Mutations and Allelic Deletions of the *MEN1* Gene Are Associated with a Subset of Sporadic Endocrine Pancreatic and Neuroendocrine Tumors and Not Restricted to Foregut Neoplasms

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Endocrine pancreatic tumors (EPT) and neuroendocrine tumors (NET) occur sporadically and rarely in association with multiple endocrine neoplasia type 1 (MEN1). We analyzed the frequency of allelic deletions and mutations of the recently identified *MEN1* gene in 53 sporadic tumors including 30 EPT and 23 NET (carcinoids) of different locations and types. Allelic deletion of the *MEN1* locus was identified in 18/49 (36.7%) tumors (13/30, 43.3% in EPT and 5/19, 26.3% in NET) and mutations of the *MEN1* gene were present in 8/52 (15.3%) tumors (4/30 (13.3%) EPT and 4/22 (18.1%) NET). The somatic mutations were clustered in the 5' region of the coding sequence and most frequently encompassed missense mutations. All tumors with mutations exhibited a loss of the other allele and a wild-type sequence of the *MEN1* gene in nontumorous DNA. In one additional patient with a NET of the lung and no clinical signs or history of MEN1, a 5178-9G→A splice donor site mutation in intron 4 was identified in both the tumor and blood DNA, indicating the presence of a thus far unknown MEN1 syndrome. In most tumor groups the frequency of allelic deletions at 11q13 was 2 to 3 times higher than the frequency of identified *MEN1* gene mutations. Some tumor types, including rare forms of EPT and NET of the duodenum and small intestine, exhibited mutations more frequently than other types. Furthermore, somatic mutations were not restricted to foregut tumors but were also detectable in a midgut tumor (15.2% versus 16.6%). Our data indicate that somatic *MEN1* gene mutations contribute to a subset of sporadic EPT and NET, including midgut tumors. Because the frequency of mutations varies signifi-

cantly among the investigated tumor subgroups and allelic deletions are 2 to 3 times more frequently observed, factors other than *MEN1* gene inactivation, including other tumor-suppressor genes on 11q13, may also be involved in the tumorigenesis of these neoplasms. (Am J Pathol 1999, 154:429-436)

Well differentiated neuroendocrine (carcinoid) tumors (NET) have been divided into tumors of the foregut (lung, stomach, duodenum and pancreas), midgut (jejunum, ileum), and hindgut (colon, rectum) according to their location and embryological origin.¹ NET may cause significant morbidity by local tumor symptoms and the uncontrolled secretion of peptide hormones. Patients exhibit an overall 5-year survival rate of 50%.² Endocrine pancreatic tumors (EPT), which occur in approximately 1 of every 100,000 persons,^{3,4} are clinically important because of their potential to cause life-threatening symptoms by inadequate hormone secretion at an early stage of tumor development. Thus, 60–85% of EPTs are functionally active, producing hormones such as insulin, gastrin, glucagon, or vasoactive intestinal polypeptide (VIP),⁴ which may lead to hyperinsulinemic hypoglycemia, Zollinger-Ellison's (severe gastrointestinal ulcers), Verner-Morrison syndrome (also called WDHA [watery diarrhea, hypokalemia, achlorhydria] syndrome), glucagonoma (migratory necrotizing skin erythema, mild diabetes mellitus, anemia), or somatostatinoma syndrome (diabetes mellitus, steatorrhea, gallbladder disease including cholelithiasis), respectively. The remaining tumors are functionally inactive but may immunohistochemically express various hormones. Approximately 90% of insulinomas follow a benign clinical course and are cured by resection of the tumor. In contrast, the other types of EPT have a high malignant potential (approximately 64–90%) and up to 50% of pa-

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tients have developed metastases in regional lymph nodes or the liver by the time of diagnosis.⁴

The vast majority of NET and EPT occur sporadically, but a subset of tumors is associated with inherited syndromes such as multiple endocrine neoplasia type 1 (MEN1) and von Hippel-Lindau syndrome.^{5,6} Although the molecular basis of these familial tumors has recently been established,^{5,6} only little is known about the oncogenesis and molecular basis of progression of sporadically occurring EPT and NET.

MEN1 is an autosomal-dominant genetic disorder characterized by the development of NET in the parathyroid glands, the endocrine pancreas or duodenum, and in the anterior pituitary.⁷ Up to 9% of *MEN1* gene carriers develop NET at other locations, especially in the foregut (thymus, lung, or stomach), and may also suffer from nonneuroendocrine neoplasms such as lipomas, angiofibromas, or ependymomas.⁷ The gene for MEN1 has previously been localized to chromosome 11q13⁸ and was recently cloned.⁵ A tumor suppressor function for the *MEN1* gene has been suggested based on frequent chromosome 11q13 loss of heterozygosity (LOH) in neoplasms of affected patients.^{8,9} Allelic deletions^{10,11} and recent mutation analysis studies^{12–15} had implicated the *MEN1* gene as a tumor suppressor in a significant fraction of the sporadic counterparts of typical MEN1 neoplasms and it has been hypothesized that these tumors are restricted to the foregut.^{16,17} However, in previous studies Jakobovitz et al¹¹ demonstrated that loss of 11q13 is also encountered in 100% and 63% of sporadic NET of the midgut and hindgut, respectively, suggesting that somatic mutations of the *MEN1* gene might also contribute to the pathogenesis of these tumors. A systematic examination of both allelic deletions and mutations of the *MEN1* gene in the latter tumor types, however, is missing.

In the present study, we analyzed 53 tumor tissues, including sporadic NET of the lung and gastrointestinal tract and EPT, for inactivation of the *MEN1* gene to test the hypothesis that *MEN1* gene mutations in sporadic tumors are restricted to foregut tumors. Both allelic deletions of chromosome 11q13 and *MEN1* gene mutations were assessed.

Materials and Methods

Patient Samples

Frozen tissue samples from 53 patients with EPT or NET were obtained from the files of the Departments of Pathology at the University of Zürich and Erasmus University of Rotterdam. The samples included 30 EPT (9 insulinomas, 5 VIPomas, 3 gastrinomas, 1 glucagonomas, 1 somatostatinoma, 11 nonfunctional tumors) and 23 well differentiated NET (12 lung, 2 stomach, 3 duodenum, 3 ileum, 1 colon, 1 liver, 1 metastasis) (Table 1). Because a different pathogenic mechanism has been suggested for poorly differentiated (eg, small cell and large cell) neuroendocrine carcinomas, these neoplasms were not included in the present study.

Table 1. Materials

Tumor types and locations		
Endocrine pancreatic tumors		30
Insulinoma	9	
VIPoma	5	
Gastrinoma	3	
Glucagonoma	1	
Somatostatinoma	1	
Nonfunctioning	11	
Neuroendocrine tumors		23
Lung/Bronchus	12	
Stomach	2	
Duodenum	3	
Ileum	3	
Colon	1	
Metastasis	1	
Liver	1	
Total		53

Hematoxylin and eosin-stained and immunohistochemically stained sections of formaldehyde-fixed, paraffin-embedded samples of each tumor were used to reevaluate and classify the series of tumors according to the most recent classification.¹⁸

MEN1 Gene Mutation Analysis

Genomic DNA was isolated as recently described.^{19,20} DNA from peripheral blood leukocytes of healthy persons served as negative controls and from MEN1 patients from our files as positive controls. The 2790-bp coding region and splice sites of the *MEN1* gene were screened for mutations according to our previously published protocols.²¹ Polymerase chain reaction (PCR) fragments ranging from 244 to 409 bp in length were amplified from 100-ng tumor or germline DNA using previously published primers and conditions²¹ (see Table 2). In brief, PCR amplification of exons 3 to 9 was performed in a total amount of 50 μ l reaction mixture containing 0.2 mmol/L dATP, dTTP, dGTP, dCTP, 50 pmol of each sense and antisense primer, 1.5 mmol/L Mg²⁺, 10 mmol/L Tris-HCl, 50 mmol/L KCl, and 1 Unit Taq DNA polymerase (AmpliTaq Gold, Perkin Elmer, Norwalk, CT). For PCR amplification of exons 2 and 10, Expand High Fidelity DNA Polymerase (Boehringer Mannheim, Mannheim, Germany) and 10% DMSO were used.

For the single strand conformation polymorphism (SSCP) analysis, 10 μ l of denatured PCR products in stop buffer (95% formamide, 20 mmol/L EDTA, 0.05% xylene cyanol, 0.05% bromophenol blue) were loaded onto non-denaturing 6% polyacrylamide gels. For the heteroduplex method PCR products of tumors were admixed with those of normal control DNA (1:1), denatured, slowly cooled to room temperature, and then loaded onto a nondenaturing polyacrylamide-derived gel matrix (MDE Gel Solution, FMC BioProducts, Rockland, ME) containing 15% urea. Electrophoresis was carried out at 8 W and 500–700 V, respectively, for 16 hours at room temperature. After electrophoresis the DNA was visualized by silver staining as recently described.^{22,23} Aberrant bands from PCR-SSCP analyses were excised from additionally prepared

Table 2. PCR Primers

Primer	Position	Orientation	Sequence	Length (bp)	Temp. (°C)
M2newF	2193-2211	sense	5'-CCTTAGCGGACCCCTGGGA-3'	365	63
M2newR	2575-2537	antisense	5'-CTTAGCGGACCCTGGGAGGA-3'		
M2NNF	2436-2455	sense	5'-TCAACCGCGTCATCCCTACC-3'	405	60
M2NR	2840-2821	antisense	5'-CACCTGCCGAACCTCACAAAG-3'		
M3NF	4265-4284	sense	5'-GGACCCCTTTCATTACCTC-3'	357	57
M3NR	4631-4612	antisense	5'-TCTGTCTTCCCTTCTATGT-3'		
M4NF	4639-4658	sense	5'-CTTTCCTGGCTGTCATTCC-3'	244	62
M4NR	4900-4883	antisense	5'-TCCCACAGCAAGTCAAGTCT-3'		
M5/6NF	5125-5145	sense	5'-ACCCGTTCTCCTCCCTGTTC-3'	379	65
M5/6NR	5504-5485	antisense	5'-GAGACTGGATGGGCGATACC-3'		
M7NF	5891-5912	sense	5'-GGTGGGAGTGGAGATGGAGAGG-3'	340	67
M7R	6233-6212	antisense	5'-GGACGAGGGTGGTGGAACTG-3'		
M8NF	6581-6604	sense	5'-AGACCCCTTCAGACCTACAGAG-3'	273	67
M8R	6855-6834	antisense	5'-CCATCCCTAATCCCGTACATGC-3'		
M9F	7142-7164	sense	5'-CTGCTAAGGGGTGAGTAAGAGAC-3'	288	63
M9NR	7430-7411	antisense	5'-AAAAGTCTGACAAGCCCGTG-3'		
M10NF	7546-7565	sense	5'-TTGCTCTCACCCTTGCTCTCC-3'	409	56
M10NNR	7955-7938	antisense	5'-CTTGATGGCGCTCGAGTT-3'		
M10NNF	7896-7915	sense	5'-AAGATGAAGGGCATGAAGGA-3'	332	56
M10NR	8228-8208	antisense	5'-GCTGGAGAAAATCGTGGGTT-3'		

Sybr Green-stained SSCP polyacrylamide gels (Molecular Probes, Eugene, OR) and an aliquot of the eluted DNA was reamplified under the above described conditions, gel purified using a QIAquick Gel Extraction Kit (Qiagen AG, Basel, Switzerland), and subjected to DNA cycle sequencing in sense and antisense direction using the TaqDyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Weiterstadt, Germany) followed by gel electrophoresis, data collection, and analysis on an automated DNA sequencer (Model 373A, Applied Biosystems).

MEN1 Allelic Deletion Analysis

Fluorescent *in situ* hybridization (FISH) analysis was performed as recently described, using the cosmid clone c10B11 (40 kb) containing the *MEN1* gene as a probe.²¹ In brief, touch preparations from frozen tissue samples were fixed in 70% ethanol for 1 hour, treated with 100 μ g/ml pepsin (Sigma, St. Louis, MO) in 0.01 N HCl for 20 minutes at 37°C, and postfixed for 10 minutes in 1% formaldehyde in phosphate-buffered saline at room temperature.²⁴ Fluorescein-labeled cosmid probe (250 ng) and 20 ng of rhodamin-labeled α -repetitive DNA specific for chromosome 11 in hybridization buffer (50% formamide, 10% dextran sulfate, 2 $\times$ SSC, herring sperm DNA, yeast tRNA, Cot-1 fraction of human DNA) were hybridized overnight in a humidified chamber at 37°C. Posthybridization washes were performed at 45°C in 50% formamide/2 $\times$ SSC (3 times for 5 minutes) and 0.1 $\times$ SSC (3 times for 5 minutes). Amplification of the cosmid probe signal was achieved using rabbit anti-fluorescein (Dako, Copenhagen, Denmark) and swine anti-rabbit Ig fluorescein antibodies, followed by dehydration of the slides and mounting in Vectashield (Vector, Burlingame, CA) containing 0.5 μ g/ml 4',6-diamidino-2-phenylindole antifade (DAPI, Sigma) for nuclear counterstaining. Hybridization signals of at least 100 interphases for each tumor were scored using a Zeiss Axioplan fluorescence microscope with appropriate filter sets. Images were taken with a

CCD camera using the Vysis Quips FISH software (IG Instrumenten-Gesellschaft AG, Bern, Switzerland). The presence of only one *MEN1* cosmid signal in more than 30% of tumor cells was interpreted as an allelic deletion. Normal connective tissue in the vicinity of tumors served as internal control and exhibited nuclei with one *MEN1* signal in 3 to 7% of cells.

In 34 patients from whom DNA of nontumorous tissue or blood was available, samples were also screened for allelic deletion using three polymorphic markers on 11q13 as recently described²⁵ and the DNA was visualized by silver staining as described above. The markers included an intragenic microsatellite marker (D11S4946) at the *MEN1* 5' region and two flanking 11q13 markers, PYGM (human muscle glycogen phosphorylase) and D11S4936.²⁶

Results

Results of the *MEN1* gene mutation and LOH analyses are listed in Tables 3–5 and representative examples are illustrated in Figure 1.

We identified a total of nine tumors with *MEN1* gene mutations (Tables 4 and 5). Eight mutations proved to be of somatic origin when the wild-type sequence was identified in nontumorous tissues or blood of the affected patients. One patient with a NET of the lung (atypical carcinoid) exhibited a germline 5178–9G→A splice acceptor site mutation in intron 4 that was detectable in various nontumorous tissues and blood; he was therefore diagnosed as a *MEN1* gene carrier.²¹ This patient's tumor also exhibited an allelic deletion at the intragenic *MEN1* gene microsatellite locus D11S4946. The overall frequency of identified somatic *MEN1* gene mutations in the 52 analyzed real sporadic cases of our study was therefore 15.3% (8/52). The highest frequency of mutations was found in gastrointestinal NET (2/11; 18%) and NET of the lung (2/11; 18%) followed by EPTs (4/30; 13.3%). The

Table 3. Overview of Results

Case	Diagnosis	Sex	Polymorphism*	Mutation*	D11S4936	D11S4946	PYGM	FISH
1	Insulinoma	f			□	□	□	
2	Insulinoma	f			□	■	■	■
3	Insulinoma	f			□	■	□	
4	Insulinoma, mal	m			ni	□	□	□
5	Insulinoma	m		1674del7bp (10)	■	■	■	■
6	Insulinoma	m			□	■	■	
7	Insulinoma	m			□	□	□	□
8	Insulinoma	f						□
9	Insulinoma	f						□
10	Gastrinoma	m			□	■	□	■
11	Gastrinoma	m			□	■	■	
12	Gastrinoma	m			ni	ni	□	□
13	VIPoma	f			ni	□	□	□
14	VIPoma	f			□	■	□	■
15	VIPoma metastasis	m	D418D (9)	Q450X (9)	□	■	□	
16	VIPoma	f						■
17	VIPoma	f		L37P (2)				■
18	Glucagonoma	f		A50P (2)	ni	□	□	□
19	Somatostatinoma	m						■
20	Non-functioning EPT, mal	f			□	□	□	□
21	Non-functioning EPT, mal	f			■	■	■	■
22	Non-functioning EPT, mal	m			□	□	□	□
23	Non-functioning EPT, mal	m			ni	□	□	□
24	Non-functioning EPT, mal	m			□	ni	ni	■
25	Non-functioning EPT	m			□	□	□	
26	Non-functioning EPT, mal	m			ni	□	□	□
27	Non-functioning EPT	m						□
28	Non-functioning EPT	f						■
29	Non-functioning EPT, mal	m						□
30	Non-functioning EPT, mal	m						□
31	NET Lung, mal	m		5178-9G→A [†]	ni	■	□	■
32	NET Lung, mal	m		D172V (3)	■	■	■	■
33	NET Lung	m			□	□	□	□
34	NET Lung	f		434ins29 (2)	■	■	■	■
35	NET Lung, mal	f			□	□	□	
36	NET Lung	f						□
37	NET Lung	f						□
38	NET Lung	m						
39	NET Lung	f						□
40	NET Lung	f						□
41	NET Lung	m	S145S (2)					□
42	NET Lung	f						□
43	NET Stomach	m			□	■	□	□ [‡]
44	NET Stomach	m			ni	□	■	□
45	NET Duodenum	m			□	ni	ni	■
46	NET Duodenum	f		Q209X (3)	□	■	□	
47	NET Duodenum	f						□
48	NET Ileum	f			□	□	□	□
49	NET Ileum	m		V53I (2)				
50	NET Ileum	f			□	ni	□	□
51	NET Colon, mal	f	R171Q (3)		ni	□	□	□
52	NET metastasis	m						
53	NET liver	f			ni	□	□	□

EPT, endocrine pancreatic tumor; NET, neuroendocrine tumor; PYGM, human muscle glycogen phosphorylase; FISH, fluorescent *in situ* hybridization; mal, malignant; ni, not informative; ■, loss of heterozygosity; □, retention of constitutional heterozygosity.

*Numbers in parentheses identify the exons involved.

[†]Germline mutation in intron 4.

[‡]25% of tumor cells exhibited loss of one MEN1 cosmid signal.

identified somatic mutations consisted of 2 stop codon mutations (Q450X, Q209X), 4 missense mutations (L37P, A50P, D172V, V53I), one deletion of 7 bp (1674del7), and one 29-bp insertion (434ins29). The greatest number of mutations was encountered in exon 2 (four tumors), followed by exon 3 (two tumors); one mutation each was found in exons 9 and 10. All tumors with somatic *MEN1* gene mutations also exhibited an allelic deletion at the

MEN1 locus, either by microsatellite analysis using the marker D11S4946 or by FISH, with the exception of one tumor from which no normal tissue or sufficient fresh material was available for analysis (sample 49, Tables 2 and 3). Furthermore, three NETs exhibited the recently published polymorphisms D418D in exon 9 (one VIPoma), S145S in exon 2 (one NET of the lung) and R171Q in exon 3 (one NET of the colon).²⁷

Figure 1. Loss of heterozygosity analysis, PCR-SSCP analysis, and sequence analysis of the *MEN1* gene in a sporadic neuroendocrine tumor of the lung (Cd1). Note that only one of the *MEN1* genes (green spots, arrows) is detectable in the majority of tumor cells when compared with the chromosomes 11 (red dots) and that one allele of the three 11q13 markers is lost in the tumor DNA (T) when compared to normal tissue (N). The PCR-SSCP of exon 3 shows a band shift (line 4, red arrowheads) in the tumor DNA caused by a D172V missense mutation. In the sporadic endocrine pancreatic tumor P149 the LOH-FISH analysis shows only one signal of chromosome 11 (red) and the *MEN1* locus (green), indicating the loss of one chromosome 11 in the majority of tumor cells. PCR-SSCP analysis reveals a band shift in exon 2 (line 3, red arrowheads) caused by an A50F missense mutation.

PCR-LOH and FISH analysis performed in 49 sporadic tumors (PCR-LOH in 8, FISH in 16, and both analyses in 25 tumors) revealed an allelic deletion of the *MEN1* locus in 18 tumors (36.7%) and at 11q13 in 19 tumors (38.7%). This additional tumor exhibited an isolated allelic deletion of the PYGM locus. The frequency in EPT was 43.3% (13/30) and in NET 26.3% (5/19). Thus a two- to threefold higher frequency of allelic deletions in comparison to

detected *MEN1* gene mutations was found in all analyzed tumor groups.

Among the different pancreatic tumor groups, the frequencies of allelic deletions and mutations in insulinomas were 4/9 and 1/9, in gastrinomas 2/3 and 0/3, in VIPomas 4/5 and 2/5, in glucagonomas 0/1 and 0/1, in somatostatinomas 1/1 and 1/1, and in nonfunctioning EPT 3/11 and 0/11. Thus the combined frequency of allelic deletions

Table 4. Allelic Deletions and Mutations of the *MEN1* Gene

Tumor type (no.)	LOH	Mutation
Insulinomas (9)	4/9 (44.4%)	1/9 (11.1%)
Gastrinomas (3)	2/3 (66.6%)	0/3
VIPomas (5)	4/5 (80%)	2/5 (40%)
Glucagonomas (1)	0/1	0/1
Somatostatinomas (1)	1/1 (100%)	1/1 (100%)
Non-functioning (11)	3/11 (27.2%)	0/11
All EPT (30)	13/30 (43.3%)	4/30 (13.3%)
Lung (11)	2/10 (20%)	2/11 (18.1%)
Stomach (2)	1/2 (50%) [†]	0/2
Duodenum (3)	2/3 (66.6%)	1/3 (33.3%)
Small intestine (3)	0/2	1/3 (33.3%)
Colon (1)	0/1	0/1
Liver (1)	0/1	0/1
Metastasis (1)	0/0	0/1
All NET (22)	5/19 (26.3%)	4/22 (18.1%)
Total (52)*	18/49 (36.7%)	8/52 (15.3%)

LOH, loss of heterozygosity.

*One additional tumor of the series proved to be associated with a *MEN1* phenotype and is therefore not included in this list.

[†]An additional tumor with an isolated LOH at the *PYGM* locus.

and mutations in EPT of our series were 13/30 (43.3%) and 4/30 (13.3%), respectively.

In the analyzed sporadic NET, allelic deletions and mutations occurred in 7/19 (26.3%) and 4/22 (18.1%) samples, respectively (lung 2/10 and 2/11, stomach 2/2 and 0/2, duodenal gastrinomas 2/3 and 1/3, small intestine 0/2 and 1/3, colon 0/1 and 0/1, liver 0/1 and 0/1, and metastasis of a gastrointestinal NET 0/0 and 0/1).

Allelic deletions and mutations were found in 18/45 (40%) and 7/46 (15.2%) of foregut tumors and in 0/4 and 1/6 (16.6%) of midgut and hindgut tumors.

Discussion

The results of the present study on allelic deletions and mutations of the *MEN1* gene imply a role for the *MEN1* gene in the development of 15.3% of sporadic EPT and NET. Few studies have been published in which sporadic EPT were investigated for mutations of the *MEN1* gene.^{14,17,28,29} Collectively, the previously published and

present results on a total of 61 EPT indicate that the frequency of allelic deletions of the *MEN1* gene in these tumors is 51% (25/49) and the mutation rate is 20.9% (13/62). Among subtypes of EPT, the highest mutation frequencies are encountered in the rare types of EPT⁴ such as VIPomas, somatostatinomas and glucagonomas. The more frequently encountered insulinomas, nonfunctioning EPT, and gastrinomas generally exhibit a lower frequency rate of somatic *MEN1* gene mutations, indicating that the molecular mechanisms of the latter tumors might be different from those of the former.

So far, only one study of NET of the gastrointestinal tract and one of NET of the lung have been published.^{12,17} Present and recently published data suggest that the overall frequency of allelic deletion at the *MEN1* locus in these tumors appears to be 58.4% (31/53) and the mutation rate 23.2% (17/73). Thus, the rates of allelic deletion and mutations of the *MEN1* gene in sporadic EPT and NET appear to be very similar, indicating that the *MEN1* gene plays a role in the tumorigenesis of approximately one fifth of the sporadically encountered tumors. The highest frequencies of *MEN1* mutations in sporadic NET were encountered in the duodenum (31%) and the lung (27%), whereas tumors of the stomach, small intestine, and colorectum exhibit mutations less frequently. These findings indicate that, like tumors of the endocrine pancreas, an organ- or cell type-specific pattern of *MEN1* gene mutations might exist. However, this assumption may be biased by the small numbers of tumors examined for certain tumor types.

Combining our results with those recently published, it appears that virtually all examined tumor groups exhibit an overall LOH rate of 50% at the *MEN1* locus and that the frequencies of encountered allelic deletions is usually 2 to 3 times higher than the frequency of mutations of the *MEN1* gene. This suggests that LOH at 11q13 per se is not an indicator of *MEN1* mutations. Such findings can easily be explained by other modes of *MEN1* gene inactivation, such as methylation of the promotor or noncoding regions or the presence of mutations in unexamined gene regions. It seems also likely that the higher frequency of allelic deletions is an indicator for the inactivation of yet another tumor suppressor gene on 11q13 in these tumors. Thus, two recent deletion mapping studies

Table 5. Results: Characterization of *MEN1* Gene Mutations

Organ	Tumor	Mutation	Type	Exon	Allelic deletion	Frequency
Pancreas	Insulinoma	1674del7	frameshift	10	yes	4/30 (13.3%)
	VIPoma	Q450X	stop codon	9	yes	
	VIPoma	L37P	missense	2	yes	
	Somatostatinoma	A50P	missense	2	yes	
Lung	Bronchial NET	5178-9G→A*	splice donor	intron 4	yes	2/11 (18%) [†]
	Bronchial NET	D172V	missense	3	yes	
	Bronchial NET	434ins29	frameshift	2	yes	
Intestine	Duodenal NET	Q209X	stop codon	3	yes	2/11 (18%)
	Ileal NET	V53I	missense	2	na	

NET, neuroendocrine (carcinoid) tumor; na, not analyzed.

*Germline mutation in a newly diagnosed *MEN1* gene carrier.

[†]Frequency in real sporadic tumors.

of 11q13 on 59 sporadic neuroendocrine neoplasms of different locations provided evidence that a second tumor suppressor gene in the interval between D11S4907 and D11S987 telomeric of the *MEN1* gene locus might also be involved in the tumorigenesis of sporadic neuroendocrine neoplasms.^{30,31}

Another interesting finding of our study is that the *MEN1* gene mutations encountered were not restricted to foregut tumors, as recently reported by Toliat et al¹⁷ and suggested by Debelenko et al.¹⁶ Indeed, the frequencies of mutations in foregut and mid/hindgut tumors were very similar (15.2% versus 16.6%), despite the fact that the number of investigated mid/hindgut tumors was much smaller than that of the foregut tumors (6 versus 46). It remains to be seen, however, if NET of the hindgut (colon and rectum) also contain somatic *MEN1* gene mutations, because we have investigated only one colonic neuroendocrine carcinoid tumor and Toliat et al investigated only four tumors, including one NET of the appendix.

Analysis of the published data and that presented here makes it clear that missense mutations are the most frequently encountered mutation type (14 tumors), followed by deletions (8 tumors), stop codon mutations (6 tumors), and insertions (3 tumors). Furthermore, the majority of alterations are encountered in the 5' part of the coding region of the *MEN1* gene. No correlation seems to exist between tumor type and a particular type or location of mutation in the *MEN1* gene. Likewise, no association between type or location of the mutation and clinical course could be established.

From all of the evidence discussed above, it appears that the most useful application of *MEN1* gene mutation analysis would be to identify *MEN1* gene carriers among patients with clinically sporadic EPT or NET. As a case in point, one individual with a NET of the lung in our series turned out to be a *MEN1* gene carrier. Based on clinical experience it is estimated that the *de novo* mutation rate of *MEN1* is approximately 10% and that 1.5% of patients suffering from gastrointestinal NET are in fact *MEN1* gene carriers.³² Thus, we recommend testing all patients suffering from multiple NET or a combination of NET or EPT and other *MEN1*-associated lesions of the skin, adipose tissue, or the adrenal cortex for *MEN1* gene mutations.

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