

Genotype-Phenotype Analysis in Multiple Endocrine Neoplasia Type 1

Maria A. Kouvaraki, MD, PhD; Jeffrey E. Lee, MD; Suzanne E. Shapiro, MS; Robert F. Gagel, MD; Steven I. Sherman, MD; Rena V. Sellin, MD; Gilbert J. Cote, PhD; Douglas B. Evans, MD

Hypothesis: Multiple endocrine neoplasia type 1 (MEN 1) syndrome is an autosomal dominant disorder caused by germline mutations in the *MEN1* gene and characterized by multiple endocrine tumors, most notably in the parathyroid glands, pituitary, and pancreas. The syndrome demonstrates variable expressivity and considerable genetic heterogeneity. Patient data were examined for possible associations between genotype and phenotype.

Design: We reviewed recorded medical data from 1975 to 2001 on patients with MEN 1 and compared specific types and locations of *MEN1* gene mutations with manifestations of the syndrome.

Patients and Results: We identified 109 affected patients from 24 MEN 1 kindreds. The phenotypic expression of MEN 1 in affected individuals included hyper-

parathyroidism in 74%, pancreatic endocrine tumors in 51%, and pituitary tumors in 35%. Twelve of 14 insulinomas occurred in patients with pituitary tumors. Mutation analysis was completed in 14 of 24 kindreds (80 of the 109 patients). Mutations were most common in exons 2 (31%), 9 (15%), and 10 (23%). All 21 patients with frameshift mutations (and known pancreatic endocrine tumor status) had such tumors. Pituitary tumors were associated with frameshift mutations in exon 2.

Conclusions: The type and location of *MEN1* mutations may be associated with the phenotypic expression of specific tumors. Such information may assist in the genetic counseling and surveillance of at-risk patients. A specific genotype-phenotype correlation is unlikely because of the heterogeneity of the mutations in the *MEN1* gene.

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MULTIPLE endocrine neoplasia type 1 (MEN 1) is an autosomal dominant disease that demonstrates near-complete penetrance and has an estimated prevalence between 1 and 10 per 100 000 individuals.¹⁻³ Patients with MEN 1 develop various combinations of endocrine tumors involving the parathyroid glands (90%-97%), pancreatic islet cells and duodenum (30%-80%), and anterior pituitary gland (15%-50%).^{1,2,4} Facial angiofibromas, lipomas, carcinoid tumors, thyroid neoplasms, adrenocortical adenomas, pheochromocytomas, malignant melanomas, and testicular teratomas are also seen, although less frequently, in patients with MEN 1.⁵⁻⁷

The prognosis of MEN 1 is related to the development of pancreatic endocrine tumors (PETs): metastatic neuroendocrine carcinoma is the leading cause of disease-specific mortality in patients with MEN 1.⁸ Up to 50% of patients with MEN 1 and PETs will develop liver metastasis at some point during their lives.⁹⁻¹¹ Thymic or bronchial carcinoid tumors also may metastasize.

Genetic linkage analysis and studies of loss of heterozygosity have been success-

ful in mapping the *MEN1* gene to chromosomal locus 11q13,¹²⁻¹⁷ and recently this gene has been identified by positional cloning.^{3,18} The *MEN1* gene spans 9 kilobases and consists of 10 exons; its product is a 610-amino acid protein known as menin.³ It has been suggested that menin is involved with the JunD, Pem, and NF- κ B signaling pathways and thus is likely to regulate gene transcription.^{3,19-22} Menin directly binds to the activated protein 1-transcription factor JunD and thus inhibits JunD-dependent transcription. Menin behaves as a tumor suppressor gene.^{3,19,20} Consistent with Knudson's 2-hit model of tumor suppression,²³ in individuals who inherit a mutated *MEN1* allele, inactivation of the remaining *MEN1* allele results in tumor development in specific tissues.²³

Germline mutations have been identified in approximately 77% of families with MEN 1.²⁴ Mutation analysis has disclosed 294 unique mutations, distributed throughout the gene and most often leading to a truncated protein.^{3,14,24-29} At present, there does not appear to be a correlation between specific mutations in the *MEN1* gene and the type of tumor, the biological behavior (tumor progression) of

From the Departments of Surgical Oncology (Drs Kouvaraki, Lee, and Evans and Ms Shapiro) and Endocrine Neoplasia and Hormonal Disorders (Drs Gagel, Sherman, Sellin, and Cote), The University of Texas MD Anderson Cancer Center, Houston.

PATIENTS AND METHODS

STUDY GROUP

The study included 109 affected individuals from 24 unrelated MEN 1 kindreds retrieved from the MEN 1 database in the Department of Surgical Oncology at The University of Texas MD Anderson Cancer Center. This database was developed by review of medical records from 1975 to 2001. The clinical diagnosis of MEN 1 was based on accepted criteria as previously published.³⁶ Forty-seven (43%) of the 109 patients were seen in consultation at our institution; information from the remaining 62 patients (57%) was obtained by patient interview and telephone follow-up.

For the 47 patients examined at our institution, the presence or absence of hyperparathyroidism, PETs, pituitary neoplasms, carcinoid tumors, neoplasms of the adrenal glands, and other phenotypic characteristics of MEN 1 was determined from review of operative and pathology reports, radiographic imaging studies, and/or laboratory analysis. The presence of hyperparathyroidism was confirmed by (1) elevated levels of serum calcium and intact parathyroid hormone with or without a history of clinical symptoms secondary to hypercalcemia or (2) histologic documentation of parathyroid hyperplasia after parathyroidectomy. Patients judged to have a PET included those who had a pancreatic neoplasm found at operation or identified on computed tomographic images. In the absence of operative/pathologic or computed tomographic evaluation of the pancreas, the presence or absence of a PET was not confirmed. The type of PET was defined as functioning or nonfunctioning. Functioning tumors were those associated with a clinical syndrome or an elevation (greater than 2 times the upper limit of normal) in serum levels of pancreatic peptides. In the absence of a clinical syndrome attributable to peptide hypersecretion, patients with mild elevations (less than 2 times

the upper limit of normal) of gastrin, pancreatic polypeptide, glucagon, or vasoactive intestinal peptide were considered to have nonfunctioning tumors. The diagnosis of insulinoma was based on results of an observed fast with serum glucose level less than 45 mg/dL (2.5 mmol/L) and a concomitant insulin level greater than 6 μ U/mL (42 pmol/L).

For the 62 patients determined to have MEN 1 by patient interview, confirmation by laboratory, radiographic, and pathologic data was incomplete. It is reasonable to assume that the frequency of MEN 1 and specific manifestations of MEN 1 in the kindreds studied is underestimated.

MUTATION ANALYSIS OF MEN1 GENE

Mutation analysis was limited to the proband within each kindred; once a mutation was identified in the proband, all blood relatives with documented MEN 1 were assigned the same genotype. For mutation analysis, blood was collected from affected individuals with informed consent. DNA was isolated from whole blood with a kit (QIAGEN blood or tissue kit; QIAGEN Inc, Chatsworth, Calif). Polymerase chain reaction assays and sequence analysis were performed as previously described.²⁸ In all cases, a detected mutation was confirmed by sequencing the opposite strand of a second sample and by restriction digestion where possible.

STATISTICAL ANALYSIS

The statistical associations of the site (exon) or the type of mutations with clinicopathologic variables were assessed by χ^2 and Fisher exact tests. Associations of continuous variables with different groups of mutations were assessed by the nonparametric Mann-Whitney test. All analyses were performed with the StatView (version 5.01) statistical software package (Abacus Concepts Inc, Berkeley, Calif). Differences were considered statistically significant at $P < .05$.

specific tumors, or the presenting clinical features of the disease (such as age or sites of tumor involvement).^{2,30-32} The phenotypic expression of MEN 1 may vary extensively between families and even among affected members within the same family.^{26,33-35}

The present study was undertaken to investigate the mutations in the *MEN1* gene of 24 independent families with MEN 1 referred to our institution between 1975 and 2001. We analyzed the phenotypic expression of MEN 1 in 109 affected kindred members in an effort to define the relationship between mutations in the *MEN1* gene and the associated neuroendocrine neoplasms.

RESULTS

Of the 109 affected individuals, 67 (61%) were alive, 28 (26%) were dead (most deaths were from MEN 1-related complications), and vital status was unknown for the remaining 14 (13%). The mean age of the 67 living individuals was 43 years (median, 42 years; range, 19-69 years). The mean age at the time of death for the 24 individuals in whom the age at death was known was 50 years (median, 50 years; range, 27-86 years); the age at death was unknown for 4 individuals.

The phenotypic expression of MEN 1 in all 109 individuals is summarized in **Table 1**. The most common manifestations of disease were hyperparathyroidism (74%), PETs (51%), and pituitary tumors (35%). As seen in Table 1, the frequency of observed phenotype was based on the extent of evaluation. For the 47 patients treated at our institution, the mean age at the time of clinical presentation with a first manifestation of MEN 1 was 29 years (median, 29 years; range, 9-53 years). The first manifestation of MEN 1 was hyperparathyroidism in 30 (64%) of these 47 patients.

The presence or absence of a PET was confirmed in 66 of the 109 individuals (in 43 of the 47 patients treated at our institution and in 23 of the remaining 62 affected relatives). Among these 66 individuals, PETs were found in 56 (85%). The type of PET was known for 45 of 56 patients. Fifty-two different types of PETs were found in these 45 patients, including 24 gastrinomas (46%), 15 insulinomas (29%), 9 nonfunctioning tumors (17%) (5 with elevation of pancreatic polypeptide), and 4 glucagonomas (8%).

The associations of hyperparathyroidism, pituitary tumors, carcinoid tumors, adrenal neoplasms, lipomas, and cutaneous angiofibromas with the types of PETs are

Table 1. MEN 1 Manifestations in 109 Individuals With MEN 1*

Patient Group	Manifestation					
	HPT	PETs	Pituitary Tumors	Lipomas	Adrenal Tumors	Carcinoid Tumors
Patients examined at MD Anderson, No. (n = 47)	44	33	22	10	8	7
Patient information from interview, No. (n = 62)	37	23	16	3	0	1
Total, No. (%) (n = 109)	81 (74)	56 (51)	38 (35)	13 (12)	8 (7)	8 (7)
Total with verified data, No. (%)	81 (98)	56 (85)	38 (61)	13 (26)	8 (17)	8 (17)
n†	83	66	62	50	47	47

*Some patients had multiple tumors. MEN 1 indicates multiple endocrine neoplasia type 1; HPT, hyperparathyroidism; and PETs, pancreatic endocrine tumors.
†For each manifestation of MEN 1, n represents the number of patients in whom the presence or absence of disease was known rather than the whole patient set.

Table 2. Other MEN 1–Associated Tumors With Various Types of PETs*

Type of PET	No. of Patients Affected/No. of Patients With Verified Status					
	HPT	Pituitary Tumors	Lipomas	Adrenal Tumors	Carcinoid Tumors	Cutaneous Angiofibromas
Gastrinoma (n = 24)	22/22	9/19	3/16	5/16	1/14	2/16
Insulinoma (n = 15)	14/15	12/14	4/11	0/11	2/11	0/11
Nonfunctioning (n = 9)	8/9	5/9	4/9	3/9	2/9	0/9
Glucagonoma (n = 4)	4/4	4/4	1/4	0/4	0/4	0/4
Total (%)	48/50 (96)	30/46 (65)	12/40 (30)	8/40 (20)	5/38 (13)	2/40 (50)

*Includes only verified data (ie, patients in whom the presence or absence of disease was known). Abbreviations are explained in the first footnote to Table 1.

Table 3. MEN 1 Mutations in 14 MEN 1* Kindreds

Family	Mutation	Exon	Type of Mutation	No. of MEN 1–Affected Relatives
A	R460X	10	Nonsense	4
B	E477X	10	Nonsense	7
D	354_356delGAA	2	Deletion	5
E	W341X	7	Nonsense	14
F	247_250delCTGT	2	Frameshift	3
G	Y276X	6	Nonsense	5
J	1216_1217insA	9	Frameshift	2
L	None found	1
M	S427R	9	Missense	5
N	E473X	10	Nonsense	2
O	Y227X	4	Nonsense	9
Q	625_628delCAGA	3	Frameshift	4
S	210_211delCC	2	Frameshift	18
V	275_286delGCTTACCGCCC	2	Deletion	1

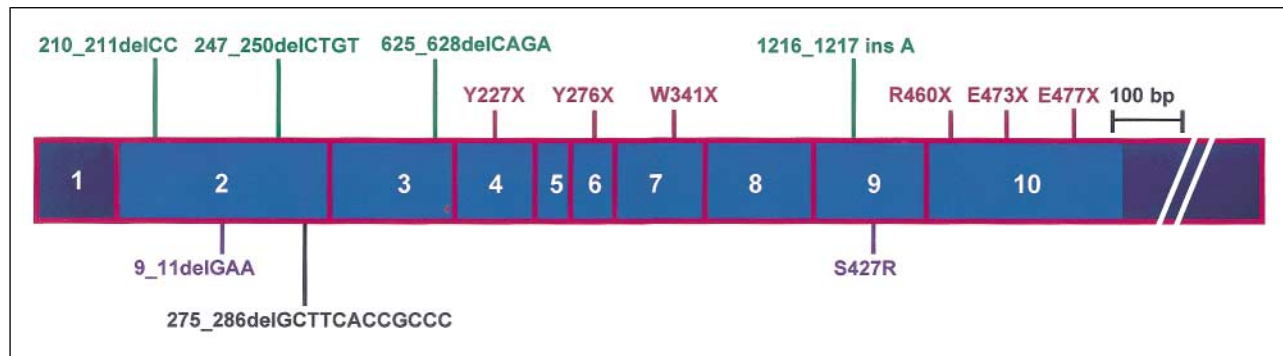
*MEN 1 indicates multiple endocrine neoplasia type 1.

shown in **Table 2**. Table 2 includes tumors from individuals in whom the presence or absence of disease was verified by medical evaluation or interview. Of interest, 12 (86% [representing 8 different kindreds]) of 14 insulinomas occurred in patients with pituitary tumors ($P = .03$).

At present, mutation analysis is complete in 14 of the 24 kindreds (80 [73%] of the 109 patients). A germline mutation in *menin* was identified in 79 of the 80 patients. **Table 3** summarizes the 13 identified *MEN1* germline mutations, as well as the type of mutation and exon location. Among the 13 kindreds with an identified *MEN1* mutation, mutations were most common in exons 2 (31%), 9 (15%), and 10 (23%). The positions of the mutations in the *MEN1* gene are illustrated in the **Figure**.

The mutation type and location in patients for whom both the *MEN1* mutation and the presence or absence of a specific type of tumor were known are shown in **Table 4**. The presence or absence of hyperparathyroidism was known in 83 (76%) of the 109 patients; hyperparathyroidism was present in 81 (98%). The *MEN1* mutation was known for 60 of the 83 patients with known parathyroid status; hyperparathyroidism was present in 59 (98%). Hyperparathyroidism was not associated with any specific type or site of *MEN1* mutation.

The presence or absence of PETs was known in 66 (61%) of the 109 patients; PETs were present in 56 (85%). The *MEN1* mutation was known for 49 of the 66 patients with known PET status; PETs were present in 43 (88%). All (100%) of the 21 patients with known PET



Schematic representation of the *MEN1* gene and localization of germline mutations found in 23 families. Green-labeled mutations correspond to frameshift; red, to nonsense; and purple, to missense mutations; black labels represent deletions. bp indicates base pairs.

Table 4. Mutation Types and Locations in Affected Individuals With and Without Specific MEN 1 Manifestations*

Manifestation	Total	No. of Patients							
		<i>MEN 1</i> Mutation Type				Exon of <i>MEN 1</i> Mutation			
		Frameshift (n = 27)	Nonsense (n = 41)	Missense (n = 5)	Deletion (n = 6)	2 (n = 27)	9 (n = 7)	10 (n = 13)	Other (n = 32)
HPT									
Present	59	20	31	3	5	20	4	10	25
Absent	1	1	0	0	0	0	1	0	0
PET									
Present	43	21	17	2	3	18	4	7	14
Absent	6	0	6	0	0	0	0	3	3
Pituitary tumors									
Present	29	16	10	2	1	15	2	2	10
Absent	20	2	17	0	1	1	2	8	9
Carcinoid tumor									
Present	5	0	4	0	1	1	0	3	1
Absent	31	11	18	1	1	9	3	6	13
Adrenal tumor									
Present	8	3	4	0	1	2	2	2	2
Absent	27	8	17	1	1	8	1	7	11

*Includes only patients in whom both the *MEN 1* mutation and the presence and absence of the specific manifestation were known. Abbreviations are explained in the first footnote to Table 1.

and *MEN1* mutation status who had frameshift mutations had PETs, whereas 22 (79%) of 28 patients with all other types of *MEN1* mutations had PETs ($P = .03$).

The presence or absence of a pituitary tumor was known in 62 (57%) of the 109 patients; pituitary tumors were present in 38 (61%). The *MEN1* mutation was known for 49 of the 62 patients with known pituitary status; pituitary tumors were present in 29 (59%). Fourteen (48%) of the 29 patients with pituitary tumors had frameshift mutations in exon 2 (representing 2 of 9 kindreds); frameshift mutations in exon 2 were not found in any of the 20 patients without pituitary tumors ($P < .001$).

The presence or absence of a bronchial or thymic carcinoid tumor was known in 47 (43%) of the 109 patients; bronchial or thymic carcinoid tumors were present in 8 (17%). The *MEN1* gene mutation was known for 36 of the 47 patients with known carcinoid status; bronchial or thymic carcinoid tumors were present in 5 (14%).

The presence or absence of an adrenal tumor was known in 47 (43%) of the 109 patients; adrenal tumors were present in 8 (17%). The *MEN1* mutation was known

for 35 of the 47 patients with known adrenal status; adrenal tumors were present in 8 (23%). There was an even distribution of mutation type and location among the 8 adrenal tumors.

The type and location (exon) of *MEN1* mutations present in the 43 patients with PETs are shown in **Table 5**. These 43 patients had a total of 50 PETs (some patients had more than 1 type of PET). Of these PETs, 26 (52%) were associated with frameshift mutations, 19 (38%) with nonsense mutations, 3 (6%) with deletions, and 2 (4%) with missense mutations. Of interest, all 4 glucagonomas were associated with frameshift mutations in exon 2 ($P = .004$).

Twenty-five patients with PETs were treated surgically at our institution. Surgical procedures consisted of distal pancreatectomy with or without enucleation of tumors in the head of the pancreas or the uncinate process in 18 patients, enucleation or nonanatomic resection of tumors in 2 patients, pancreaticoduodenectomy in 2 patients, and total pancreatectomy in 3 patients. Because of tumor recurrence in the pancreatic head, completion total pancreatectomy was performed in 5 (28%) of the 18 pa-

Table 5. Mutation Types and Locations in 43 Patients With 50 Pancreatic Endocrine Tumors (PETs)*

PET Tumor Type	No. of Tumors								
	Total (N = 50)	MEN 1 Mutation Type				Exon of MEN 1 Mutation			
		Frameshift (n = 26)	Nonsense (n = 19)	Missense (n = 2)	Deletion (n = 3)	2 (n = 23)	9 (n = 4)	10 (n = 8)	Other (n = 15)
Gastrinoma	20	10	8	0	2	8	1	3	8
Insulinoma	9	5	3	1	0	5	1	0	3
PPomas	5	3	2	0	0	2	1	1	1
Nonfunctioning	4	0	3	0	1	1	0	3	0
Glucagonoma	4	4	0	0	0	4	0	0	0
Function status unknown	8	4	3	1	0	3	1	1	3

*Includes 50 individual tumors from 43 patients in whom *MEN 1* mutation analysis was known. PPomas indicates pancreatic polypeptide tumors.

tients who underwent initial distal pancreatectomy. Lymph node metastases were found in 10 (45%) of the 22 patients who underwent surgery that included resection of 1 or more lymph nodes for pathologic analysis. Synchronous liver metastases (at the time of surgery) were found in 2 (8%) of the 25 patients who had surgery, and metachronous liver metastases developed in 2 (9%) of the remaining 23 surgically treated patients.

The presence or absence of metastatic neuroendocrine carcinoma in either regional lymph nodes or distant organs was known for 26 (58%) of the 45 patients in whom the type of PET was known. Metastatic disease in regional lymph nodes and/or distant organs was found in 16 (62%) of these 26 patients. Twelve (86%) of 14 patients with gastrinoma had lymph node or distant metastases. In contrast, metastases were found in only 4 (33%) of 12 patients with PETs other than gastrinoma ($P = .01$). The mutation type and location was known for 22 of the 26 patients. No significant correlation was found between the presence or absence of metastatic disease (lymph node or distant organ) and the type or site of mutation in patients with PETs.

COMMENT

In the present study, we determined the phenotypic expression of *MEN 1* in 109 affected kindred members from 24 unrelated families. We attempted to define the relationship between the mutations in the *MEN 1* gene and the associated neuroendocrine neoplasms. We identified a mutation in 13 families, each with a unique germline mutation (Table 3). We did not detect a mutation in 1 of the 14 probands in whom analysis was completed. The inability to detect a *MEN 1* germline mutation in some patients with *MEN 1* is expected, as large deletions and/or mutations in the promoter regions or in untranslated regions of the gene may not be detected. Of the mutations we identified, Y276X is a novel nonsense mutation in exon 6, and 275_286delGCTTCAC-CGCC is a novel deletion in exon 2. The remaining 11 mutations identified in our patients were previously reported.^{3,7,14,26,29,37} Mutations 354_356delGAA, 247_250delCTGT, 1216_1217insA, and 210_211delCC have been renamed by means of standardized nomenclature³⁸ and were previously reported as K119del, 357del4, 1325insA, and 320del2, respectively.

Consistent with previous reports, many *MEN 1* mutations in this study were nonsense or frameshift mutations, which eliminate the function of 1 copy of the gene and result in a truncated menin protein.^{14,26,29} As expected, the mutations found in our patients were scattered throughout the coding region of the *MEN 1* gene.^{3,14,24-29} Bassett and colleagues²⁶ have suggested that mutation hot spots exist in exons 2, 3, and 10; in the present study, *MEN 1* mutations were most common in exons 2, 9, and 10.

Several previous reports have failed to demonstrate a direct genotype-phenotype correlation in patients with *MEN 1*. There are 2 possible reasons for this. First, a wide spectrum of mutations in the *MEN 1* gene has been identified in different families with the same clinical manifestations of *MEN 1*. For example, several kindreds from Newfoundland and Tasmania have been identified as having a distinct *MEN 1* phenotype characterized by a high prevalence of prolactinomas, late-onset hyperparathyroidism, and a rare occurrence of pancreatic neoplasms.^{26,33-35,39,40} This variant of *MEN 1* (*MEN 1*Burin) has been reported in at least 5 kindreds. However, these 5 kindreds do not all have the same *MEN 1* mutation. Second, previous studies have observed the same *MEN 1* mutation in different kindreds with different clinical manifestations of *MEN 1*.^{26,33-35}

Bartsch et al³⁰ suggested that the type or site of mutation might predict the biological behavior of PETs in patients with *MEN 1*. In their study, 55% of patients with nonsense or frameshift mutations of the C- or N-terminal regions of exons 2, 9, or 10 had malignant tumors, compared with only 10% of patients with all other mutations of the *MEN 1* gene. However, most patients considered to have metastatic disease had lymph node metastases but no distant organ metastases. In fact, only 1 patient with a nonsense or frameshift mutation in exon 2, 9, or 10 had a distant organ metastasis (lung).

Other studies suggesting a possible genotype-phenotype association in patients with *MEN 1* include a report by Calender et al⁴¹ in which patients with *MEN 1* and triple-organ involvement or aggressive phenotypes had truncating mutations. In addition, a mild variant form of *MEN 1* called *familial isolated hyperparathyroidism* has been associated with missense mutations occurring mainly between exons 3 and 7.^{31,32,42}

In the present report, all patients with frameshift mutations had PETs (Table 4). Furthermore, glucagonomas appeared to be associated with frameshift mutations in exon

2. A significant association was also found between pituitary tumors and frameshift mutations in exon 2, while bronchial and thymic carcinoids were more frequently associated with mutations in exon 10 (Table 4). However, our data did not demonstrate a significant association between the specific type or location of *MEN1* mutation and the development of metastatic disease in patients with PETs. The small number of patients with lymph node and distant organ metastases in our study limits this analysis.

In contrast to MEN 1, MEN 2 is characterized by a strong genotype-phenotype correlation. The biological aggressiveness of medullary thyroid carcinoma can be predicted on the basis of the specific mutation in the *RET* proto-oncogene.⁴³ In addition, specific codon mutations are associated with classic MEN 2B, MEN 2A, and familial medullary thyroid carcinoma.^{36,44} Most important, knowledge of the specific *RET* mutation guides the recommendation for the timing of thyroidectomy in at-risk patients. Such data are not yet available for patients with MEN 1, specifically those with PETs.

Our findings suggest that the specific mutation type and location in an individual family with MEN 1 may be associated with the clinical manifestations of the MEN 1 syndrome. A more specific genotype-phenotype correlation is probably not possible because of the heterogeneity of the mutations reported in the *MEN1* gene. Initial reports such as this will improve our overall understanding of the molecular genetics of MEN 1. At present, guidelines for the operative treatment of patients with MEN 1-associated neoplasms are not based on information about the specific *MEN1* mutation; however, this information may be helpful for the screening and genetic counseling of at-risk patients.

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Corresponding author and reprints: Douglas B. Evans, MD, Department of Surgical Oncology, Campus Box 444, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030 (e-mail: devans@mdanderson.org).

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DISCUSSION

Jon A. Van Heerden, MD, Rochester, Minn: This presentation and the ensuing manuscript that is excellent are no exception. As emphasized in the preceding presentation this morning, we as surgeons continue to understand the diseases we deal with on a daily basis better by an improved understanding, by us, of the molecular biology and the genetic mutations associated with these diseases. Because of this better understanding, we are today able to, for example, advise a total thyroidectomy on a healthy preschool child, with no physical abnormalities, based solely on genetic mutations, in this case, a positive *RET* proto-oncogene, and fully expect a 100% long-term cure. What a wonderful advance, and wouldn't it be surgical utopia if this became a reality for a wide array of malignancies we deal with on a daily basis.

The Houston group has told us, however, that we are not doing nearly as well in those rare patients with MEN 1, who, as you know, have a propensity to develop pancreatic endocrine tumors. MEN patients are in fact prone to premature death, with an important cause of death being metastatic islet cell tumors. Liver metastases were present in 15% of their patients and lymph node metastases in a staggering 45% of their patients. We, and others, have in fact found that the 20-year survival of MEN patients was about 64% in comparison to 81% of a matched non-MEN patient population group. Almost one third of the deaths in this group were due to metastatic islet cell tumors.

Most importantly, I think, the authors have shown a correlation between frameshift mutations, in particular exons, with specific pancreatic endocrine tumors, glucagonomas and PPomas in particular. This interesting finding is in keeping with the recent publication in *Surgery* by investigators from Germany, headed by Mathias Rothmand, who found that patients with truncating nonsense or frameshift mutations in the N- or C-terminal regions of the *MEN1* gene, which are exons 2, 9, and 10, had a higher rate of malignancy, 55% vs 10%, and a shorter disease-free interval, 26 vs 92 months, than those MEN patients without these genetic mutations.

May I ask the authors 2 simple questions? What is your advice to a 19-year-old totally asymptomatic MEN 1 patient who has a normal CT scan of the abdomen, whose father died of metastatic glucagonoma at age 40, and who has a frameshift mutation on exon 2? Is this patient a candidate for a prophylactic total pancreatectomy?

Secondly, because of the rarity of these patients and in view of the small numbers in your study, and the small numbers worldwide, should we perhaps assemble all MEN 1 patients, perhaps with the help of the International Association of Endocrine Surgeons, in an attempt to determine the relationship between MEN 1 phenotype and tumor aggressiveness?

Richard A. Prinz, MD, Chicago, Ill: I would like to ask a variation of Dr Van Heerden's first question. Have you done this gene mutation analysis prospectively in patients with MEN 1 who are at risk of developing islet cell tumors? If you have, how has it helped to tailor your screening and imaging in these patients to facilitate early diagnosis and treatment of an islet cell tumor?

Goffrey B. Thompson, MD, Rochester: You grouped all the gastrinomas under the heading of pancreatic endocrine

tumors. Generally, in MEN 1 patients, we think of the gastrin excess as coming from duodenal microcarcinoids. That has been our experience as well as others elsewhere. I am curious what the breakdown was for these tumors (duodenal vs pancreatic), and is there any correlation between the duodenal and pancreatic gastrin-producing tumors and the type or types of mutations seen?

Edwin L. Kaplan, MD, Chicago: I share Dr Van Heerden's point of view that we should have an international database. I want to ask one question. When studied by immunohistochemistry, many pancreatic endocrine tumors contain more than one peptide. How do you classify these tumors?

Dr Lee: Thank you all for your kind comments and cogent questions. I will answer some of the easier questions first. Dr Kaplan asked how we classified patients whose tumors stained for multiple peptides by immunohistochemistry. These patients were classified on the basis of clinical hormone production rather than on the basis of immunohistochemical markers.

We were asked how many of our gastrinoma patients had duodenal tumors. Duodenotomy was performed in 4 gastrinoma patients and islet cell tumors were identified and enucleated in 3 of these 4 patients. Duodenal tumors were found in an additional 5 patients who underwent pancreaticoduodenectomy or total pancreatectomy.

Dr Van Heerden asked about an international registry of MEN 1 patients. We certainly would support the development of an international registry as Dr Van Heerden has described. The number of patients and kindreds seen at any individual institution, even those such as ours with a dedicated clinical and basic science research program in MEN 1, is relatively small. Our presentation today, as well as recent publications of other groups, demonstrate that it is very difficult to get at genotype-phenotype correlations using data from any single institution, so we would certainly support development of such an international registry.

Dr Prinz and Dr Van Heerden asked related questions regarding screening and prophylactic surgery for patients from MEN 1 kindreds, particularly in kindreds in which the pancreatic neuroendocrine disease has been relatively aggressive. The issue of prophylactic surgery goes to the heart of our presentation and the difficulty in getting good data about associations between genotype and phenotype. We think the example of MEN 2 is an excellent one where prophylactic thyroidectomy has really changed the way that we manage these patients and the natural history of the disease. We don't think we are there yet with regard to MEN 1 and surgical treatment of pancreatic neuroendocrine disease. For MEN 1 patients who have hyperparathyroidism, where the aggressiveness of hyperparathyroidism does appear to be kindred-specific, we and others have begun to tailor our surgical treatments for those patients based on the aggressiveness of the hyperparathyroidism in the kindred and the individual patient. However, we do not recommend yet prophylactic total pancreatectomy for any of our patients with MEN 1. The way that we would manage a patient such as Dr Van Heerden described would be to do an initial thin-cut, high resolution, contrast-enhanced CT scan. We would also add endoscopic ultrasound to look at the pancreas and the duodenum at the time of initial presentation. We would follow a patient like this, in the absence of better data, with annual CT scans as well as measurements of plasma peptide hormone levels. At the first clinical evidence for a pancreatic endocrine tumor, we would consider operation. We would not wait until the tumor reached an arbitrary size, 2 cm for example. The operation that we would do would be an operation such as Dr Norman Thompson has described, that is, a distal pancreatectomy, enucleation of tumors in the pancreatic head, and a peripancreatic and portal lymph node dissection.