

# Multiple Endocrine Neoplasia Type 2

## Evaluation of the Genotype-Phenotype Relationship

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**Hypothesis:** Multiple endocrine neoplasia type 2 (MEN 2) is caused by *RET* proto-oncogene mutations and has a strong penetrance for medullary thyroid carcinoma (MTC). Subtypes are defined by the presence or absence of pheochromocytomas, hyperparathyroidism, and characteristic clinical stigmas. We hypothesize that specific *RET* mutations correlate with the MEN 2 phenotype and aggressiveness of MTC.

**Design:** Review of endocrine surgery database from 1951 through 2002.

**Setting:** Tertiary referral center.

**Patients:** Eighty-six patients from 47 kindreds were identified with MEN 2A, MEN 2B, or familial MTC. Patients were classified into 3 *RET* mutation risk groups: level 1, low risk for MTC (codons 609, 768, 790, 791, 804, and 891); level 2, intermediate risk (codons 611, 618, 620, and 634); and level 3, highest risk (codons 883 and 918).

**Main Outcome Measures:** Stage of MTC at diagno-

sis and at last follow-up and frequency of pheochromocytomas and hyperparathyroidism.

**Results:** *RET* analysis was complete for 71 patients from 39 kindreds. Multivariate analysis identified an increased likelihood of stage III or IV MTC at diagnosis with increasing age (odds ratio, 1.12 per year of age at thyroidectomy; 95% confidence interval, 1.07-1.17;  $P < .001$ ) and increasing risk group (odds ratio, 14.23 per incremental increase in MTC risk group from 1 to 3; 95% confidence interval, 3.05-66.55;  $P < .001$ ). Pheochromocytomas were found in 21 patients from 12 kindreds; 20 of 21 patients had codon 634 or 918 mutations. Hyperparathyroidism was found in 10 patients from 7 kindreds; 7 of 10 patients had codon 634 mutations.

**Conclusion:** Specific *RET* mutations predict the phenotypic expression of disease and the MTC aggressiveness in patients with MEN 2, guiding the timing of thyroidectomy and screening for pheochromocytoma.

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**M**ULTIPLE endocrine neoplasia type 2 (MEN 2) is an autosomal dominant syndrome that is characterized by medullary thyroid carcinoma (MTC), pheochromocytoma, and hyperparathyroidism (HPT). MEN 2 is divided into subtypes depending on the presence or absence of tissue-specific tumors and phenotypic characteristics, and the number of affected family members (**Table 1**).<sup>1</sup> Rare variants of MEN 2 have been associated with Hirschsprung disease (HSCR) and cutaneous lichen amyloidosis (CLA).<sup>2,3</sup> Medullary thyroid carcinoma, which has a penetrance of greater than 90% and is usually the first manifestation of MEN 2,<sup>4,5</sup> has been confirmed in thyroidectomy specimens from patients as young as 17 months.<sup>6</sup> The prognosis of MEN 2 is related to the aggressiveness of the MTC,

which can develop early lymph node metastases. These metastases have been found in patients as young as 3 years.<sup>7</sup> Germline mutations in the *RET* proto-oncogene cause MEN 2.<sup>8,9</sup> Analysis of the *RET* mutation status of at-risk patients allows for effective screening and improved clinical management.<sup>10</sup>

The *RET* gene is located on chromosome 10 and includes 21 exons. It encodes a transmembrane tyrosine kinase, the ret receptor, which is primarily expressed by neuroendocrine and neural cells.<sup>11-14</sup> Rearrangements of *RET* have been identified in papillary thyroid carcinomas, while inactivating *RET* mutations play a role in the development of HSCR.<sup>15-20</sup> Missense mutations that result in a constitutively active receptor are responsible for MEN 2.<sup>21-23</sup> These mutation hot spots are located in the extracellular cysteine-rich region involved in receptor dimeriza-

**Table 1. Classification of MEN 2 Subtypes**

Subtype	MTC	Pheochromocytoma	HPT	No. of Affected Family Members
MEN 2A	Yes	Yes*	Yes*	Any
MEN 2B†	Yes	Yes	No	Any
FMTC	Yes	No	No	≥4
Unclassified	Yes	No	No	≤3

Abbreviations: FMTC, familial medullary thyroid carcinoma; HPT, hyperparathyroidism; MEN, multiple endocrine neoplasia; MTC, medullary thyroid carcinoma.

\*Diagnosis of a pheochromocytoma and/or HPT is required.

†Characteristic mucosal neuromas on the tongue, lips, subconjunctival areas, and gastrointestinal tract are required.

tion that is encoded by exons 10 and 11, and in the intracellular tyrosine kinase domain encoded by exon 16.<sup>24-26</sup>

The specific *RET* codon mutation can be correlated with the phenotypic expression of MEN 2. For example, pheochromocytoma and HPT are most common in patients with codon 634 mutations, and HPT does not occur in patients with codon 918 mutations.<sup>27-32</sup> Recently, it has been suggested that the biological aggressiveness of MTC can also be associated with the specific *RET* mutation.<sup>33</sup> *RET* mutations have been stratified into 3 groups (levels 1-3) based on the biological aggressiveness of MTC observed in patients with different *RET* mutations. Patients with level 1 mutations (codons 609, 768, 790, 791, 804, and 891) have the lowest risk for the development and growth of MTC. Patients with level 2 mutations (codons 611, 618, 620, and 634) are at intermediate risk, and patients with level 3 mutations (codons 883 and 918) are at the highest risk for the early development and growth of MTC.<sup>34</sup> In the present study, we examined the impact of both tumor biological characteristics and age at thyroidectomy on the staging of MTC at both diagnosis and last follow-up to confirm this risk-group stratification in our MEN 2 population. In addition, we examined the frequency and characteristics of pheochromocytoma and HPT to determine whether *RET* mutation-specific screening can be applied to this patient population.

## METHODS

A review of the endocrine surgery database at The University of Texas M. D. Anderson Cancer Center, Houston, a tertiary referral center, covering 1951 to 2002 identified 86 individuals with MEN 2 in 47 kindreds who had been evaluated at the hospital (institutional review board protocols RCR01-485 and RCR01-096). The diagnosis and subtype of MEN 2 were confirmed according to previously published criteria.<sup>1</sup> Briefly, kindreds with MTC and at least 1 family member presenting with pheochromocytoma and either with or without HPT were categorized as having MEN 2A. Kindreds with MTC and characteristic mucosal neuromas on the tongue, lips, subconjunctival areas, and gastrointestinal tract and either with or without pheochromocytoma were categorized as having MEN 2B. Kindreds with MTC in 4 or more members and with objective evidence against adrenal and parathyroid gland involvement were categorized as having familial MTC (FMTC). Kindreds with MTC in 3 or fewer members and with no evidence of pheochromocytoma, HPT, or MEN 2B were categorized as unclassified. Follow-up information was obtained during routine outpatient visits, telephone interviews, or written communication with the patient and/or family member.

For each patient, we determined the phenotypic characteristics of MEN 2 by reviewing the original laboratory results, radiographic imaging studies, and operative and pathology reports. Staging was based on the TNM classification.<sup>35</sup> The stage of disease at diagnosis and at last follow-up was used as a measure of the biological aggressiveness of MTC; patients with advanced disease (stage III, IVA, IVB, or IVC) were assumed to have more aggressive disease than those with small, localized MTC (stage I or II). The diagnosis of MTC with or without local regional lymph node metastasis was made after the original histology reports of surgical specimens were reviewed. Distant metastases were confirmed by means of the original radiographic imaging studies and/or biopsy reports. A plasma basal calcitonin level above the upper limit of normal was considered evidence of persistent MTC. Transcutaneous cervical ultrasonography was used to exclude the presence of residual thyroid tissue (incomplete thyroidectomy) as a cause of post-thyroidectomy elevations in plasma calcitonin level.

The diagnosis of pheochromocytoma was made on the basis of the original histologic review of adrenalectomy specimens or biochemical evidence of elevated levels of serum or 24-hour urinary fractionated metanephrines or catecholamines. The diagnosis of HPT was made on the basis of the original histologic review of parathyroidectomy specimens or on biochemical evidence of hypercalcemia and inappropriate elevation of serum parathyroid hormone levels. Additional nonendocrine manifestations of MEN 2 were determined by clinical examination with or without histologic confirmation.

Mutation analyses of the *RET* proto-oncogene were performed on blood collected from affected and at-risk individuals before 1994 by manual DNA sequencing or allele-specific hybridization and confirmed by a secondary method as previously described.<sup>36</sup> Informed consent was obtained at the time of blood collection. Mutation analyses for affected and at-risk individuals after 1994 were performed on phenol-chloroform-extracted DNA with a fluorescence sequence DNA analyzer (ABI Prism 3700; Applied Biosystems, Foster City, Calif). *RET* sequencing was limited to exons 10, 11, and 16 and, if necessary, expanded to include exons 13, 14, and 15. Patients were stratified to 1 of 3 risk groups (levels 1-3) on the basis of their genotype: level 1 mutations included codons 609, 768, 790, 791, 804, and 891; level 2 mutations included codons 611, 618, 620, and 634; and level 3 mutations included codons 883 and 918.

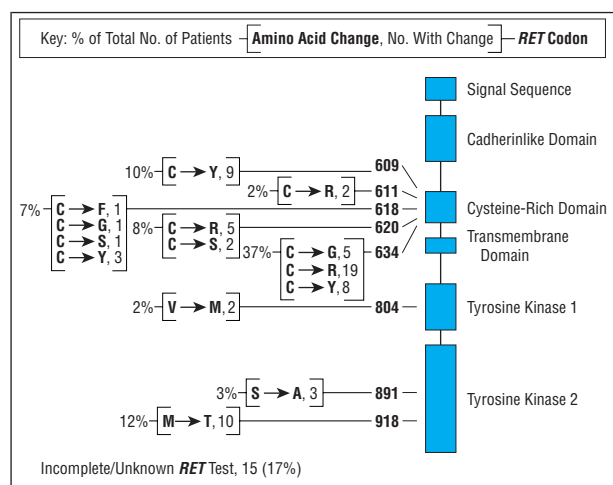
Statistical analyses were performed with SAS statistical software (SAS Institute Inc, Cary, NC). Logistic regression was used to estimate the likelihood of aggressive MTC disease with age at diagnosis and risk group as variables in univariate and multivariate models. Correlation among kindred members was accounted for by implementing logistic regression in the framework of generalized linear models with the use of generalized estimating equations for correlated outcomes. Variable effects were estimated as odds ratios with 95% confidence intervals. Results were considered statistically significant at  $P < .05$ .

## RESULTS

Eighty-six patients from 47 kindreds were identified with MEN 2. Eighty-one (94%) were alive at the time of analysis and 5 (6%) had died. Two patients had died of progressive metastatic MTC, 1 patient had died after a probable hypertensive crisis attributed to bilateral pheochromocytomas, and 1 patient had died of metastatic bladder carcinoma; the cause of death in 1 patient was unknown. Among the 47 kindreds, 19 (40%) were categorized as having MEN 2A; 10 (21%), MEN 2B; and 6 (13%), FMTC. The remaining 12 kindreds (26%) were categorized as unclas-

sified and included FMTC kindreds with 3 or fewer affected individuals and MEN 2A kindreds with affected individuals who had inconclusive or incomplete biochemical evidence of pheochromocytomas and HPT.

We completed the *RET* mutation analysis for 71 patients (83%) in 39 kindreds (83%). The distribution of the identified *RET* mutations is shown in **Figure 1**. Most of the mutations were in codons 609, 611, 618, 620, and 634, which encode the cysteine-rich domain. The most frequent mutation was a missense mutation at codon 634 resulting in an amino acid change from cysteine to arginine. There were 10 patients from 9 kindreds with level 3 (highest-risk) *RET* mutations, all involving codon 918. Fifty-three patients from 22 kindreds had level 2 (intermediate-risk) mutations involving codons 611, 618, 620, and 634. Fifteen patients from 9 kindreds had level 1 (lowest-risk) mutations involving codons 609, 804, and 891.



**Figure 1.** Distribution of *RET* mutations (n=86).

The frequency and characteristics of MTC within each risk group are given in **Table 2**. In the level 1 risk group, 1 kindred (11%) was categorized as having MEN 2A and 3 kindreds (33%) were categorized as having FMTC. The remaining 5 kindreds (56%) were categorized as unclassified. All 15 patients were alive at last follow-up. Thyroidectomy was performed in 14 of the 15 patients. The median age at thyroidectomy was 40.7 years (range, 15.2-78.2 years). The median age at thyroidectomy for the 12 patients with MTC in the surgical specimen (patients with premalignant C-cell hyperplasia were excluded) was 41.5 years (range, 17.4-78.2 years). Lymph node metastases within the initial surgical specimen were found in 3 (25%) of the 12 patients. Three of the 9 remaining patients had no mention of lymph node status in the pathology report. At the time of diagnosis, 8 patients (53%) had stage 0, I, or II disease and 3 patients (20%) had stage III or IV disease. The stage at the time of diagnosis for 4 patients (27%) was unknown. Recurrent disease in the neck developed in 4 (33%) of the 12 patients at a median of 3.1 years after initial thyroid surgery. One (7%) of the 15 patients in this lowest-risk group developed distant metastases 6.5 years after thyroidectomy at age 29.3 years, and at last follow-up 9 patients (60%) had persistent disease, as evidenced by elevated basal calcitonin levels.

In the level 2 intermediate-risk group, 15 (68%) of the 22 kindreds were categorized as having MEN 2A and 3 kindreds (14%) were categorized as having FMTC; the remaining 4 kindreds (18%) were categorized as unclassified. Two (4%) of 53 patients died: 1 at age 38 years of a probable hypertensive crisis due to bilateral pheochromocytomas and the other at age 57 years of metastatic MTC. Thyroidectomy was performed in 52 of the 53 patients. The median age at thyroidectomy was 21.8 years (range, 3.0-60.4 years). The median age at thyroidectomy for the 44 patients with MTC in the thyroid specimen (patients

**Table 2. Patient Risk Group and MTC Characteristics**

MTC Characteristics	Patient Risk Group		
	Level 1: Codon 609, 804, 891 Mutations (n = 15)	Level 2: Codon 611, 618, 620, 634 Mutations (n = 53)	Level 3: Codon 918 Mutations (n = 10)
Kindreds, No.	9	22	9
MEN 2 subtype, No. (% of kindreds in risk group)	MEN 2A, 1 (11) FMTC, 3 (33) Unclassified, 5 (56)	MEN 2A, 15 (68) FMTC, 3 (14) Unclassified, 4 (18)	MEN 2B, 9 (100)
Deceased, No. (% of patients in risk group)	0	2 (4)	0
Thyroidectomy, No. (% of patients in risk group)	14 (93)	52 (98)	9 (90)
MTC present at initial thyroidectomy, No. (% of thyroidectomies)	12 (86)	44 (85)	9 (100)
Median age at initial thyroidectomy for patients with MTC present, y (range)	41.5 (17.4-78.2)	22.9 (5.2-60.4)	13.5 (5.0-25.5)
Nodal metastases at initial thyroidectomy, No. (% of patients with MTC at initial thyroidectomy)	3 (25)	16 (36)	4 (44)
Stage after initial thyroidectomy, No. (% of patients in risk group)	0, I, II, 8 (53) III, IV, 3 (20) Unknown, 4 (27)	0, I, II, 22 (42) III, IV, 16 (30) Unknown, 15 (28)	0, I, II, 1 (10) III, IV, 5 (50) Unknown, 4 (40)
Recurrent neck disease, No. (% of patients with MTC at initial thyroidectomy)	4 (33)	9 (20)	4 (44)
Distant metastases, No. (% of patients in risk group)	1 (7)	10 (19)	4 (40)
Median age at diagnosis of distant metastases, y (range)	29.3	33.6 (18.9-57.1)	16.4 (9.6-22.6)
Persistent disease, No. (% of patients in risk group)	9 (60)	37 (70)	10 (100)

Abbreviations: FMTC, familial medullary thyroid carcinoma; MEN, multiple endocrine neoplasia; MTC, medullary thyroid carcinoma.

**Table 3. Univariate and Multivariate Odds Ratios for Advanced MTC (Stage III or IV) at Diagnosis**

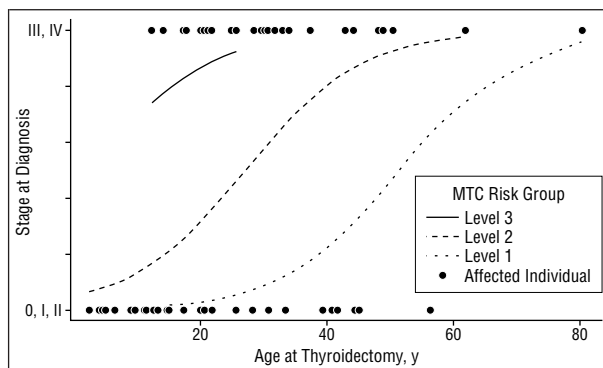
Variable	Odds Ratio (95% Confidence Interval)	
	Univariate	Multivariate
Age at thyroidectomy	1.06 (1.02-1.10)	1.12 (1.07-1.17)
P value	.004	<.001
MTC risk group	3.06 (1.01-9.28)	14.23 (3.05-66.55)
P value	<.001	<.001

Abbreviation: MTC, medullary thyroid carcinoma.

with premalignant C-cell hyperplasia were excluded) was 22.9 years (range, 5.2–60.4 years). Lymph node metastases were found within the initial surgical specimen in 16 (36%) of the 44 patients. Thirteen of the 28 remaining patients had no mention of lymph node status in the pathology report. At the time of diagnosis, 22 (42%) of the 53 patients had stage 0, I, or II disease and 16 patients (30%) had stage III or IV disease. The stage at the time of diagnosis for 15 patients (28%) was unknown. Recurrent disease in the neck developed in 9 (20%) of the 44 patients at a median of 5.9 years after initial thyroid surgery. Ten patients (19%) in this intermediate-risk group developed distant metastases at a median of 8.6 years after thyroidectomy and at a median age of 33.6 years (range, 18.9–57.1 years). At last follow-up, calcitonin levels were elevated in 37 (70%) of the 53 patients.

All 10 patients in the level 3 highest-risk group were classified as having MEN 2B. All patients were alive at last follow-up. Nine of the 10 patients had undergone thyroidectomy at a median age of 13.5 years (range, 5.0–25.5 years). All had MTC in the surgical specimen. Four (44%) of the 9 patients had lymph node metastases at the time of initial surgery. Four of the 5 remaining patients had no mention of lymph node status in the pathology report. At the time of diagnosis, 1 patient (10%) had stage 0, I, or II disease and 5 patients (50%) had stage III or IV disease. The stage at the time of diagnosis was unknown for 4 patients (40%). Recurrent disease in the neck developed in 4 (44%) of the 9 patients at a median of 5.7 years after initial thyroid surgery. Four (40%) of the 10 patients in this highest-risk group developed distant metastases at a median of 2.6 years after thyroidectomy and at a median age of 16.4 years (range, 9.6–22.6 years). At last follow-up, all 10 patients (100%) had evidence of residual MTC, as evidenced by an elevated basal level of serum calcitonin.

Univariate and multivariate logistic regression was used to correlate the age at thyroidectomy and risk group (levels 1–3) with the aggressiveness (stage) of MTC at diagnosis (**Table 3**). On the basis of the univariate analysis, older age at thyroidectomy was significantly associated with the likelihood of having stage III or IV MTC (odds ratio, 1.06 per year of age at thyroidectomy; 95% confidence interval, 1.02–1.10;  $P=.004$ ). In addition, the likelihood of having stage III or IV MTC at the time of diagnosis increased 3-fold for each incremental increase in the MTC risk group from level 1 to level 3 (95% confidence interval, 1.01–9.28;  $P<.001$ ). On the basis of the multivariate analysis, the relationship between advanced MTC (stage III or IV) at the time of diagnosis and age at thy-



**Figure 2.** Multivariate analysis of medullary thyroid carcinoma (MTC) stage at diagnosis and association with risk group and age at thyroidectomy. Lines are parametric splines of predicted values.

roidectomy and risk group became more pronounced. The risk of having stage III or IV MTC at the time of diagnosis increased 12% per year of age at thyroidectomy (95% confidence interval, 1.07–1.17;  $P<.001$ ). The likelihood of having stage III or IV MTC at the time of diagnosis increased 14-fold for each incremental increase in the MTC risk group from level 1 to level 3 (95% confidence interval, 3.05–66.55;  $P<.001$ ). **Figure 2** represents the multivariate model and depicts the estimated effects of age at thyroidectomy and MTC risk group on the likelihood of having advanced-stage MTC at diagnosis.

The frequency of pheochromocytomas and HPT in kindreds categorized by the MEN 2 subtype and genotype is shown in **Table 4**. Pheochromocytomas were diagnosed in 21 patients (24%) from 12 kindreds (26%). One patient (5%) died of a catecholamine crisis attributed to bilateral pheochromocytomas. The median age at diagnosis was 33.1 years (range, 19.0–60.4 years). Sixteen patients (76%) had mutations in codon 634. Bilateral tumors were found at initial presentation in 11 (52%) of the 21 patients. Contralateral pheochromocytomas developed in 3 (30%) of the 10 patients with a unilateral tumor at initial presentation at a median of 2.6 years after their initial adrenalectomy. Fifteen cortical-sparing adrenalectomies were performed in 11 of the 21 patients. Four ipsilateral recurrences (27%) occurred, at a median of 5.1 years after the initial subtotal adrenalectomy. None of the patients who had undergone adrenalectomy developed a catecholamine crisis during the period of follow-up.

Hyperparathyroidism was diagnosed in 10 patients (12%) from 7 kindreds (15%). The median age at diagnosis was 36.2 years (range, 17.5–49.4 years). Seven patients (70%) had mutations in codon 634. In 4 (40%) of the 10 patients, recurrent HPT required at least 1 reoperation.

The frequency and genotype of other phenotypic characteristics of MEN 2 are shown in **Table 5**. Of the 10 patients from 5 kindreds with CLA, all had a codon 634 *RET* mutation. One member of an MEN 2A kindred had manifestations of both MEN 2A and HSCR. This patient had a *RET* mutation in codon 620. Enlarged corneal nerves were diagnosed in 9 patients from 9 kindreds. This characteristic was most common (6 patients [67%]) in patients with MEN 2B. Patients with codon 609 and 891 mutations were also identified with enlarged corneal nerves. However, not all patients were examined for this characteristic.

**Table 4. Frequency of Pheochromocytomas and Hyperparathyroidism With Associated MEN 2 Subtype and Genotype**

	No. (%) (N = 86)	Kindreds, No. (%) (N = 47)	Median Age at Diagnosis, y (Range)	MEN 2 Subtype, No. (% of Total in Group)	RET Codon Mutation, No. (% of Total in Group)
Pheochromocytomas	21 (24)	12 (26)	33.1 (19.0-60.4)	MEN 2A, 17 (81) MEN 2B, 4 (19)	634, 16 (76) 918, 4 (19) Unknown, 1 (5)
Hyperparathyroidism	10 (12)	7 (15)	36.2 (17.5-49.4)	MEN 2A, 9 (90) Unclassified, 1 (10)	634, 7 (70) Unknown, 1 (50) 609, 1 (10) Unknown, 2 (20)

Abbreviation: MEN, multiple endocrine neoplasia.

**Table 5. Frequency and Genotype of Other Phenotypic Characteristics of MEN 2**

	No. (%) (N = 86)	Kindreds, No. (%) (N = 47)	Phenotype, No. (% of Total in Group)	RET Codon Mutation, No. (% of Total in Group)
Cutaneous lichen amyloidosis	10 (12)	5 (11)	MEN 2A, 10 (100)	634, 10 (100)
Hirschsprung disease	1 (1)	1 (2)	MEN 2A, 1 (100)	620, 1 (100)
Marfanoid habitus	5 (6)	4 (9)	MEN 2B, 5 (100)	918, 4 (80) Unknown, 1 (20)
Intestinal neuromas	2 (2)	2 (4)	MEN 2B, 2 (100)	918, 1 (50) Unknown 1 (50)
Enlarged corneal nerves	9 (10)	9 (19)	MEN 2B, 6 (67) FMTC, 1 (11) Unclassified, 2 (22)	609, 1 (11) 918, 5 (56) 891, 2 (22) Unknown, 1 (11)

Abbreviations: FMTC, familial medullary thyroid carcinoma; MEN, multiple endocrine neoplasia.

### COMMENT

Recently, it has been suggested that patients with MEN 2 can be stratified into 3 groups based on the risk for development and growth of MTC.<sup>33,34</sup> We sought to determine whether the apparent difference in MTC phenotype between the different MTC risk groups was due to a difference in age at onset or in tumor biological characteristics. Our multivariate analyses estimated statistically significant and independent associations of both greater age at thyroidectomy and increasing MTC risk group and the likelihood of having advanced MTC (stage III or IV) at the time of diagnosis. These results confirm the hypothesis that MTC risk group is an independent predictor of MTC aggressiveness.

Our statistical modeling was supported by the clinical impression that patients with level 1 mutations are often older at presentation and have more indolent tumors than do patients with level 2 or 3 mutations. For example, 1 patient with a V804M (level 1) mutation presented at age 55 years with a palpable thyroid mass (MTC). After thyroidectomy and central and bilateral neck dissection, the patient was found to have multifocal, bilateral MTC yet no local regional metastases in the 60 lymph nodes examined. After surgery, calcitonin levels in this patient fell to less than 1 pg/mL. In contrast, a patient with an M918T (level 3) mutation presented at age 17 years with the mucosal neuromas that are characteristic of MEN 2B and with a normal thyroid by palpation. After thyroidectomy and neck dissection, however, this patient was found to have multifocal MTC with bilateral local regional metastases in 32 of 71 lymph nodes and within 5 years had developed

bone and liver metastases. These examples suggest that patients with lowest-risk mutations develop MTC with a different metastatic potential and also have a delay in the neoplastic transformation of thyroid C cells.

Because age is an independent predictor of aggressive disease at thyroidectomy, early thyroidectomy in at-risk patients with level 2 and 3 mutations can potentially reduce the risk of MTC to that for patients with lower-risk mutations. The importance of early thyroidectomy cannot be overemphasized.<sup>34,37-40</sup> The data in **Table 6**, which lists all thyroidectomies performed in the pediatric subgroup of this study population, demonstrate that MTC can be found in young patients who have undergone prophylactic thyroidectomy. One patient with a C634R mutation had undergone prophylactic thyroidectomy at age 5 years and was found to have a 0.4-cm MTC in the right thyroid lobe.

If thyroidectomy is delayed, patients with level 1 mutations can also develop lymph node and distant metastases. One patient with an S891A mutation presented with bone metastases at 29 years of age and within 6 years had developed metastases in the liver, lung, and breast. Although unusual for a carrier of a level 1 mutation, this case emphasizes the heterogeneity in biological behavior that may complicate the treatment of patients who postpone thyroidectomy.

Pheochromocytoma, which has been reported in all RET mutations except those in codons 768, V804M, and 891, is most frequently associated with mutations in codons 634 and 918.<sup>34,40</sup> These latter 2 mutations were found in most of the patients with pheochromocytomas in our study. Pheochromocytomas were unilateral at the time of diagnosis in 10 (48%) of 21 patients. Three (33%)

**Table 6. Thyroidectomies in Pediatric Patients With MEN 2**

Risk Group	Patient No.	RET Codon Mutation	Age at Thyroidectomy, y	Indication for Surgery*	Thyroid Histologic Findings
Level 1	1	609	15.2	Prophylactic	Benign
	2	609	17.4	Prophylactic	Benign
Level 2	1	618	9.4	Prophylactic	Benign
	2	618	12.9	Prophylactic	Benign
	3	620	4.6	Prophylactic	CCH
	4	620	6.9	Prophylactic	Benign
	5	634	3.0	Prophylactic	Benign
	6	634	4.9	Prophylactic	Benign
	7	634	5.2	Prophylactic	CCH
	8	634	5.2	Prophylactic	MTC
	9	634	5.5	Prophylactic	Benign
	10	634	7.0	Prophylactic	MTC
	11	634	9.9	Prophylactic	CCH
	12	634	10.0	Prophylactic	MTC
	13	634	11.5	Therapeutic	MTC
	14	634	11.8	Prophylactic	MTC
	15	634	14.3	Therapeutic	MTC
	Level 3	16	634	17.5	Therapeutic
1		918	5.9	Therapeutic	MTC
2		918	9.3	Therapeutic	MTC
3		918	12.3	Therapeutic	MTC
4		918	12.6	Therapeutic	MTC
5		918	13.5	Therapeutic	MTC
6		918	14.0	Therapeutic	MTC
7	918	17.4	Therapeutic	MTC	

Abbreviations: CCH, C-cell hyperplasia; MEN, multiple endocrine neoplasia; MTC, medullary thyroid carcinoma.

\*Prophylactic is defined as a referral for surgery prompted by young age rather than by abnormal physical examination results, cervical ultrasound, or a marked elevation in the calcitonin level.

of the 10 developed a contralateral recurrence within the first 3 years of follow-up. Previous reports suggested an increasing rate of contralateral recurrence as patients age.<sup>41,42</sup> We currently screen all patients with MEN 2, except those in FMTC kindreds, by a yearly evaluation of either plasma or 24-hour urinary fractionated metanephrines and catecholamines.

At last follow-up, none of the patients in this study had been diagnosed as having metastatic pheochromocytoma. The absence of metastatic pheochromocytoma in patients with hereditary pheochromocytoma and the risk of morbidity and mortality from adrenal insufficiency in patients who undergo bilateral total adrenalectomy support the surgical practice of cortical-sparing adrenalectomy. In our study, 4 (27%) of 15 cortical-sparing adrenalectomies subsequently required reoperation because of ipsilateral recurrence. This rate of recurrence was consistent with those reported previously.<sup>43-45</sup>

Our current management of unilateral familial pheochromocytoma is to perform a laparoscopic adrenalectomy. For bilateral disease or a contralateral recurrence, the main surgical objective is to preserve a portion of the adrenal cortex in situ, which is often facilitated by an open surgical procedure.<sup>46</sup>

Hyperparathyroidism occurs in 10% to 35% of patients with MEN 2A.<sup>47</sup> It is associated most commonly with codon 634 mutations and less frequently with codon 609, 611, 618, 620, 790, and 791 mutations. Ten (12%) of the patients with MEN 2 evaluated in this study received the diagnosis of HPT. Six of the 7 for whom the codon mutation analysis was complete had mutations in codon 634.

None of the patients with codon 918 mutations were diagnosed as having HPT. This pattern was consistent with previous reports that patients with a mutation in codons 918, 883, or 922 have no risk of HPT.<sup>48</sup>

Other phenotypic characteristics of MEN 2 were rare. Twelve percent of our patients with MEN 2 had the diagnosis of CLA, and all had a mutation in codon 634. The pathophysiologic mechanism underlying the development of CLA may be related to a sensory abnormality in the C6 through T6 dermatomes and resulting pruritus and chronic irritation.<sup>49,50</sup> In general, not all patients with codon 634 mutations developed CLA, and not all patients with CLA had *RET* mutations. One patient with a C620R mutation also had a history of HSCR, which is a rare variant of MEN 2. In the families with a history of both diseases, not all mutation carriers developed manifestations of HSCR, and HSCR has also been associated with germline mutations in other genes. The underlying molecular mechanisms causing HSCR are likely multifactorial as well as multigenic.<sup>51,52</sup> Enlarged corneal nerves were identified in only 10% of our patients with MEN 2. However, not all patients were examined for this abnormality. Takai et al<sup>53</sup> examined 22 patients in 10 families with MEN 2A and identified pathologic corneal nerve thickening in 60%. Although most common in families with MEN 2B, enlarged corneal nerves have also been reported in families with FMTC.<sup>54</sup> It is likely that slitlamp examination of all patients with MEN 2 will increase the identification of this phenotypic characteristic.

The results of this study confirmed that the biological behavior of MTC can be stratified by the specific *RET*

mutation in our MEN 2 population. Our findings also emphasize the importance of DNA-based mutation analysis of the *RET* proto-oncogene for at-risk patients. Current genetic-testing methods can detect 99% of *RET* mutations. Because of the importance of early intervention in the management of MTC and of screening for pheochromocytoma, the identification of mutation carriers can significantly affect clinical decision making in these patients. The comprehensive care of identified families and patients should also include appropriate genetic counseling and long-term surveillance.

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## DISCUSSION

**Neil Yeston, MD, Hartford, Conn:** If I am an adult who has the MEN 2 disease and I come to you for counseling with a newborn who has the chromosomal abnormality that you described, when are you going to recommend to me that my child needs a thyroidectomy? A similar question: Is the risk level for the mutation consistent within kindreds, or have you found that there is varying risk within kindreds? A follow-up to Dr Yeston—when do you actually begin testing kindreds in terms of the age of the offspring?

**Barbara Kinder, MD, New Haven, Conn:** I wonder if you could tell me any information you might have about those 12, I believe, unclassified patients in terms of the genetic issues, and tell us a little bit about your surgical approach to the pheochromocytomas. Do you do the cortical-sparing procedure laparoscopically, and, if so, if you have had to go back, can you then repeat it laparoscopically or do you have to go to open? Also, how do you manage the parathyroids in these patients?

**Peter J. Deckers, MD, Farmington, Conn:** I hope this society will bear with me just a second on this particular comment. As some of you know, for the past 10 years I have had the privilege of being a dean of the School of Medicine, and every August I welcome a new class of students into our school. Given all the things the students hear about health care and health care financing and what is going on in medicine today, they need the talk that I give, which is to tell them that they are entering medicine at its most exciting time: the era of molecular medicine. I tell them that gene profiling, understanding genes will allow decision making like what you saw in this particular presentation and that eventually drugs will be developed that will allow us to turn off these genes. I won't go any further on that, except to say, Dr Yip, I would like to invite you to our school next year to give that talk to our students when they come in.

It is important—and I am going to say this because I know that her mentor at M. D. Anderson has to leave this meeting and go someplace else this afternoon for all the right kinds of personal reasons, but I would like the society to recognize Dr Doug Evans. Dr Evans, please stand up [applause].

**[Unidentified Speaker]:** I would just like to ask about those low-risk and medium-risk patients. There was a persistence of disease on one of your slides of 60% to 70% in even these favorable patients. How do you work up these slightly elevated calcitonins, even if they are just slightly above normal? Have you reoperated on those patients?

**Dr Yip:** To address the first question, when should patients be tested and how should thyroidectomy decisions be made after a patient has been determined to have a gene carrier, at our institution we test all children of known gene carriers. If these children are found to have mutations, then the current recommendation is for thyroidectomy to be performed as soon as possible. Preferably the highest-risk mutation carriers should have thyroidectomy during infancy, within a few months of their birth. Usually the current recommendation for intermediate-risk carriers is before 5 years of age, and for the lowest risk carriers, those patients should have thyroidectomy as soon as possible. However, the consensus recommendations are still somewhat controversial. Many of these people choose to wait and have calcitonin levels monitored, and once those begin to show an elevation then they elect to undergo prophylactic thyroidectomy at that time.

Within our modeling, we adjusted for the fact that patients within kindreds tend to have similar expression of disease, so in a kindred that tends to have hyperparathyroidism in their family, these patients also have a high risk of hyperparathyroidism. A thorough family history always is important in following these patients in order to determine what their specific tumor risk will be.

In the 12 unclassified patients that we had, these patients are patients with basically incomplete kindreds. They either have not yet been classified as FMTC kindreds or MEN 2A kindreds, and these patients should be monitored carefully for pheochromocytoma and hyperparathyroidism, since they have not yet been classified into one or the other.

To address the question about the management of the parathyroids, in patients—could I have the slide projector back on, please? On this slide is basically our algorithm for managing the parathyroids. In a patient with MEN 2A or FMTC with a normal parathyroid hormone level or calcium level, the superior glands are left in situ and the inferior glands are removed with the nodal dissection at the time of thyroidectomy. The parathyroid is then autografted to the forearm and all identified parathyroid tissue is also cryopreserved. If the family has a history of hyperparathyroidism, only if there is questionable vascularity of the in situ glands or there is a low intraoperative PTH [parathyroid hormone] is autografting performed. However, if the operation is being done for known hyperparathyroidism, any grossly abnormal glands are removed, the extent of resection and the need for autografting are guided by intraoperative PTH, a transcervical thymectomy is performed to remove any ectopic parathyroids, and any identified parathyroid tissue is cryopreserved.

This is our current management of familial pheos. If a unilateral pheo is identified with a known normal contralateral gland on the CT or MR, a laparoscopic adrenalectomy is performed. If, however, a bilateral pheo or a contralateral recurrence has been identified, then the goal of preserving a rim of cortex is the key to the surgical procedure and a cortical-sparing adrenalectomy is performed. This is facilitated by an open procedure.

And the final question regarding the patients with low and intermediate risk who have persistent elevated calcitonin levels, these patients are followed with yearly monitoring of their calcitonin levels, and further management is dependent on the progression of their calcitonin levels.