[CANCER RESEARCH 57, 4682–4686, November 1, 1997]

*Advances in Brief*

# Somatic Mutations of the *MEN1* Tumor Suppressor Gene in Sporadic Gastrinomas and Insulinomas

**Zhengping Zhuang, Alexander O. Vortmeyer, Svetlana Pack, Steve Huang, Thu A. Pham, Chaoyu Wang, Won-Sang Park, Sunita K. Agarwal, Larisa V. Debelenko, MaryBeth Kester, Siradanahalli C. Guru, Pachiappan Manickam, Shodimu-Emmanuel Olufemi, Fang Yu, Christina Heppner, Judy S. Crabtree, Monica C. Skarulis, David J. Venzon, Michael R. Emmert-Buck, Allen M. Spiegel, Settara C. Chandrasekharappa, Francis S. Collins, A. Lee Burns, Stephen J. Marx, Robert T. Jensen, Lance A. Liotta, and Irina A. Lubensky[1]**

*Laboratory of Pathology, National Cancer Institute [Z. Z., A. O. V., S. P., S. H., T. A. P., C. W., W-S. P., L. V. D., M. R. E-B., L. A. L., I. A. L.]; Metabolic Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases [S. K. A., M. K., C. H., A. M. S., A. L. B., S. J. M.]; Laboratory of Gene Transfer, National Human Genome Research Institute [S. C. G., P. M., S-E. O., J. S. C., S. C. C., F. S. C.]; Digestive Diseases [F. Y., R. T. J.] and Diabetes Branches [M. C. S.], National Institute of Diabetes and Digestive and Kidney Diseases; and Biostatistics and Data Management Section, National Cancer Institute [D. J. V.], NIH, Bethesda, Maryland 20892*

## Abstract

**Gastrinomas and insulinomas are frequent in multiple endocrine neoplasia type 1 (MEN1). The *MEN1* tumor suppressor gene was recently identified. To elucidate the etiological role of the *MEN1* gene in sporadic enteropancreatic endocrine tumorigenesis, we analyzed tumors (28 gastrinomas and 12 insulinomas) from 40 patients for *MEN1* gene mutations and allelic deletions. One copy of the *MEN1* gene was found to be deleted in 25 of 27 (93%) sporadic gastrinomas and in 6 of 12 (50%) sporadic insulinomas. *MEN1* gene mutations were identified in 9 of 27 (33%) sporadic gastrinomas and 2 of 12 (17%) insulinomas and were not seen in corresponding germ-line DNA sequence. A specific *MEN1* mutation was detected in one gastrinoma and in the corresponding germ-line DNA of a patient who had no family history of MEN1. Somatic *MEN1* gene mutations and deletions play a critical role in the tumorigenesis of sporadic gastrinomas and may also contribute to the development of a subgroup of insulinomas.**

## Introduction

Gastrinomas and insulinomas occur sporadically (1) and as a part of an autosomal dominant syndrome, MEN1[2] (2). Insulinomas usually follow a benign clinical course and are cured by resection of the tumor. In contrast, gastrinomas have high malignant potential, with regional lymph node or liver metastases developing in up to 90% of the cases, and are curable in the long term in only 30% of the cases.

The role of tumor suppressor genes/oncogenes in enteropancreatic endocrine tumorigenesis remains uncertain (3, 4). The Knudson tumor suppressor gene hypothesis states that hereditary cancers develop due to the inheritance of a mutated tumor suppressor gene and that a somatic mutational event involving the wild-type allele of the gene leads to a neoplasm (5). The model predicts that sporadic tumors of the same cell type would arise after somatic inactivation of both copies of the gene responsible for the familial tumor (5). The *MEN1* gene was linked to chromosome 11q13 in 1988 (6). Previous studies demonstrated LOH with polymorphic markers on 11q13 in sporadic gastrinomas and insulinomas, suggesting that the *MEN1* gene may play a role in sporadic enteropancreatic endocrine tumorigenesis (3, 7).

The *MEN1* tumor suppressor gene was recently identified (8), and germ-line mutations in the gene were detected in 47 of 50 kindreds with familial MEN1 (9). To evaluate whether sporadic gastrinomas and insulinomas are associated with functional inactivation of the *MEN1* gene, the gene was analyzed for deletions and mutations in 40 sporadic enteropancreatic tumors.

## Patients and Methods

**Subjects.** Tumors from 40 patients who underwent exploratory laparotomy for pancreatic or duodenal endocrine tumors at the NIH were included in the study. All patients gave informed consent in a protocol approved by the NIDDK Institutional Review Board. Clinical and family history were reviewed in each case, and all 40 patients were thought to have a sporadic neoplasm at the time of surgery. The diagnosis of insulinoma required a fasting blood sugar of less than 45 mg/dl, hypoglycemia symptoms, and a simultaneous elevated plasma insulin level of >5 microunits/ml (10). The diagnosis of ZES (gastrinoma) was established as described previously (11) and required meeting at least two of the following criteria: an elevated fasting serum gastrin level; a basal acid output of more than 15 mEq/h if the patient had not had previous gastric surgery or of more than 5 mEq/h if the patient had had previous gastric surgery; and an increase in the serum gastrin level of 200 pg/ml after i.v. secretin, an increase in serum gastrin level of 395 pg/ml after an i.v. calcium infusion, or a positive histological diagnosis of gastrinoma.

A diagnosis of sporadic insulinoma was made by an absence of a family history of endocrinopathies associated with MEN1 (renal stones, hyperparathyroidism, pituitary disease, or pancreatic endocrine tumors) and normal serum calcium and albumin measurements. If results were unclear, serum prolactin and gastrin measurements and family screening were done in some cases. A diagnosis of sporadic gastrinoma required a negative family history (as described above) and normal values for total serum calcium, ionized calcium, albumin, plasma PTH level (mid-PTH portion), plasma PTH (intact PTH), serum prolactin, urinary cortisol, and normal computed tomography or magnetic resonance imaging of the sella turcica (12).

**Tumors.** Twenty-eight frozen gastrinomas (4 pancreatic, 8 duodenal, and 1 common bile duct) and metastases (14 lymph node and 1 liver) and 12 benign pancreatic insulinomas were obtained from the file of the Laboratory of Pathology, National Cancer Institute, NIH (Bethesda, MD). All tumors were frozen in liquid nitrogen at the time of surgery and stored at −70°C. Paired normal DNA was obtained from frozen normal exocrine pancreas, duodenum, lymph node, or blood of each patient. Histological evaluation of tumors revealed characteristic neuroendocrine features and positive staining for chromogranin A (Boehringer Mannheim, Indianapolis, IN) and/or synaptophysin (Zymed, San Diego, CA) by immunohistochemistry. The clinical diagnoses of gastrinoma and insulinoma were confirmed by positive immunostain for gastrin (DAKO Corp., Carpenteria, CA) and insulin (BioGenex, San Ramon, CA), respectively.

Tumor and normal cells were selected from 5-μm-thick H&E-stained histological slides, microdissected under direct light microscope visualization as

Received 7/31/97; accepted 9/22/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at Laboratory of Pathology, National Cancer Institute, NIH, Building 10, Room 2N212, 10 Center Drive, Bethesda, MD 20892. Phone: (301) 496-0549; Fax: (301) 402-0043.

[2] The abbreviations used are: MEN1, multiple endocrine neoplasia type 1; LOH, loss of heterozygosity; ZES, Zollinger-Ellison syndrome; PTH, parathyroid hormone; SSCP, single-strand conformation polymorphism; FISH, fluorescence *in situ* hybridization.

described previously (13), and used for PCR-SSCP analysis (14). For this study, dissections were not performed to obtain pure populations of tumor cells but to enrich tumor samples sufficiently to perform mutational analysis by SSCP. Procured cells were resuspended in 30 μl solution containing Tris-HCl (pH 8.0), 0.1 M EDTA (pH 8.0), 1% Tween 20, and 0.1 mg/ml proteinase K and incubated overnight at 37°C. Following thermal inactivation of proteinase K (95°C for 5 min), 1–1.5 μl of the DNA extract were used for PCR amplification.

***MEN1* Allelic Deletion Analysis.** FISH was performed using cosmid clone c10B11 (size, 40 kb) containing the *MEN1* gene as a probe (15). Touch preparations of frozen tumor specimens were made on glass slides and used for FISH analysis as described previously (16, 17). Hybridization signals were scored using a Zeiss Axiophot epifluorescence microscope, and two-color images were captured on a Photometrics charge-coupled device camera (Photometrics, Ltd., Tucson, AZ) using IP Lab image software (Signal Analytics Corporation, Vienna, VA). At least 100 interphases with strong hybridization signals were scored for each tumor. The presence of cells with one MEN1 signal at a frequency of more than 30% was interpreted as an allelic deletion (loss of one copy of the *MEN1* gene). Seventy % of the cases with allelic deletion detected by FISH showed, upon touch preparation, cells with one MEN1 signal at a frequency of more than 70%, and 30% of the cases showed cells with one MEN1 signal at a frequency of 35–70%. The percentage of tumor and somatic cells in each frozen tissue imprint correlated with percentage of tumor and somatic cells in the H&E-stained frozen tissue sections from which the touch preparations had been prepared. Normal frozen pancreatic (exocrine and endocrine) tissue controls showed cells with one MEN1 signal at a frequency of 3%.

In cases with no detectable *MEN1* allelic deletion by FISH, DNA from precisely microdissected tumor and normal samples (13) was screened for LOH using three polymorphic markers on 11q13 as described previously (3, 7). The markers included intragenic marker *D11S4946*, located in the *MEN1* 5′-flanking region, and the flanking markers *PYGM* and *D11S449* (18, 19).

***MEN1* Mutation Analysis by SSCP and Sequencing.** PCR-SSCP analysis was performed using 13 primer sets (Table 1) with intronic sequences designed to amplify exons 2–10 from tumor and normal DNA. After detection of a mutant allele, DNA was extracted from the excised aberrant band of the SSCP gel by overnight incubation in 100 μl of distilled water at room temperature and subsequently reamplified by PCR (14, 20). The PCR products were directly sequenced (Cyclin Sequencing kit, Perkin-Elmer), and normal and tumor DNA sequences were compared. Tumor and peripheral blood DNA from familial MEN1 patients with known germ-line mutations (8) in each exon were run in parallel with each SSCP assay gel as positive controls.

Table 1 *PCR primer sets used for MEN1 gene mutation analysis*

| Exon | Primer sequence | Size (bp) | Annealing temperature (°C) |
|---|---|---|---|
| 2A[a] | 5′-ttgccttgcaggccgccgcc-3′<br>5′-tggtagggatgacgcggttg-3′ | 201 | 60 |
| 2B[a] | 5′-ggcttcgtggagcattttct-3′<br>5′-ctcgaggatagagggacagg-3′ | 202 | 60 |
| 2C[a] | 5′-ttcaccgcccagatccgagg-3′<br>5′-taagattcccacctactggg-3′ | 297 | 58 |
| 3 | 5′-attacctccccttccacag-3′<br>5′-ctgggggggagggaacaatac-3′ | 249 | 55 |
| 4 | 5′-cataatgatctcatcccccc-3′<br>5′-attggctcagccctcacctg-3′ | 171 | 58 |
| 5 | 5′-gttccgtggctcataactct-3′<br>5′-tggccacttccctctactga-3′ | 98 | 58 |
| 6 | 5′-ggcagcctgaattatgatcc-3′<br>5′-ttctgcaccctccttagatg-3′ | 142 | 58 |
| 7 | 5′-ggactccttgggatcttcctgtg-3′<br>5′-atcctcactcctggatgacagtg-3′ | 183 | 60 |
| 8 | 5′-cagagaccccactgctctca-3′<br>5′-ggctggagctccagcctttc-3′ | 181 | 65 |
| 9 | 5′-ctgctaaggggtgagtaagagac-3′<br>5′-gtctgacaagcccgtggctgctg-3′ | 225 | 60 |
| 10A[a] | 5′-tcaccttgctctccccactg-3′<br>5′-ccaggcccttgtccagtgct-3′ | 189 | 60 |
| 10B[a] | 5′-ccaagaagccagcactggac-3′<br>5′-cactctggaaagtgagcact-3′ | 183 | 62 |
| 10C[a] | 5′-ctgaaggtggcagcacggct-3′<br>5′-gtagtcactaggggtggaca-3′ | 219 | 60 |

[a] Several primer sets were used to amplify exons 2 and 10 due to their large size.

## Results

Gastrinomas from 28 patients (15 males and 13 females; mean age = 50 years, range = 15–71 years) and insulinomas from 12 patients (7 males and 5 females; mean age = 47 years, range = 17–81 years) were examined for allelic deletions and mutations of the *MEN1* gene.

One copy of the *MEN1* gene was found to be deleted in 25 of 27 (93%) sporadic gastrinomas and in 6 of 12 (50%) sporadic insulinomas as determined by FISH using a probe containing the *MEN1* gene (Fig. 1). *MEN1* gene somatic mutations were identified in 33% (9 of 27) of sporadic gastrinomas and 17% (2 of 12) of sporadic insulinomas analyzed (Table 2 and Fig. 2). All but two of the tumors with mutations also showed *MEN1* gene deletion by FISH, indicating that both copies of the gene were inactivated (Table 2). The absence of allelic deletion in tumors G-1236 and In-1257 was confirmed by LOH analysis using an intragenic microsatellite marker (*D11S4946*) at the *MEN1* 5′ region and two flanking 11q13 markers, *PYGM* and *D11S449* (data not shown). No mutation in the *MEN1* gene was detected by SSCP analysis in any exons from 17 of 27 sporadic gastrinomas and 5 of 12 insulinomas that showed allelic deletion. Observed *MEN1* gene mutations were limited to tumor DNA and were not detected in germ-line DNA in any case, except for one patient who showed an exon 8 mutation in both tumor (G-1229) and germ-line DNA (Table 2). This patient had no family history of MEN1. Preoperatively, this patient's MEN1 status was unclear because his serum calcium, prolactin, and (mid-PTH portion) PTH levels were normal; however, he had a slightly elevated intact PTH level and a history of neck surgery for an unknown reason 15 years prior to admission.

Mutations observed among enteropancreatic endocrine tumors in the study included four deletions, two insertions, and five missense mutations (Table 2 and Fig. 2). No tumor was found to have more than one *MEN1* gene mutation. Mutations were identified in exons 2, 3, 4, 8, and 10 (Table 2). Five of nine (56%) somatic mutations in sporadic gastrinomas occurred in exon 2. Two insulinomas showed mutations, one in exon 2 and one in exon 10. Deletions or insertions resulting in a frameshift of the protein coding sequence accounted for 55% (6 of 11) of the mutations. These mutations produce altered transcripts, resulting in protein truncation with predicted loss of function. The five missense mutations resulted in amino acid substitutions. These were not polymorphisms because matched normal DNA from the same individuals showed only wild-type *MEN1* sequence (Fig. 2*C*), and the missense mutations were not seen in an analysis of 142 normal chromosomes (8). Characterization of the functional domains of menin will be necessary to define the significance of the observed missense mutations. *MEN1* mutations and deletions were detected in both localized (duodenal and pancreatic) gastrinomas and metastatic (lymph node) gastrinomas examined (Table 2).

We identified three benign polymorphisms R171Q (CGG/CAG), D418D (GAC/GAT), and A541T (GCA/ACA) in exons 3, 9 and 10, respectively, in three sporadic insulinoma cases. These polymorphisms were identical to the three most common polymorphisms observed previously (8, 9).

## Discussion

The role of the *MEN1* gene in sporadic enteropancreatic endocrine tumorigenesis was suggested previously by LOH studies on 11q13 (3, 7). Our previous work using 10 polymorphic markers flanking the area of the putative *MEN1* gene on chromosome 11q13 demonstrated that 44% of sporadic gastrinomas showed LOH (3, 7). The closest flanking markers used were *PYGM* (centromeric) and *D11S449* and *PPP1CA* (telomeric), each located more than 100 kb from the *MEN1* gene. Here, we tested for allelic deletions at the precise location of the

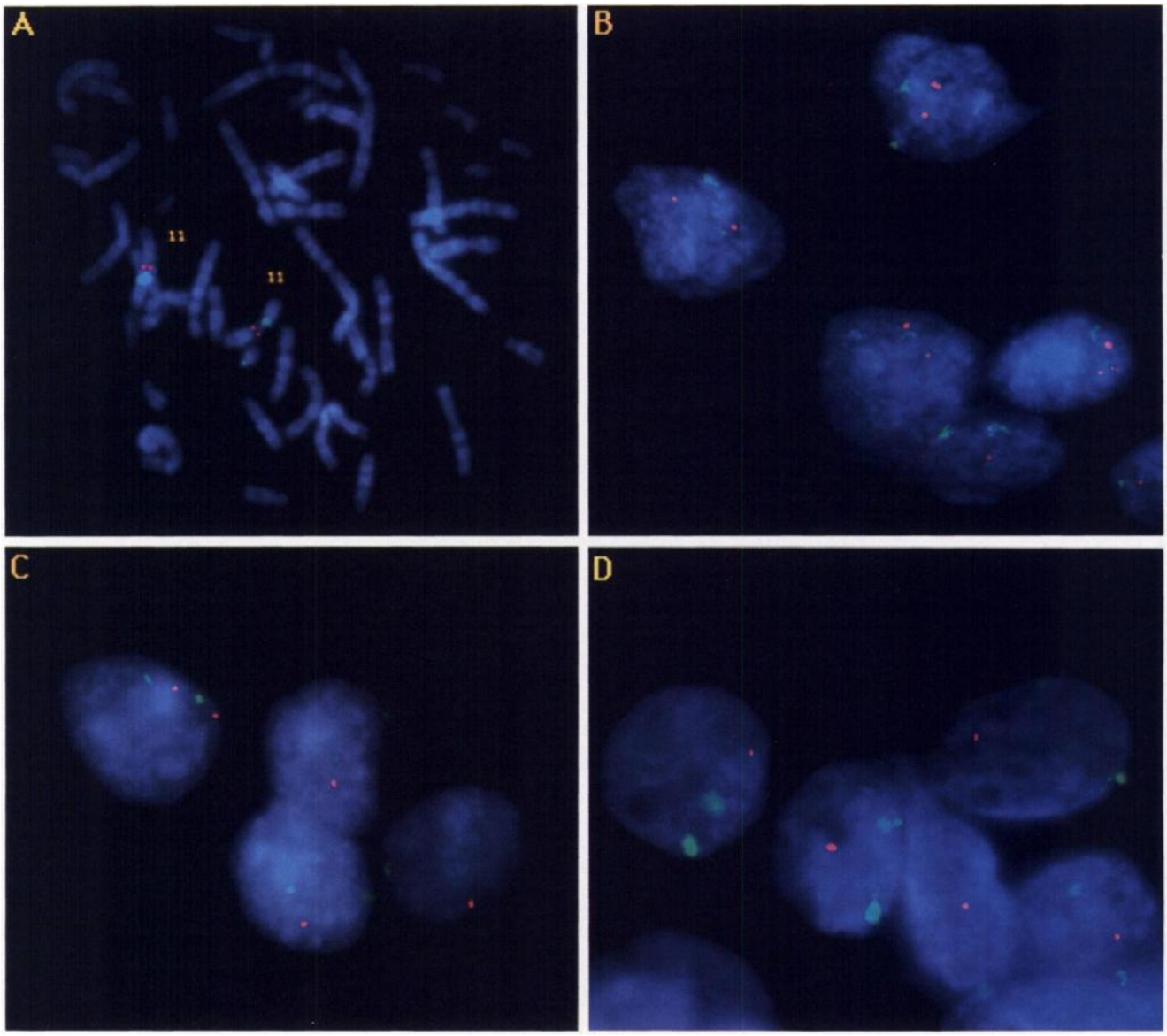


Fig. 1. Representative results of FISH in lymphoblast cell line and sporadic enteropancreatic endocrine tumors. *Green signal*, α-satellite centromeric marker; *red signal*, chromosome 11q13 probe containing the *MEN1* gene (cosmid c10B11). *A*, the *MEN1* gene localized to 11q13 on metaphase chromosome preparation from a normal lymphoblast cell line by FISH. *B–D*, interphase touch preparations of sporadic enteropancreatic endocrine tumors. *B*, sporadic insulinoma (In-756) showing no deletion of the *MEN1* gene by FISH. Two *red signals*, two alleles of the *MEN1* gene. *C*, allelic deletion of the *MEN1* gene detected in sporadic insulinoma cells (In-1147; one *red signal*) as compared to a normal somatic cell with two *red signals* (*left*). *D*, allelic deletion of the *MEN1* in sporadic gastrinoma cells (G-1544; one *red signal*).

gene. The significant increase in deletion rate, 93% *versus* 44%, indicates that a substantial subset of gastrinomas may contain limited interstitial deletions of a 300 kb or less in size that would not be evident at flanking markers *PYGM*, *D11S449*, and *PPP1CA*. Interestingly, the LOH profile observed in gastrinomas differs from that seen in MEN1-associated parathyroid tumors, which exclusively show large deletions of several megabases or more (13). The significance, if any, of the different deletion profiles between the two tumor types is currently not clear.

The high frequency of *MEN1* mutations (33%) and allelic deletions (93%) in sporadic gastrinomas indicates that, analogous to MEN1-associated tumors, *MEN1* gene alterations are critical events in sporadic gastrinoma tumorigenesis. Mutational inactivation of one *MEN1* allele coupled with deletion of the second allele strongly implicates the *MEN1* gene in the pathogenesis of these tumors, as defined in the two-hit recessive (loss-of-function) mechanism (5). *MEN1* mutations were detected in localized duodenal and pancreatic gastrinomas and

Table 2 *MEN1 gene mutations and allelic deletions in gastrinomas and insulinomas*

| Exon | Tumor code[a] | Tumor location | Mutation[b] | Allelic deletion[c] |
|---|---|---|---|---|
| 2 | G-344 | Duodenum | 358del4[d] | + |
| 2 | G-58 | Lymph node[e] | 358del25[f] | + |
| 2 | G-187 | Duodenum | I86F (ATC → TTC) | + |
| 2 | G-1351 | Pancreas | 483delAT | + |
| 2 | G-1236 | Pancreas | W126G (TGG → GGG) | − |
| 2 | In-1147 | Pancreas | 545insT | + |
| 3 | G-40 | Lymph node[e] | F159C (TTT → TGT) | + |
| 4 | G-520 | Lymph node[e] | 875insA | + |
| 8 | G-1491 | Lymph node[e] | 1212del7[g] | + |
| 8 | G-1229 | Lymph node[e] | E363del[(germ-line)] | + |
| 10 | In-1257 | Pancreas | A535V (GCT → GTT) | − |
| 10 | G-555 | Lymph node[e] | S543L (TCA → TTA) | + |

[a] G, gastrinoma; In, insulinoma.
[b] Mutation abbreviations follow standard nomenclature (27).
[c] Presence (+) or absence (−) of *MEN1* allelic deletion as detected by FISH.
[d] Deleted nucleotides are CTGT.
[e] Metastasis.
[f] Deleted nucleotides are TGTCTA TCATCGCCGC CCTCTATGC.
[g] Deleted nucleotides are GCCAATG.

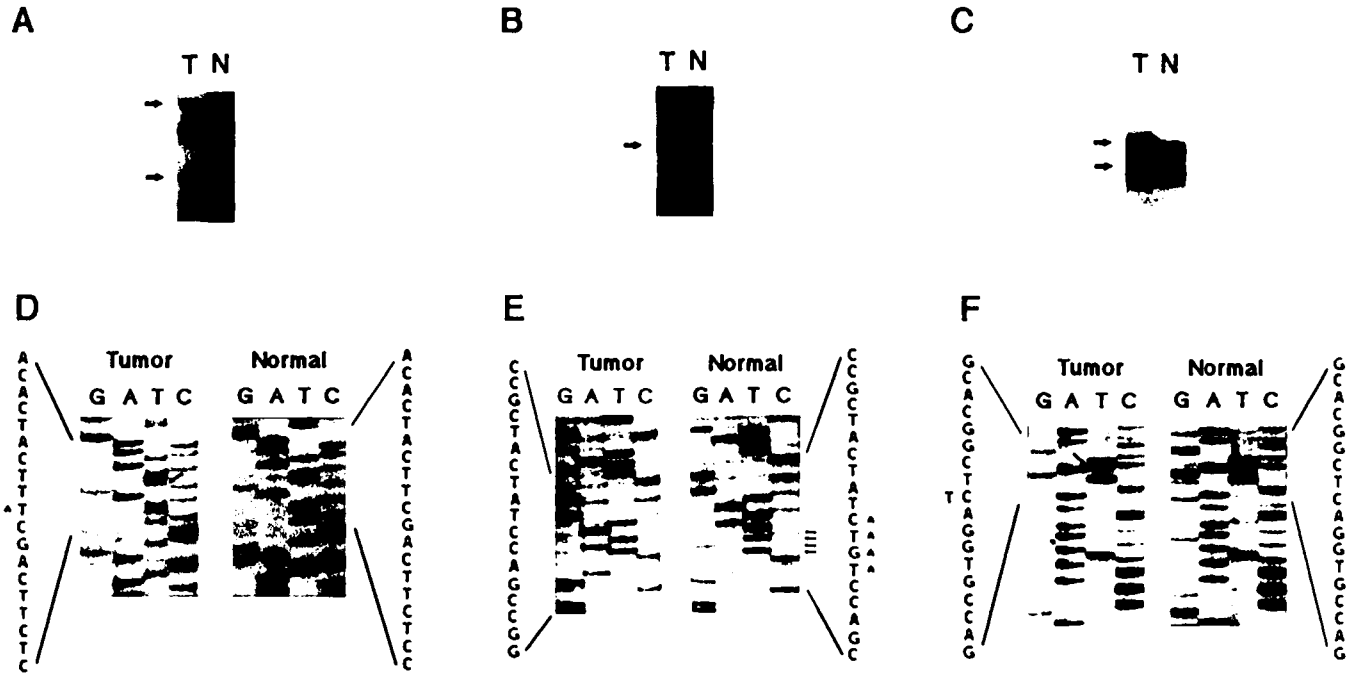


Fig. 2. Detection of somatic mutations in the *MEN1* gene in sporadic enteropancreatic endocrine tumors. *A–C*, SSCP changes for three *MEN1* gene mutations in tumors In-1147 (*A*), G-344 (*B*), and G-555 (*C*) are shown. *Arrows*, mutant alleles. The mutations were found in the tumor (*Lanes T*) but not in the corresponding normal (*Lanes N*) tissue. SSCP gels (*A* and *C*) show the presence of both normal and mutated allele in the tumor (*Lanes T*) samples due to normal tissue contamination. *D–F*, corresponding DNA sequence of aberrant band from SSCP gel. *Arrowheads*, changes in tumor DNA sequence as compared to normal tissue DNA. *D*, mutation 545insT in insulinoma In-1147. *E*, mutation 358del4 in gastrinoma G-344. *F*, missense mutation TCA→TTA (S543L) in gastrinoma G-555. In this instance, clear excision of the abnormal band was not possible, so the sequence reveals both the mutant and wild-type allele.

metastatic (lymph node) gastrinomas examined (Table 2), suggesting that they occur at an early stage of sporadic gastrinoma tumorigenesis.

In the present study, we observed an apparent clustering of mutations in exon 2 of the *MEN1* gene in sporadic gastrinomas. Exon 2 is the first coding exon in the *MEN1* gene, and four of the five exon 2 mutations observed in the gastrinomas produce frameshifts resulting in a protein that, even if biologically stable, contains little of the wild-type amino acid sequence. Thus, it is possible that tumors which share essentially complete knockout of menin may also share phenotypic characteristics. Clearly, the number of cases examined is small and needs to be expanded before firm genotypic/phenotypic conclusions can be drawn.

Here, a *MEN1* gene mutation was found in exon 8 in germ-line DNA in a patient who had no family history of MEN1. The patient had ZES with normal serum calcium and prolactin levels, conflicting levels of plasma PTH values, and an unclear past history of neck surgery. Interestingly, the E363del mutation in this patient was identical to a germ-line mutation previously described in two unrelated MEN1 families (8, 9). Patients with MEN1 can initially present with enteropancreatic endocrine tumors as the sole manifestation of their disease (12, 21) and can, therefore, be difficult to distinguish from patients with sporadic tumors without MEN1. In one study, one-third of the 28 patients with ZES with MEN1 initially presented with ZES and only developed hyperparathyroidism or pituitary disease years later (12). Because 20–25% of patients with ZES have MEN1 and because their clinical management differs significantly from that of patients with the sporadic form of the disease, the ability to screen patients with ZES for *MEN1* germ-line mutations will be a valuable tool for evaluation of ZES patients in the future.

Somatic *MEN1* mutations were detected in 2 of 12 (17%) sporadic insulinomas. One of the two insulinomas also showed a loss of the second copy of the *MEN1* gene. Five insulinoma cases had a loss of one copy of the *MEN1* gene without a detectable *MEN1* gene mutation in the other copy. In 50% (6 of 12) of insulinomas, neither *MEN1* mutation nor deletion could be detected. Thus, the *MEN1* gene appears to be altered in a subset of sporadic insulinomas. Other, as yet unidentified tumor suppressor genes/oncogenes may contribute to the pathogenesis of the majority of insulinomas (22). The data reported here for insulinomas are similar to recent data obtained in a group of sporadic parathyroid tumors analyzed for *MEN1* gene mutations (23). *MEN1* mutations were identified in 7 of 33 (21%) sporadic parathyroid tumors, and LOH on 11q13 was detected in 13 of 33 (39%) parathyroid tumor samples.

No mutation in the *MEN1* gene was detected by SSCP analysis in any exons from 17 of 27 sporadic gastrinomas and 5 of 12 insulinomas that showed allelic deletion. This work may, in fact, underestimate the prevalence of *MEN1* somatic mutations in sporadic enteropancreatic endocrine tumors, as the sensitivity rate of SSCP analysis for detection of single base substitutions is estimated at 80% (24). Moreover, some of these tumors may contain a mutation in regions of the *MEN1* gene that were not screened, such as the promoter. In the absence of a mutation or deletion, an alternative mechanism, such as hypermethylation of a CpG island as described in other tumor suppressor genes (25, 26), may inhibit transcription of the second copy of the *MEN1* gene. Lastly, another as yet unidentified tumor suppressor gene that is important for pathogenesis of some enteropancreatic endocrine tumors may exist near the *MEN1* locus. Further studies are necessary to investigate such alternative mechanisms in enteropancreatic endocrine tumorigenesis.

In summary, a high frequency of *MEN1* mutations and allelic deletions was observed in localized and metastatic sporadic gastrinomas. The finding that the *MEN1* tumor suppressor gene has an etiological role in sporadic gastrinoma should lead to a better understanding of the molecular pathogenesis of this malignant tumor and should aid in methods of diagnosis and treatment of patients with gastrinoma. The study suggests that *MEN1* mutations and allelic

deletions may also contribute to the development of a subgroup of benign insulinomas.

## Acknowledgments

We thank Drs. Richard H. Alexander and Douglas L. Fraker, Surgery Branch, National Cancer Institute (Bethesda, MD), who performed surgery on the subjects studied. We also thank Dr. John L. Doppman and Warren G. Magnuson, Clinical Center Radiology Department, NIH, for localization of islet cell tumors. We are grateful to Drs. Phillip Gorden and Simeon Taylor, National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD), and to many past and present members of the National Institute of Diabetes and Digestive and Kidney Diseases who participated in the care of the subjects studied.

## References

1. Jensen, R. T., and Norton, J. A. Endocrine tumors of the pancreas. *In:* T. Yamada, B. H. Alpers, C. Owyang, D. W. Powell, and F. E. Silverstein (eds.). Textbook of Gastroenterology, Ed. 2, pp. 2131–2166. Philadelphia: J. B. Lippincott Co., 1995.
2. Padberg, B., Schröder, S., Capella, C., Frilling, A., Klöppel, G., and Heitz, P. H. Multiple endocrine neoplasia type 1 (MEN1) revisited. Virchows Arch., *426:* 541–548, 1995.
3. Debelenko, L. V., Zhuang, Z., Emmert-Buck, M. R., Settara C. Chandrasekharappa, S. C., Manickam, P., Guru, S. C., Marx, S. J., Skarulis, M. C., Spiegel, A. M., Collins, F. S., Jensen, R. T., Liotta, L. A., and Lubensky, I. A. Allelic deletions on chromosome 11q13 in MEN1-associated and sporadic gastrinomas and pancreatic endocrine tumors. Cancer Res., *57:* 2238–2243, 1997.
4. Weber, H. S, and Jensen, R. T. Pancreatic endocrine tumors and carcinoid tumors: recent insights from genetic and molecular biologic studies. *In:* C. G. Dervenis (ed.). Advances in Pancreatic Disease: Molecular Biology, Diagnosis and Treatment, pp. 55–75. Stuttgart, Germany: Georg Thieme Verlag, 1996.
5. Knudson, A. G. Hereditary cancer, oncogenes, and antioncogenes. Cancer Res., *45:* 1437–1443, 1985.
6. Larsson, C., Skogseid, B., Oberg, K., Nakamura, Y., and Nordenskjold, M. Multiple endocrine neoplasia type 1 maps to chromosome 11 and is lost in insulinoma. Nature (Lond.), *332:* 85–87, 1988.
7. Emmert-Buck, M. R, Lubensky, I. A., Dong, Q., Manickam, P., Guru, S., Kester, M. B., Olufemi, S-E., Agarwal, S., Burns, A. L., Spiegel, A. M., Collins, F. S., Marx, S. J., Zhuang, Z., Liotta, L. A., Chandrasekharappa, S., and Debelenko, L. V. Localization of the multiple endocrine neoplasia type 1 (*MEN1*) gene based on tumor LOH analysis. Cancer Res., *57:* 1855–1858, 1997.
8. Chandrasekharappa, S. C., Guru, S. C., Manickam, P., Olufemi, S-E., Collins, F. S., Emmert-Buck, M. R., Debelenko, L. V., Zhuang, Z., Lubensky, I. A., Liotta, L. A., Crabtree, J. S., Wang, Y., Roe, B. A., Weisemann, J., Boguski, M. S., Agarwal, S. K., Kester, M. B., Kim, Y. S., Heppner, C., Dong, Q., Spiegel, A. M., Burns, A. L., and Marx, S. J. Positional cloning of the gene for multiple endocrine neoplasia-type 1. Science (Washington DC), *276:* 404–407, 1997.
9. Agarwal, S., Kester, M. B., Debelenko, L. V., Heppner, C., Emmert-Buck, M. R., Skarulis, M., Doppman, J., Kim, Y., Lubensky, I. A., Zhuang, Z., Boguski, M. S., Weisemann, J., Green, J., Guru, S., Manickam, P., Olufemi, S-E., Liotta, L. A., Chandrasekharappa, S., Collins, F. S., Spiegel, A. M., Burns, A. L., and Marx, S. J. Germline mutations of the MEN1 gene in familial MEN1 and related states. Hum. Mol. Genet., *6:* 1169–1175, 1997.
10. Gorden, P., Skarulis, M. C., Roach, P., Comi, R. J., Fraker, D. L., Norton, J. A., Alexander, H. R., and Doppman, J. L. Plasma proinsulin-like component in insulinoma: a 25 year experience. J. Clin. Endocrinol. Metab., *80:* 2884–2887, 1995.
11. Fishbeyn, V. A, Norton, J. A, Benya, R. V, Pisegna, J. R., Venzon, D. J., Metz, D. C., and Jensen, R. T. Assessment and prediction of long-term cure in patients with Zollinger-Ellison syndrome: the best approach. Ann. Intern. Med., *119:* 199–206, 1993.
12. Benya, R. V., Metz, D. C., Venzon, D. J., Fishbeyn, V. A., Strader, D. B., Orbuch, M., and Jensen, R. T. Zollinger-Ellison syndrome can be the initial endocrine manifestation in patients with multiple endocrine neoplasia-type 1. Am. J. Med., *97:* 436–444, 1994.
13. Lubensky, I. A., Debelenko, L. V., Zhuang, Z., Emmert-Buck, M. R., Dong, Q., Chandrasekharappa, S., Guru, S., Manickam, P., Olufemi, E-S., Marx, S. J., Spiegel, A., Collins, F. S., and Liotta, L. A. Allelic deletions on chromosome 11q13 in multiple tumors from individual MEN1 patients. Cancer Res., *56:* 5272–5278, 1996.
14. Orita, M., Iwahara H., Kanazawa, H., Hayashi, K., and Sekiya, T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc. Natl. Acad. Sci. USA, *86:* 2766–2770, 1989.
15. Guru, S., Olufemi, S-E., Manickam, P., Cummings, C., Gieser, L. M., Pike, B. L., Bittner, M. L., Jiang, Y., Chinault, A. S., Novack, N. J., Brozowska, A., Crabtree, J., Wang, Y., Roe, B., Weisemann, J., Boguski, M., Agarwal, S., Burns, A. L., Spiegel, A. M., Marx, S. J., Flejter, W. L., de Jong, P. J., Collins, F. S., and Chandrasekharappa, S. A 2.8 Mb clone contig of the multiple endocrine neoplasia type 1 (MEN1) region at 11q13. Genomics, *42:* 436–445, 1997.
16. Pinkel, D., Straune, T., and Gray, J. W. Cytogenetic analysis using quantitative, high sensitivity fluorescence hybridization. Proc. Natl. Acad. Sci. USA, *83:* 2934–2938, 1986.
17. Ried, T., Lengauer, C., Lipp, M., Fischer, C., Cremer, T., and Ward, D. C. Evaluation of the utility of interphase cytogenetics to detect residual cells with a malignant genotype in mixed cell populations: a Burkitt lymphoma model. DNA Cell Biol., *12:* 637–643, 1993.
18. James, M. R., Richard, C. W., III, Schott. J-J., Yousry, C., Clark, K., Bell, J., Terwilliger, J. D., Hazan, J., Dubay, C., Vignal, A., Agrapart, M., Imai, T., Nakamura, Y., Polymeropoulos, M., Weissenbach, J., Cox, D. R., and Lathrop, G. M. A radiation hybrid map of 506 STS markers spanning human chromosome 11. Nat. Genet., *8:* 70–76, 1994.
19. Manickam, P., Guru, S., Debelenko, L. V., Agarwal, S., Olufemi, S-E., Weisemann, J., Boguski, M., Crabtree, J., Roe, B., Wang, Y., Lubensky, I. A., Zhuang, Z., Kester, M. B., Burns, A. L., Spiegel, A. M., Marx, S. J., Liotta, L. A., Emmert-Buck, M. R., Collins, F. S., and Chandrasekharappa, S. Eighteen new polymorphic markers in the multiple endocrine neoplasia type 1 (MEN1) region. Hum. Genet., *101:* 102–108, 1997.
20. Gnarra, J., Tory, K., Weng, Y., Schmidt, L., Wei, M., Li, H., Latif, F., Liu, S., Chen, F., Duh, F., Lubensky, I., Duan, D. R., Florence, C., Pozzatti, R., Walther, M. M., Bander, N. H., Grossman, H. B., Brauch, H., Pomer, S., Brooks, J. D., Isaacs, W. B., Lerman, M. I., Zbar, B., and Linehan, W. M. Mutations of the VHL tumour suppressor gene in renal carcinoma. Nat. Genet., *7:* 85–90, 1994.
21. Shepherd, J. J. The natural history of multiple endocrine neoplasia type I: highly uncommon or highly unrecognized. Arch. Surg., *126:* 935–952, 1991.
22. Chung, D. C., Smith, A. P., Louis, D. N., Graeme-Cooke, F., Warshaw, A. L., and Arnold, A. A novel pancreatic endocrine tumor suppressor gene locus on chromosome 3p with clinical prognostic implications. J. Clin. Invest., *100:* 404–410, 1997.
23. Heppner, C., Kester, M. B., Agarwal, S., Debelenko, L. V., Emmert-Buck, M. R., Guru, S., Manickam, P., Olufemi, S-E., Skarulis, M., Doppman, J., Alexander, R., Kim, Y., Saggar, S., Lubensky, I. A., Zhuang, Z., Boguski, M., Weisemann, J., Liotta, L. A., Chandrasekharappa, S., Collins, F. S., Spiegel, A. M., Burns, A. L, and Marx, S. J. Somatic mutation of the *MEN1* gene in sporadic parathyroid tumors. Nat. Genet., *16:* 375–378, 1997.
24. Sheffield, V. C., Beck, J. S, Kwitek, A. E, Sandstrom, D. W., and Stone, E. M. The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. Genomics, *16:* 325–332, 1993.
25. Merlo, A., Herman, J. G., Mao, L., Lee, D. J., Gabrielson, E., Burger, P. C., Baylin, S. B., and Sidransky, D. 5′ CpG island methylation is associated with transcriptional silencing of the tumor suppressor *p16/CDKN2/MTS1* in human cancers. Nat. Med., *1:* 686–692, 1995.
26. Herman, J. G, Latif, F., Weng, Y., Lerman, M. I., Zbar, B., Liu, S., Samid, D., Duan, D. S., Gnarra, J. R., Linehan, W. M., and Baylin, S. B. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc. Natl. Acad. Sci. USA, *91:* 9700–9704, 1994.
27. Beaudet, A. L., and Tsui, L. A suggested nomenclature for designating mutations. Hum. Mutat., *2:* 245–248, 1993.