A or FMTC phenotypes. Accordingly, all patients with these mutations should be screened for pheochromocytoma.
§ Mutations associated exclusively with FMTC.
†† 844 mutation, see footnotes 1 & 2 in text.
not identified early in life by genetic testing (Brandi 2001). MTC appears earlier or at the same time as pheochromocytoma and hyperparathyroidism (HPT), which are the other two neoplasms associated with the MEN2 syndromes.

MEN2A comprises over 75% of the MEN2 cases (Eng 1996; Brandi 2001). Other less common variants of MEN2 are:

1) FMTC (Siggelkow 2001)
2) MEN2A with cutaneous lichen amyloidosis (Pacini 1993)
3) MEN2A or FMTC with Hirschsprung’s disease (Blank 1996; Eng 1996)

MEN2A CARRIERS HAVE:

1) Bilateral MTC thyroid tumors before age 10
2) Pheochromocytomas (about 50% develop unilateral or bilateral tumors) (Melvin 1972; Gagel 1988; Schuffenecker 1998b; Brandi 2001)
3) Multiple parathyroid tumors that produce HPT (about 30%) (Melvin 1972; Gagel 1988; Schuffenecker 1998a; Schuffenecker 1998b)

MEN2B is the most distinctive and aggressive form of familial MTC. Only few MEN2B children undergoing thyroidectomy for MTC after the first several years of life are cured; many experience recurrence or death within several decades of initial surgery (Carney 1978; Skinner 1996). The causes of MEN2B deaths in one study (Carney 1978) were MTC (15 deaths), pheochromocytoma (10 deaths) and alimentary tract complications (2 deaths). Children with MEN2B commonly develop microscopic MTC, sometimes with metastases, during the first year of life (Smith 1999; Sanso 2002).

This syndrome can be recognized at the bedside. Persons affected with MEN2B have:

1) Long bones, ribs, and skull, which results in a marfanoid habitus with a decreased upper/lower body ratio, and increased laxity of joint ligaments.
2) Thickened corneal nerves.
3) Mucosal neuromas.
4) Ganglioneuromas in the lips, tongue and conjunctiva and digestive tract, conjunctiva, lips and tongue (Fig. 1), and also in the salivary glands, pancreas, gallbladder, upper respiratory tract and urinary bladder.
5) Pheochromocytoma (half the carriers).
6) None have HPT.

FMTC is not associated with pheochromocytoma or HPT.

1) MTC has a high penetrance in all MEN2 syndromes but is manifest at an older age and is clinically more indolent in FMTC than it is in sporadic tumors or in other MEN2 syndromes.
2) MTC is usually the initial neoplastic manifestation in MEN2 syndromes, often before pheochromocytoma develops, and it is easy to mistake a small MEN2A kindred for an FMTC kindred, with the resulting danger that the diagnosis of pheochromocytoma may not be considered prior to surgery (Brandi 2001).

Categorization of an MEN kindred as FMTC, according to the 1999 International Multiple Endocrine Neoplasia meeting in Gubbio, Italy (Brandi 2001), requires fulfillment of all of the following criteria:

1) More than 10 carriers in the kindred.
2) Multiple carriers or affected members over age 50 years.
3) An adequate medical history, particularly in older kindred members.

These conservative criteria deliberately categorize small FMTC kindreds as MEN2A to avoid missing occult pheochromocytomas. Others (Moers 1996) suggest that MEN2
should not be subclassified as MEN2A or FMTC, but rather should be subclassified according to their specific RET mutation because the difference in outcome among kindreds with different RET mutations is so unique, which is important in making decisions about prophylactic thyroidectomy in children with a RET mutation.

**RET Mutations**

RET, the predisposing gene for inherited MTC, is located in the pericentric region of chromosome 10q11.2 and consists of 21 exons encoding a plasma membrane-bound tyrosine kinase enzyme termed ret. This membrane-associated protein contains an extracellular domain, a transmembrane domain and an intracellular domain (Fig. 2). Familial
MTC results from single RET point mutations that change one amino acid that activates the ret tyrosine kinase receptor. Activating germline mutations of the RET protooncogene are found in 98% of families with MEN2A and FMTC (Table 1) (Brandi 2001). There is a close relationship between genotype and phenotype expression (Eng 1996).

FMTC is the most difficult of the MEN2 kindreds to identify. By 2001 (3) pheochromocytoma had been found in kindreds with all RET mutations except those in codons 609, val804met and 891, suggesting that these are the only mutations causing FMTC. At present, RET mutations in FMTC are thought to occur at exon 13 in codons 609 and at exon 14 in codons 804 (Bartsch 2000; Siggelkow 2001), 844 (Bartsch 2000) and/or with S836S polymorphism. families have a less virulent form of MEN2A that can be mistaken for FMTC (Table 1) (Machens 2001; Gimm 2002; Fitze 2002). This is important because operative deaths can occur from hypertensive crises in MEN2A patients mistakenly thought to have FMTC.

Over 95% of MEN2B cases are due to a single germline point mutation in the intracellular tyrosine kinase region of the RET protooncogene, which is frequently a \textit{de novo} mutation located on an allele inherited from the patient’s father and is associated with advanced paternal age (Carlson 1994). A few cases have been reported with other mutations (Table 1) In comparison with mutations in exon 11, those with mutations in exons 13 and 14 or with MEN2B phenotypic syndrome have MTC that occurs earlier in life and behaves more aggressively (Carlson 1994; Bolino 1995; Eng 1996).

**Genotype-phenotype Correlations in MTC Kindreds**

Correlations between tumor behavior in MEN2 kindreds and the RET mutation causing the syndrome provide powerful insight into the clinical management—screening, surveillance, and prophylaxis paradigms—of individuals with familial MTC (Eng 1996) and are more likely than CT testing to identify the correct selection and timing of patients for surgery. The clinically relevant features of specific RET mutations that induce unique clinical manifestations in large MTC kindreds provides and prognostic information that bears directly on the selection and timing of prophylactic thyroidectomy in children and young adults.

**MTC WITH HIRSCHSPRUNG’S DISEASE.** The rare cases of Hirschsprung’s disease with exon 10 germline mutations identical to those found in hereditary MTC (Blank 1996) has resulted in an international consensus recommendation that germline (blood) testing for RET mutations be done at exon 10 in codons 609, 618 and 620 in all children with Hirschsprung’s disease.

**PHEOCHROMOCYTOMA** is an important cause of morbidity and mortality in MEN2 carriers. In the past when MTC was identified late in its course with CT testing, sudden death often occurred from pheochromocytoma, perhaps as often as that from MTC (Gagel 1988). One large study (Modigliani 1995a) found that in about 25% of the cases, the manifestations of pheochromocytoma occurred 2 to 15 years before the diagnosis of MTC and were identified simultaneously with MTC in 35% and 2 to 11 years after MTC in 40%; about 68% of the pheochromocytomas were bilateral
and 4% were malignant. In a more recent prospective study (Modigliani 1995b) in which MTC was identified by genetic testing, pheochromocytomas were identified simultaneously with the MTC in half the cases but the other half was detected during follow-up after MTC had been identified. The same was true for bilaterality: adrenal tumors were initially found to be bilateral in almost 80% while bilaterality became manifest in the others during follow-up. The presentation of pheochromocytoma is thus highly variable and unpredictable necessitating regular long-term clinical and biological monitoring.

SCREENING FOR PHEOCHR OMCYTOMA. Plasma free metanephrine, which is the best test for excluding or confirming pheochromocytoma (Lenders 2002), should be done in all MEN2A and MEN2B patients and should be done as follows:

1) In carriers with high-risk codons for pheochromocytoma, screening should begin at the age when thyroidectomy would be considered or by the age of 5 to 7 years, whichever is earlier, and should be done annually thereafter (Skinner 1996).

2) In families at less risk for pheochromocytoma, especially those with codons 609, 768, val804met, and 891, screening may be initiated at an older age, depending on the familial pattern of the pheochromocytoma. There is no consensus on the best imaging studies for pheochromocytoma, although most use abdominal CT (Skinner 1996).

TREATMENT OF PHEOCHR OMCYTOMA With high metanephrine levels or symptoms consistent with pheochromocytoma, a retroperitoneal imaging study (Computed tomography or MR) should be performed, although many also use MIBG (meta-iodo benzyl guanidine) scanning for preoperative localization. All patients with evidence of excessive catecholamine production should receive appropriate medical therapy with α-adrenergic antagonist before adrenal surgery. Laparoscopic adrenalectomy is now the procedure of choice for patients with unilateral pheochromocytoma with bilateral or unilateral adrenal tumors. However, adrenal insufficiency remains a major problem.

HYPERPARATHYR OIDISM (HPT) IN MEN2A HPT occurs in 20-30% of MEN2A patients (Melvin 1972). It is found with the highest frequency in those with any codon 634 mutation. Most patients are asymptomatic, although hypercalciuria and renal calculi may occur. HPT is milder in MEN2A than it is in MEN1.

SCREENING FOR AND TREATMENT OF HPT IN MEN2A Since MEN2A carriers are more likely to have HPT if they have a mutation causing any amino acid substitution in RET codon 634 (Eng 1996; Schuffenecker 1998b), they should be screened annually. Those with mutations at codons 609, 611, 618, 620, 790, and 791, which are less often associated with HPT, require less frequent screening for HPT, perhaps every 2 years (Skinner 1996). Screening for HPT is done with plasma PTH and calcium.

TREATMENT OF HPT IN MEN2A The diagnosis of HPT and indications for parathyroid surgery are similar to those for sporadic HPT (O’Riordain 1993; Raue 1995; Kraimps 1996; Bilezikian 2002). Although fewer than four parathyroid glands may be enlarged,
the consensus is that all four glands should be identified at parathyroid surgery (Brandi 2001). The indications and operations—resection of only enlarged glands, subtotal parathyroidectomy, and parathyroidectomy with autotransplantation—should be similar to those in other patients with a potential for multiple parathyroid tumors (Brandi 2001). If the surgeon encounters one or more parathyroid tumors during surgery for MTC in a patient with MEN2, they should be excised as would be done if there is biochemical evidence of mild HPT (Brandi 2001).

**DIAGNOSIS AND MANAGEMENT OF FAMILIAL MTC**

Studies of MEN2 families have demonstrated a direct correlation between early diagnosis of MTC and outcome (Tashjian 1968; Melvin 1972; Jackson 1973; Gagel 1975; Gagel 1987; Lips 1994; Niccoli-Sire 1999; Brandi 2001). Prevention or cure of MTC is mainly dependent upon the adequacy and success of the initial operation, which in turn is dependent on early diagnosis and low tumor stage (Wells Jr 1994).

**RET Testing**

Early detection and intervention alters the clinical course of MTC (Gagel 1988; Gagel 1995). This can only be done with RET testing. Potential carriers at risk for a specific RET mutation can be identified by direct DNA blood testing, providing an opportunity for prophylactic thyroidectomy before MTC develops. In an international workshop in 1997 (Lips 1998), a consensus was reached that the decision to perform thyroidectomy in MEN2 carriers should be based predominantly on the result of a RET mutation rather than CT testing. This was reaffirmed at the Seventh International Workshop on Multiple Endocrine Neoplasia held in Gubbio, Italy in 1999 (Brandi et al, 2001). This recommendation stems from several unique features of MEN2:

1) Children operated upon in their teenage years in the era of provocative CT testing usually experienced long-term cure, but many were identified only after MTC had developed. In one long-term study, for example, when surgery was recommended for any elevation in annual provocative CT testing of MEN2 kindred, 77% of the children already had MTC at the time of surgery, some of which were macroscopic tumors; moreover, recurrent disease developed in 24% (Iler 1999).

2) Provocative CT testing of select patients for thyroidectomy is associated with an incidence of false positive tests as high as 10%, which may result in unnecessary thyroidectomy (Lips 1994; Brandi 2001).

3) RET testing has a higher true positive rate and a lower false negative rate than any other test, thus facilitating earlier thyroidectomy in carriers (Wells Jr 1998; Van Heurn 1999; Heptulla 1999; Niccoli-Sire 1999).

Table 2. Indications for RET Testing.

RET Testing is Indicated in:
- Patients with presumed sporadic MTC
- Members of known MTC kindreds
- All patients with pheochromocytoma
- Children with Hirschsprung's disease

RET Testing is not Recommended in:
- Patients with apparently sporadic hyperparathyroidism

Indications for RET Mutation Testing (Table 2)
To properly manage kindred with MEN2A or FMTC, a RET

1) RET analysis should always be first performed on the index case, even if the MTC appears to be sporadic (Olson 1992; Komminoth 1995; Fink 1996).
2) A RET analysis should always be done in patients with sporadic MTC, even when the family history appears to be negative, because such patients often have germline RET mutations identifying them as an index case for an unrecognized MEN2 kindred (Fitze 2002). The likelihood of a RET germline mutation in an individual with a supposedly sporadic MTC is between 1% and 7% (Brandi 2001). Certain RET mutations frequently present as MTC in a long-standing multinodular goiter (Niccoli-Sire 2001).
3) All patients with ostensibly sporadic pheochromocytoma or Hirschsprung's disease should be tested for germline MEN2 RET mutations (Brandi 2001; Neumann 2002).

Method of RET Testing

**TESTING THE INDEX CASE** The leukocytes of suspected carriers should be tested for MEN2-associated germline mutations by polymerase chain reaction amplification of the appropriate RET gene exons and direct DNA sequencing, which is a practical means of identifying the mutation since all known mutations are found in exons 10, 11, 13, 14, 15 and 16 (Table 1). If these exons prove to be negative, the other 15 should be sequenced, which is only available in research laboratories (Brandi 2001). It is particularly important to examine exons 13, 14 and 15 because mutations in these exons are likely to cause MTC with a low prevalence of pheochromocytoma that is likely to escape recognition as a familial disorder (Brandi 2001).

**TESTING FAMILY MEMBERS** When a RET mutation is found in an index case:

1) All first degree relatives must be screened to determine which individuals carry the gene. This is performed twice and on separate blood samples to exclude errors.
2) Theoretically, half of first degree relatives do not carry the mutated gene and their risk of developing the disease is similar to that of the general public.

A small risk of hereditary MTC remains if no germline mutation is found. The probability that a first-degree relative will inherit an autosomal dominant gene for MTC
from an individual with sporadic MTC in whom no germline mutation is found is 0.18% (Brandi 2001).

**ANALYSIS FOR RET MUTATIONS IN TUMOR TISSUE** from ostensibly sporadic cases of MTC has limited value in identifying an index case, but may provide a substitute if peripheral blood from an affected person is not available. However, somatic mutations in RET, predominantly at codon 918, and very rarely at codon 883 have been found in nearly 90% of the thyroid tumors of sporadic MTC cases in which case the peripheral blood tests are negative for germline RET mutations (Eng 1998; Gimm 1999). Somatic codon 918 mutations, which can be identified by RET immunohistochemical staining, (Eng 1998), are more aggressive and may metastasize earlier and be more lethal than other somatic MTC tumors (Eng 1998); however, whether identifying this somatic mutation will enhance management is unclear.

**Calcitonin Testing**

This peptide hormone is secreted by the C cells of both CCH and MTC, which in the past was used to detect MEN2 carriers. Although RET testing identifies carriers much earlier and more reliably (Lips 1994), there still are some indications for CT testing for CCH or MTC; however, affected individuals often have normal basal plasma CT levels, and it is necessary to use intravenous pentagastrin, calcium, or both to stimulate CT secretion from MTC or hyperplastic C-cells. Omeprazole may be used for this purpose when pentagastrin is contraindicated, unavailable, or refused because of its unpleasant side effects, but pentagastrin produces a significantly greater rise in CT (Vitale 2002). The indications for CT testing are as follows:

1) Screening MEN2 family members in which the RET mutation has not been identified.
2) Testing carriers from MEN2 kindred with MTC that has displayed an indolent course presenting later in life, so children can undergo thyroidectomy when they are in the second decade of life.
3) Pre- and postoperative CT correlates with the extent of tumor and may distinguish between macroscopic and microcarcinoma MTC or identify CCH (Cohen 2000) or may suggest metastases (Pomares 2002).

**Prognosis of Familial MTC**

Prognosis is related to tumor stage (Modigliani 1998) and the plasma CT level (Pomares 2002). When plasma CT is used preoperatively to identify indolent forms of MTC, surgery is usually indicated when the pentagastrin-stimulated CT rises $>10$ pg/ml (Niccoli-Sire 1999). Preoperative CT levels are predictive of postoperative

---

1 Pentagastrin peptide for testing is currently not available in the USA. Give 0.5 µg/Kg IV push, measure plasma calcitonin at 1, 2, 5, 10 minutes. Normal response in men is $<210$ pg/mL, women $<105$ pg/mL.

2 Calcium 2 mg/kg IV push, measure plasma calcitonin at 1, 2, 5, 10 minutes. Normal response in men is $<265$ pg/mL, women $<120$ pg/mL.

3 Combined test use calcium immediately followed by Pentagastrin, measure plasma calcitonin at 1, 2, 5, 10 minutes. Normal response is $<300$ pg/ml for men and women.
CT normalization. The French Calcitonin Tumor Study Group (Cohen 2000) found that a preoperative plasma CT level $<50 \text{ pg/mL}$ was predictive of postoperative CT $<10 \text{ pg/mL}$ although a higher level did not necessarily mean that the postoperative CT would not fall to $<10 \text{ pg/mL}$. Postoperatively, any rise in plasma CT may be indicative of persistent MTC, but false-positive CT measured by RIA is common (Lips 1994; Scheuba 1999).

**INITIAL MANAGEMENT OF MEN2 SYNDROMES BASED UPON CLASSIFICATION OF FAMILIAL SYNDROMES**

**The Goal of Management**

The goal is to prevent or cure MTC in all MEN2A carriers by performing genetic testing and thyroidectomy during early childhood (Brandi 2001). Total thyroidectomy including the posterior capsule will usually remove all normal and malignant C-cells and will prevent MTC from developing (Bachelot 2002).

**Timing of Surgery**

**GENERAL RECOMMENDATIONS BASED UPON MEN2 STRATIFICATION**

The timing of surgery in MEN2 carriers continues to be refined, because genotype-phenotype correlations and gene penetration are not always predictable, but is different among MEN2 kindreds (Fig. 3). Differing recommendations among groups, mainly on the timing of surgery, are summarized below.

**MEN2A.** MTC associated with any RET mutation in codon 634 commonly appears before the age of 10 and has been reported to occur in children as young as 17 months but is rarely metastatic before the age of 14 years (Fig. 4) (Machens 2003b). According to recent consensus guidelines (Brandi 2001), thyroidectomy should be done before age 5 years for children with MEN2A.

**FMTC.** MTC usually becomes manifest in the third or fourth decade of life and has an indolent course (Fugazzola 2002). Pentagastrin testing has been recommended every other year in gene carriers identified by genetic testing, usually starting around 10 years of age (Brandi 2001). Thyroidectomy should be performed only after the pentagastrin test becomes positive (CT $>10 \text{ pg/mL}$) or during the third or fourth decade of life when the disease is known to progress, whichever comes first (Bachelot 2002). This may be modified depending on the genotype and the clinical behavior of MTC in affected families (Oriola 1998; Siggelkow 2001; Fitze 2002).

**MEN2B.** Carriers have more advanced tumor than those with MEN 2A, in spite of presenting at a younger age (O’Riordain 1994). Children with a MEN2B with RET mutations at codons 918 or 883 are at highest risk for having aggressive MTC (O’Riordain 1994; Sanso 2002) and should ideally undergo routine total thyroidectomy, including the posterior thyroid capsule, within the first 6 months of life (Brandi 2001; Lebouleux 2002) and preferably within the first month of life (Brandi 2001). This is justified in infants regardless of serum CT levels because microscopic MTC in the first year of life is common and is sometimes associated with metastases. Still, this is difficult surgery that can be done safely in only a few centers. In a study (Lebouleux 2002) of MEN2B patients, aged 2 to 27 years in which most had a 918 mutation in exon 16 and the
identification of MTC was based on the presence of a thyroid nodule or involved neck lymph nodes or on dysmorphic features of MEN2B, most had Stage 3 or 4 tumors at surgery, further confirming the need for early treatment of MTC.

**THYROID MANAGEMENT BASED ON STRATIFIED GENETIC INFORMATION** Machens et al. (2001) on the basis of careful follow-up of a cohort of 63 patients recommended a more individualized approach to the timing and extent of prophylactic surgery. They devised three MTC risk groups according to genotype:

1. **High risk** (codons 634 and 618) with the youngest ages being 3 and 7 years at MTC diagnosis.
2. **Intermediate risk** (codons 790, 620, and 611) with ages of 12, 34, and 42 year at diagnosis.
3. **A low risk** (codons 768 and 804) with ages of 47 and 60 yr, at diagnosis, respectively.

**THE SEVENTH INTERNATIONAL WORKSHOP ON MULTIPLE ENDOCRINE NEOPLASIA RECOMMENDATIONS** The conference held in Gubbio, Italy (Brandi 2001) advocates prophylactic total thyroidectomy before the age of five years in patients with mutations in RET codon 611, 618, 620, or 634 (Table 3). However, the participants in this workshop failed to reach an agreement on the approach to children with codon 609, 768, 790, 791, 804, or 891 mutations; the recommended age for prophylactic total thyroidectomy ranged from 5 to 10 years. Moreover, they did not reach a consensus on the need for prophylactic dissection of the central cervical lymph node compartment in patients with MEN2A, with differences of opinion ranging between surgeons and internists regarding
Table 3. Recommendations for Prophylactic Testing and Surgery According to RET Mutations.

<table>
<thead>
<tr>
<th>RET mutation</th>
<th>Affected exon</th>
<th>International workshop recommendations for prophylactic thyroidectomy*</th>
<th>EUROMEN recommendations for prophylactic thyroidectomy</th>
<th>Earliest reported age of MTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>609</td>
<td>10</td>
<td>Before age 5–10 years†</td>
<td>Yes but not before age 10 years§</td>
<td>5 years</td>
</tr>
<tr>
<td>611</td>
<td>10</td>
<td>Before age 5 years</td>
<td>Not before age 5 years</td>
<td>7 years</td>
</tr>
<tr>
<td>618</td>
<td>10</td>
<td>Before age 5 years</td>
<td>Not before age 5 years</td>
<td>7 years</td>
</tr>
<tr>
<td>620</td>
<td>10</td>
<td>Before age 5 years</td>
<td>Not before age 5 years</td>
<td>11 years</td>
</tr>
<tr>
<td>630</td>
<td>11</td>
<td>No recommendation‡</td>
<td>Yes but not before age 10 years§</td>
<td>15 years</td>
</tr>
<tr>
<td>634</td>
<td>11</td>
<td>Before age 5 years</td>
<td>Before age 5 years</td>
<td>15 months</td>
</tr>
<tr>
<td>768</td>
<td>13</td>
<td>Before age 5–10 years†</td>
<td>Yes but not before age 10 years§</td>
<td>&gt;20 years</td>
</tr>
<tr>
<td>790</td>
<td>13</td>
<td>Before age 5–10 years†</td>
<td>Yes but not before age 10 years§</td>
<td>12 years</td>
</tr>
<tr>
<td>791</td>
<td>13</td>
<td>Before age 5–10 years†</td>
<td>Yes but not before age 10 years§</td>
<td>13 years</td>
</tr>
<tr>
<td>804</td>
<td>14</td>
<td>Before age 5–10 years†</td>
<td>No explicit recommendation§</td>
<td>6 years</td>
</tr>
<tr>
<td>891</td>
<td>15</td>
<td>Before age 5–10 years†</td>
<td>Not before age 10 years‡</td>
<td>13 years</td>
</tr>
<tr>
<td>918{MEN2B}</td>
<td>16</td>
<td>Before age 1 years (preferable)</td>
<td>No explicit recommendation‡</td>
<td>9 months</td>
</tr>
</tbody>
</table>

* Total thyroidectomy including the posterior thyroid capsule. No consensus was reached regarding the need for prophylactic dissection of the central lymph nodes.
** Plasma free metanephrine is the best test for pheochromocytoma and should always be done preoperatively. It should be done in all MEN2A and 2B patients. In carriers with high-risk codons for pheochromocytoma, screening should begin at the age when thyroidectomy would be considered or by the age of 5 to 7 years, whichever is earlier, and should be done annually thereafter. In families at less risk for pheochromocytoma, especially those with codons 609, 768, val804met, and 891, screening may be initiated at an older age, depending on the familial pattern of the pheochromocytoma. There is no consensus on the best imaging studies for pheochromocytoma, although most use abdominal CT (56).
† There was consensus that this group should undergo prophylactic thyroidectomy, but there was little consensus regarding the timing of surgery. Some opted for a strategy similar to the high risk group, others suggested thyroidectomy at age 10 and still others opted for periodic pentagastrin-stimulated CT testing. These patients should undergo thyroidectomy when the pentagastrin test becomes positive (CT>10 pg/mL) or during the third or fourth decade of life, whichever comes first (28).
‡ A rare mutation at exon 11 in codon 630 (Bachelot 2002) is often associated with a late appearance of MTC.
§ Includes mutations in RET codons 883 or 922 with or without somatic manifestations of MEN2B, or MTC with MEN2B phenotype.
¶ The authors conclude that their data do not support the need for prophylactic thyroidectomy in asymptomatic carriers with this mutation before the age of 10 years or from central lymph node dissection before the age of 20 years.
**** All patients should be tested for pheochromocytoma prior to surgery using plasma metanephrine levels.

whether central neck dissection should be done during the primary operative procedure. Most surgeons favored a central lymph node dissection during the primary operation because of the higher morbidity associated with reentry into the central compartment during a second procedure; whereas, internists were more concerned with the higher rate of permanent hypoparathyroidism and permanent laryngeal nerve damage associated with primary central node dissection (Brandi 2001). The consensus was to stratify
management of hereditary MTC into three levels on the basis of genetic information (Table 3) (Brandi 2001).

**Level 1** (lowest risk): Children with RET codon 609, 768, 790, 804, and 891 mutations have the least high risk among the three RET codon mutation stratifications (Shan 1998; Bartsch 2000). There was consensus that this group should undergo prophylactic thyroidectomy, but there was little consensus on the management of these mutations. Some opted for a strategy similar to the high risk group, others suggested thyroidectomy at age 10 and still others opted for periodic pentagastrin-stimulated CT testing. These patients should undergo thyroidectomy when the pentagastrin test becomes positive (CT>10 pg/mL) or during the third or fourth decade of life, whichever comes first (Bachelot 2002).

**Level 2** (intermediate risk): Children with any RET codon 611, 618, 620 or 634 mutation are classified as having high risk for MTC and should undergo thyroidectomy, including removal of the posterior capsule, before the age of 5 years.

**Level 3** (highest risk): Children with MEN2B and/or RET codon 918 or 883 mutation should have a total thyroidectomy within the first 6 months of life, preferably within the first month of life. Thyroid surgery should include a central neck dissection. If metastases are identified, more extensive neck dissection is appropriate.

**EUROPEAN MULTIPLE ENDOCRINE NEOPLASIA (EUROMEN) STUDY GROUP RECOMMENDATIONS**

A 2003 report (Machens 2003b) by the EUROMEN Study Group gives another opinion on the selection of patients for surgery and management of familial MTC. This group collected data from several European countries on 207 carriers of a RET mutation who were under age 20 years and who had undergone total thyroidectomy for MTC ≤10 mm confined to the thyroid. The most common RET codon was 634 (62.8%) followed by codon 618 (9.2%), codons 620 and 790 (6.8% each), codon 791 (2.4%), codons 609, 611, 804, and 918 (1.9% each), and codon 630 (0.5%).

There was a significant age-related progression from CCH to MTC, and eventually, to nodal metastases in patients whose RET mutations were grouped according to the extracellular- and intracellular-domain codons affected (Fig. 4). The mean age at the time of diagnosis was 8.3 years among patients who had CCH and extracellular-domain mutations and was 11.2 years among those with intracellular-domain mutations (P = 0.01). Among patients with node-negative MTC, the mean age at diagnosis was 10.2 years in those with extracellular-domain mutations and 16.6 years in those with intracellular-domain mutations (P = 0.002). The mean age at diagnosis among patients with node-positive MTC was 17.1 years in those with extracellular-domain mutations, and none of the patients with MTC and intracellular-domain mutations had nodal metastases during the first two decades of life. Still, they found that grouping the rare RET mutations as extracellular- and intracellular-domain mutation is not a useful way of identifying the optimal age at which asymptomatic carriers should undergo prophylactic thyroidectomy. The authors opine that as more clinical information emerges, some of the rare RET mutations may need to be reclassified if they turn out to behave differently from the others in that group. The EUROMEN report demonstrates the following:
Figure 4. Earliest reported age at onset of familial MTC. From the data of Machens et al. (2003b). Dark bars represent extracellular codons and light bars intracellular codons of RET (see Fig. 2).
WITH ANY CODON 634 MUTATION, REGARDLESS OF THE AMINO ACID SUBSTITUTION (FIG. 4)

1) MTC commonly appears before the age of 10 years but perhaps of greatest importance, it may occur in children as young as 17 months.
2) MTC is rarely metastatic before the age of 14 years, regardless of the amino acid substitution.
3) Nodal metastases were found an average of 6.6 years after MTC had appeared.
4) These observations support the recommendation for prophylactic thyroidectomy at least by 5 years if not earlier for carriers of the 634 RET mutation.

AMONG ASYMPTOMATIC CARRIERS OF MUTATIONS IN CODON 611, 618, OR 620

1) None had evidence of MTC before the age of 5 years, suggesting early thyroidectomy is not necessary.

AMONG ASYMPTOMATIC CARRIERS OF MUTATIONS IN CODON 609, 630, 768, 790, 791, OR 891

2) The data do not support the need for prophylactic thyroidectomy before the age of 10 years or for central lymph-node dissection before the age of 20 years.

Summary of Timing of Surgery for MTC

While these data are reassuring, they are somewhat in conflict with prior studies. A slightly different perspective is gained by combing results among the studies and showing the earliest age at which MTC has been reported for a particular RET mutation (Fig. 4), which was suggested by Cote and Gagel (2003). For example, the recommendation made at the Gubbio consensus conference of performing thyroidectomy by the age of 5 years for children with a 634 codon mutation would have missed the window of opportunity to operate on the patient before the MTC had appeared according to the data shown in Figure 4 from EUROMEN group (Machens 2003b), which shows that occasionally a child may develop MTC as young as 17 months of age. Cote and Gagel (2003) suggest that a broader experience will be required before specific recommendations can be made for each mutation, but for now, the question is whether the decision regarding thyroidectomy should be based on the average behavior of MTC in MEN-2 kindreds, or on the earliest reported age at which metastasis occurs. Cote and Gagel (2003) advise that for now physicians must chart a course that balances the risks of early metastases and the small risks and sequelae of surgery in young children against the biologic behavior of MTC in other family members. The EUROMEN recommendation and the Gubbio Workshop recommendation are thus the same for 634 carriers for surgical intervention before age 5; however, they differ and for most of the other less common RET mutations (Table 3) often beginning at 10 years of age, although such an approach would likely
be associated with metastases at the time of treatment in a small number of children (Fig. 4).

SUMMARY

Successful treatment of MTC depends heavily upon early diagnosis and treatment. Although this is not usually possible for sporadic MTC, it is achievable in MTC carriers who have genetic testing and undergo surgery before their C cells undergo malignant transformation. The following represents the highlights of management of patients with this disease:

1) All patients with MTC should be tested for RET mutations, including putative sporadic cases.
2) The leukocytes of suspected carriers and sporadic MTC cases should be tested for MEN2-associated germline mutations by polymerase chain reaction amplification of the appropriate RET gene exons including 10,11,13,14,15 and 16 (Table 1).
3) When a RET mutation is found, all first degree relatives must be screened to determine which individuals carry the gene. If these exons are negative, the other 15 should be sequenced but this is only available in research laboratories.
4) There is a 0.18% probability that a first-degree relative will inherit an autosomal dominant gene for MTC from an individual with ostensibly sporadic MTC in whom no germline mutation is found.
5) Patients with MEN2B and/or RET codon 883 or 918 mutation should have a total thyroidectomy within the first 6 months of life, preferably within the first month of life.
6) Patients with 634 mutations, which comprise about 70% of all MTC mutations, should undergo thyroidectomy by age 5 years or younger; children with this mutation as young as 17 months are reported with MTC.
7) The recommendations for the timing of prophylactic thyroidectomy are not consistent for less common mutations (Table 3, Fig. 3). There is a balance between performing prophylactic thyroidectomy earlier than the youngest age at which MTC has been reported to occur for a specific RET mutation and the high risk of complications, mainly permanent hypoparathyroidism and laryngeal nerve damage, that occur when thyroidectomy is done on very young children.
8) Initial treatment of MTC is total thyroidectomy, regardless of its genetic type or putative sporadic nature, because surgery offers the only chance for a cure.
9) Treatment with I-131 has no place in the management of MTC.
10) Plasma CT measurements provide an accurate estimate of tumor burden, and are especially useful in identifying patients with residual tumor.
11) Pentagastrin–or calcium–stimulated plasma CT testing is useful in identifying CCH or early MTC in carriers of RET mutations associated with late onset MTC, but Pentagastrin is not available in the USA.
12) Pheochromocytoma may occur before or after MTC, and is an important cause of mortality, even in young patients.
13) When a diagnosis of familial MTC has been made, preoperative measurement of plasma free metanephrine should always be done, even in FMTC kindreds.

14) HPT is an important aspect of MEN2A and requires surgery according to current guidelines for the management of primary HPT.

REFERENCES


