Microadenomatosis of the Endocrine Pancreas in Patients With and Without the Multiple Endocrine Neoplasia Type 1 Syndrome

Martin Anlauf, MD, Regina Schlenger, MD,*† Aurel Perren, MD,‡ Juliane Bauersfeld,* Christian A. Koch, MD,§ Henning Dralle, MD,⊥ Andreas Raffel, MD,¶ Wolfram T. Knoefel, MD,¶ Eberhard Weihe, MD,# Philippe Ruszniewski, MD,** and Anne Couvelard, MD,†† Paul Komminoth, MD,‡‡‡ Philipp U. HeitzMD,‡ and Günter Klöppel, MD**

Abstract: It has been suggested that microadenomatosis of the endocrine pancreas is a hallmark of the multiple endocrine neoplasia type 1 syndrome (MEN1). This study attempts to elucidate the relationship between pancreatic microadenomatosis and the MEN1 and von Hippel-Lindau (VHL) syndromes. Pancreatic tissue specimens from 37 patients (with either microadenomatosis or the MEN1 syndrome) were analyzed using immunohistochemistry, confocal laser scanning microscopy, and morphometric methods. The MEN1 and the VHL status were assessed on the basis of clinical criteria (all patients) and PCR-based mutational analysis (15 and 5 patients, respectively). Pancreatic microadenomatosis was found in 35 of 37 patients, 28 of whom fulfilled the clinicopathologic criteria and 13 the genetic criteria for MEN1, whereas none of the patients had evidence of a VHL syndrome. Microadenomas were present in 26 of the 28 MEN1 patients, and all these tumors were consistently multihormonal. Five of the 9 patients with microadenomatosis and no clinical evidence for MEN1 or VHL also lacked mutations for the respective genes. Five of these 9 patients suffered from hyperinsulinism and revealed multiple insulin-positive tumors. The other patients were nonsymptomatic and showed multiple glucagon-expressing neoplasms. In microadenomatosis patients with and without the MEN1 syndrome, a subset of morphologically normal-appearing islets showed increased endocrine cell proliferation. In conclusion, endocrine multihormonal microadenomatosis of the pancreas is a feature of MEN1. In addition, a monohormonal type of pancreatic microadenomatosis was identified that consisted of either insulinomas or glucagon-producing tumors and was not associated with MEN1 or VHL.

Key Words: pancreas, endocrine tumors, microadenomatosis, MEN1, menin gene

(*Am J Surg Pathol* 2006;30:560–574)

From the Departments of *Pathology, and †Forensic Medicine, University of Kiel, Kiel, Germany; ‡Department of Pathology, University Hospital of Zürich, Switzerland; §Division of Endocrinology and Nephrology, University of Leipzig, Leipzig, Germany; ⊥Department of General and Visceral Surgery, University of Halle, Halle, Germany; ¶Department of General and Visceral Surgery, University of Düsseldorf, Düsseldorf, Germany; #Department of Molecular Neuroscience, Institute of Anatomy and Cell Biology, University of Marburg, Marburg, Germany; **Service of Gastroenterology, Hospital Beaujon, Paris, France; ††Department of Pathology, Hospital Beaujon, Paris, France; and ‡‡Department of Pathology, Hospital of Baden, Baden, Switzerland.

Martin Anlauf and Regina Schlenger both contributed equally to this study.

Supported by grants from the German Society of Pathology, the Werner and Clara Kreitz Stiftung, Kiel (F 371435), and the Swiss National Foundation (SNF 31-61884.00). Some of the results of this study are part of Regina Schlenger's and Juliane Bauersfeld's MD theses.

Reprints: Martin Anlauf, MD, Department of Pathology, University of Kiel, Michaelisstr. 11, 24105 Kiel, Germany (e-mail: manlauf@path.uni-kiel.de).

The presence of multiple small endocrine tumors in the pancreas (ie, up to 5 mm in diameter) has been referred to as microadenomatosis[11,21] and has been observed in association with the multiple endocrine neoplasia type 1 (MEN1) syndrome. In MEN1, pancreatic microadenomatosis is usually accompanied by one or more macrotumors (diameter > 5 mm), some of which may be functionally active.[11,21]

Although the identification of the MEN1 gene on 11q13[10,12,17,25,27] has led to extensive studies of the genetic and clinical features of the MEN1 syndrome,[1,5,9,11,18,19,29,36,37] our knowledge of the development and pathology of pancreatic endocrine neoplasms in MEN1 is mainly based on reports of single cases.[8,31] Only few studies on a series of MEN1 patients have been published.[21,26,34] These reports suggested that endocrine microadenomatosis is a hallmark of the MEN1 pancreas. They showed that microadenomatosis in MEN1 patients is highly variable as far as the number of tumors and their hormonal profiles are concerned. Although these microadenomas express pancreatic hormones such as insulin and glucagon, they are thought to be nonsymptomatic.

A clinically apparent hormonal syndrome was only found in association with macrotumors.

Multiple endocrine tumors have also been observed in the pancreas of patients with the von Hippel-Lindau (VHL) syndrome. These tumors typically show a clear cell pattern and many produce a variety of hormones.[28]

Opinions vary as to which type of endocrine cell lesion precedes the development of pancreatic endocrine tumors in MEN1. On the basis of morphology, it has been suggested that islet cell hyperplasia is a forerunner of pancreatic endocrine tumors in MEN1,[34] but this could not be confirmed.[21] On a molecular level, Vortmeyer et al[35] recently analyzed microdissected pancreatic tissue from patients with MEN1 for loss of the wild-type MEN1 allele and found LOH for the MEN1 locus on 11q13 in both pancreatic endocrine macrotumors and ductuloinsular complexes and small microadenomas that they called "atypical structures types 1 and 2." These lesions were considered precursors of the tumors because islets and acinar tissue nodules showed no LOH on 11q. On the other hand, in MEN1 knockout mice, it was shown that islet hyperplasia consistently precedes tumor development, suggesting that the endocrine tumors may originate from islet cells.[6,7,13,14]

This investigation was conducted to correlate endocrine microadenomatosis of the pancreas with clinical features and genetic changes that characterize the MEN1 or VHL syndrome. We studied pancreatic specimens surgically removed from 37 patients in whom pancreatic endocrine tumors were suspected and who often had the clinical features of the MEN1 syndrome. In particular, we were interested in answering the following questions: 1) Is pancreatic microadenomatosis a feature of MEN1? 2) Is there a type of microadenomatosis that occurs independently of inherited conditions such as MEN1 or VHL? 3) Can microadenomatosis cause the clinical symptoms of inappropriate hormone secretion? 4) What phenotypic changes in endocrine cells precede tumor development in association with microadenomatosis?

MATERIALS AND METHODS

Patients

The study was performed on tissue blocks from pancreatic resection specimens from 37 patients (16 male and 21 female patients; mean age, 42.3 years; range 21–69 years) collected between 1970 and 2005. In all cases, the patients were either clinically suspicious for an MEN1 syndrome or the specimens showed microadenomatosis histologically. The tissue blocks were retrieved from the surgical and consultation files of the Departments of Pathology of the Universities of Kiel and Leipzig, Germany, and Zürich, Switzerland. The occurrence of extrapancreatic endocrine tumors and the presence of symptoms of inappropriate hormone secretion from tumors associated with the MEN1 syndrome were recorded. A patient was considered to have MEN1 clinically if he/she had at least two MEN1-related endocrine tumors (pituitary adenoma, parathyroid adenoma, duodenopancreatic endocrine tumor, lung carcinoid, adrenocortical tumor) or one of these tumors together with a first-degree relative with MEN1.[11] Eight of these patients were included in earlier studies investigating the histopathology of duodenal gastrinomas and pancreatic neoplasms in MEN1.[3,21,32]

Tissues

Sections (3 μm) were cut at 100-μm intervals from paraffin-embedded tissue blocks fixed in either 4% formaldehyde or Bouin's solution. The sections were stained with hematoxylin and eosin and periodic acid-Schiff. Pancreatic specimens (from the head, body, and tail of the pancreas) from 18 age-matched patients (14 men and 4 women) with no clinically or morphologically apparent pancreatic disease, obtained from the Department of Forensic Medicine, Budapest, Hungary, were used as control tissue for the evaluation of the proliferative activity of islet cells, as previously described.[32]

Tumor Classification

In accordance with the recently published WHO criteria for endocrine tumors of the pancreas, we distinguished between microadenomas (≤ 5 mm) and macrotumors (> 5 mm).[20] In macrotumors, the size, angioinvasion, proliferation index, immunohistochemical phenotype, and evidence of metastatic spread were assessed and the tumors were classified as well-differentiated pancreatic endocrine tumor (wdPET), wdPET with uncertain behavior (wdPETub), or well-differentiated pancreatic endocrine carcinoma (wdPEC).[20] Hormonal syndromes were recorded on the basis of chart reviews.

Criteria for distinguishing microadenomas less than 1 mm in size from enlarged islets were loss of the normal cellular composition and the presence of a trabecular growth pattern and/or a dense connective tissue mantle. For each patient, the number of microadenomas was counted, and their size, growth pattern, hormonal phenotype, and proliferation index were determined.

Immunohistochemistry

Hormone expression was examined by staining with the antibodies listed in Table 1. Cell proliferation was studied with the Ki-67 antibody.

Deparaffinized 3- to 4-μm-thick sections were rehydrated and subjected to heat-induced epitope retrieval procedures, as described previously.[16,33] Prior to application of the primary antibody, blocking with nonimmune serum was performed for 20 minutes. The sections were then incubated with the primary antibody for 45 minutes, followed by another incubation period of 45 minutes with species-specific biotinylated secondary antibodies (Dianova, Hamburg, Germany). After several washes, they were incubated with the ABC reagents for 30 minutes (Vectastatin Elite ABC kit, Boehringer, Ingelheim, Germany). The immunoreaction was visualized with 3′3-diaminobenzidine (DAB, Sigma,

TABLE 1. List of Primary Antibodies

Antigen	Code	Source/Reference	Dilution	Species
ACTH	M3501	DAKO, Hamburg, Germany	1:500	Mouse, monoclonal
Chromogranin A	E 001	Linaris, Wertheim, Germany	1:2/1:1*	Mouse, monoclonal
Gastrin	A 568	DAKO, Hamburg, Germany	1:3000	Rabbit, polyclonal
GHRH	A0570	DAKO, Hamburg, Germany	1:4000	Rabbit, polyclonal
Glucagon	039P	Biogenex, San Ramon, CA	1:60	Rabbit, polyclonal
Glucagon	SC-7780	Santa Cruz Technology, CA	1:600*	Goat, polyclonal
Insulin	HB125/02911	Biogenex, San Ramon, CA	1:40	Mouse, monoclonal
Insulin	045A	DAKO, Hamburg, Germany	1:6000/1:600*	Guinea pig, monoclonal
Ki-67	M0722	DAKO, Hamburg, Germany	1:100	Mouse, monoclonal
Neurotensin	HC-8	Carraway, Worcester, MA	1:15 000	Rabbit, polyclonal
PP	95095318	Paesel and Lorei, Hanau, Germany	1:5000	Rabbit, polyclonal
PP	PH512	Binding Site, Birmingham, UK	1:60*	Sheep, polyclonal
Serotonin	5 HT H 209	DAKO, Hamburg, Germany	1:20	Rabbit, polyclonal
Somatostatin	A0566	DAKO, Hamburg, Germany	1:200	Rabbit, polyclonal
Somatostatin	BM 715	Biermann, Bad Nauheim, Germany	1:40*	Rat, monoclonal
Synaptophysin	A 0010	DAKO, Hamburg, Germany	1:50	Rabbit, polyclonal
VIP	18-0080	Zymed, San Francisco, CA	1:10	Rabbit, polyclonal

ACTH, adrenocorticotrophic hormone; GHRH, growth hormone releasing hormone; PP, pancreatic polypeptide; VIP, vasoactive intestinal peptide.
*Dilution for immunofluorescence.

Deisenhofen, Germany), and counterstained with hematoxylin. To monitor the staining specificity, appropriate controls were performed. The sections were analyzed and photographed with an Axioskop 50 microscope (Zeiss, Oberkochen, Germany).

Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy was used to study the proportions of the various hormonal cell types detected in microadenomas and in normal-appearing endocrine tissue. Double immunofluorescence detection was performed by covering the sections with a mixture of the two different primary antibodies in appropriate dilutions (Table 1) and by subsequent labeling with the species-specific secondary antibodies bearing the Alexa fluorochromes A647, A594, or A488 (MoBiTec, Göttingen, Germany). Furthermore, streptavidin coupled with Alexa fluorochromes A647, A488, or A594 (MoBiTec, Göttingen, Germany) was used in combination with biotinylated species-specific secondary antisera bearing the appropriate fluorochrome. Sections were analyzed and digital images were generated with AX70 Fluoview microscope (Olympus, Hamburg, Germany).

Mutation Analysis of the MEN1 and von Hippel-Lindau (VHL) Gene

DNA was extracted using a commercially available kit (Puregene, DNA Purification System, Gentra Systems, Minneapolis, MN), as recommended by the manufacturer. DNA was resuspended in Tris-EDTA buffer (10 mmol Tris, pH 8.0, 1 mmol EDTA) at a concentration of 0.2 to 1.5 μg/μL and stored at 4°C.

Primers with GC clamps for amplification of exons 2 to 10 of the MEN1 gene were designed for denaturing gradient gel electrophoresis (DGGE) analysis as described previously.[3] In addition, in 5 patients with multicentric pancreatic endocrine tumors who revealed no clinical and mutational evidence of MEN1, a mutational analysis of the VHL gene was performed using the primers summarized in Table 2.

TABLE 2. Primers, PCR Conditions, and Methods Used to Detect VHL Mutations

Primer (code)	Sequence	Length (bp)	Condition	Gel Condition
VHL 1aF gc	5′-*… AGC GCG TTC CAT CCT CTA C-3′	255	1.5 mM $MgCl_2$,	DGGE U/F: 50%–90%,
VHL 1aR gc	5′-† ... AGG GCC GTA CTC TTC GAC-3′		10% DMSO	15 h 60 V
VHL 1bF gc	5′-‡ ... GCG GAG AAC TGG GAC GAG-3′	418	1.5 mM $MgCl_2$,	DGGE U/F: 50%–90%,
VHL 1bR gc	5′-† ... GCT TCA GAC CGT GCT ATC GT-3′		10% DMSO	15 h 60 V
VHL 2F gc	5′-§ … CTT TAA CAA CCT TGC TT-3′	266	1.5 mM $MgCl_2$	DGGE U/F: 50%–90%,
VHL 2R gc	5′-⊥ … GTC TAT CCT GTA CTT AC CAC-3′			15 h 100 V
VHL 3F gc	5′-‡ … TTC CTT GTA CTG AGA CCC TAG T-3′	316	1.5 mM $MgCl_2$	DGGE U/F: 50%–90%,
VHL 3R gc	5′-† … AGC TGA GAT GAA ACA GTG TAA GT-3′			15 h 100 V

DGGE, denaturing gradient gel electrophoresis; U/F, urea and formamide; DMSO, dimethyl sulfoxide.
*gcgcgc.
†cgc ccg ccg cgc ccc gcg ccc ggc ccg ccg ccc ccg ccc g.
‡GC12: gcg cg.
§GC8: cgc ccg ccg cgc ccc gcg ccc ggc ccg ccg ccc ccg ccc gaa ata ata aa.
⊥cgt ccc gc.

TABLE 3. Clinicopathologic Features and Mutational Analysis of Patients With MEN 1

Patient No.	Age (yr)	Sex	Surgical Intervention	Macrotumors/ Microadenomas	Metastasis	Extrapancreatic MEN1-associated NET	Postoperative Findings	Clinical MEN1 Status	Family History	MEN1 Mutation
Patients with hyperinsulinemic hypoglycemia										
1	25	F	EN, LSPR	2/10	Ln (Ins)	ParAd	Ins↑	Yes	Yes	Na
2	26	F	LSPR	4/31	No	ParAd	Asympt	Yes	Yes	Exon 2, Codon 109, 2 bp insertion (frameshift)
3	28	F	LSPR	2/3	No	ParAd	Asympt	Yes	Nk	Na
4	54	M	LSPR, WH	7/8	Ln (Ins)	AdrAd	Ins↑	Yes	No	Exon 10, Codon 466, 14 bp deletion (frameshift)
5	57	F	WH	8/3	No	ParAd, PitAd, AdrAd, DuoGas (m)	Asympt	Yes	Nk	Na
Patients with Zollinger-Ellison syndrome										
6	21	F	WH	0/10	No	ParAd	Nk	Yes	Nk	Negative
7	31	F	EN, WH	1/0	Ln (Gas)	ParAd, DuoGas (m)	Asympt	Yes	Yes	Negative
8	33	F	LSPR	2/1	Ln (Gas)	ParAd, DuoGas (m) Prolaktin↑	Gas↑	Yes	Yes	Exon 2, Codon 64, CAG→TAG (nonsense)
9	33	F	WH	2/6	Ln (Gas)	ParAd, DuoGas (m)	Gas↑	Yes	No	Exon 10, Codon 508, CAG→TAG (nonsense)
10	35	F	LSPR	1/11	Ln (Gas)	ParAd, DuoGas (m)	Gas↑	Yes	No	Exon 3, Codon 183, TGG→TGC (missense)
11	37	M	LSPR	0/16	No	ParAd, PitAd, AdrAd, DuoGas (m), ACTH↑	Nk	Yes	Yes	Na
12	38	M	LSPR	0/13	Nk	DuoGas (m)	Gas↑	Susp	Nk	Na
13	39	M	AUT	4/50	No	DuoGas (m)	Ø	Susp	Nk	Na
14	39	M	LSPR	4/30	No	ParAd, PitAd, AdrAd, ACTH↑	Nk	Yes	Nk	Na
15	40	M	LSPR	2/30	Nk	ParAd, DuoGas (m)	Gas↑	Yes	No	Na
16	41	M	LSPR, WH	1/12	Ln (Gas)	ParAd, DuoGas (m)	Gas↑	Yes	Yes	Exon 2, Codon 108, CGA→TGA (nonsense)
17	47	M	AUT	1/0	Ln (Gas)	ParAd, AdrAd, DuoGas (m), lung carcinoid, ACTH↑	Ø	Yes	No	Intron 4, Splice-Donor-Mutation 5178-9 G→A
18	53	M	WH	0/17	Ln (Gas)	DuoGas (m)	Gas↑	Susp	Nk	Na
19	66	F	LSPR	7/22	No	ParAd, PitAd, AdrAd, DuoGas (m) ACTH↑, Prolactin↑	Gas↑	Yes	Nk	Na
20	67	M	WH, AUT	0/2	Ln (Gas)	ParAd, DuoGas (m), GHRH↑	Gas↑	Yes	Nk	Na

TABLE 3. (*continued*)

Patient No.	Age (yr)	Sex	Surgical Intervention	Macrotumors/ Microadenomas	Metastasis	Extrapancreatic MEN1-associated NET	Postoperative Findings	Clinical MEN1 Status	Family History	MEN1 Mutation
Patients with hyperinsulinemic hypoglycemia and Zollinger-Ellison syndrome										
21	51	F	EN, LSPR	2/29	No	AdrHyp, ACTH↑, PT↑	Gas↑	Yes	Nk	Na
22	62	M	WH, AUT	2/10	Ln (Gas)	ParAd, PitAd, DuoGas (m), C-Cell-Carcinoma (m), NET (lung)	Ø	Yes	Nk	Na
Patient with acromegaly and Zollinger-Ellison syndrome										
23	42	F	LSPR	1/13	Li (Gas) Ln (Gas)	ParAd, PitAd, DuoGas (m), ECLoma (m), GHRH↑	Nk	Yes	Yes	Exon 10, Codon 575, ATC→AAC (missense)
Patient with glucagonoma syndrome										
24	46	F	EN, LSPR, WH	1/2	No	ParAd	Asympt	Yes	No	Exon3, Codon 177, CTG→CCG (missense)
Patients without hormonal syndromes										
25	29	F	LSPR	1/3	No	ParAd, Prolactin↑	Asympt	Yes	Yes	Intron 7, 1-118, 1 bp G→A
26	32	M	LSPR	4/5	No	AdrAd	Asympt	Yes	Yes	Exon 2, Codon 108, CGA→TGA (nonsense)
27	38	M	LSPR	2/7	No	ParAd, AdrAd	Asympt	Yes	Yes	Exon 8, Codon 368, 13 bp deletion (frameshift)
28	64	M	WH	1/4	Ln (Som)	ParAd	Asympt	Yes	Yes	Exon 8, Codon 368, 13 bp deletion (frameshift)

EN, enucleation; LSPR, left-sided pancreas resection; WH, Whipple; AUT, autopsy; Ln, lymph node; Nk, not known; Li, liver; Ins, insulin; Gas, gastrin; Som, somatostatin; NET, neuroendocrine tumor; ParAD, parathyroid adenoma; DuoGas (m), multifocal duodenal gastrinomas; PitAd, pituitary adenoma; AdrAd, adrenal adenoma; ACTH, adrenocorticotrophic hormone; ↑, increased serum levels; PT, parathyroid hormone; GHRH, growth hormone releasing hormone; ECLoma, enterochromaffin-like cell tumor; Asympt, asymptomatic; Susp, suspicious for MEN1; Ø, autopsy findings; Na, no blood samples available for mutational analysis.

TABLE 4. Clinicopathologic Features and Mutational Analysis of Patients With Non-MEN1-Associated Insulin- or Glucagon-Producing Tumors of the Pancreas

Patient No.	Age (yr)	Sex	Surgical Intervention	Macrotumors/ Microadenomas	Metastasis	Extrapancreatic NET	Postoperative Findings	Family History	MEN1 and VHL Mutation
Patients with hyperinsulinemic hypoglycemia									
29	26	F	EN	0/4	No	No	Nk	No	Na
30	37	F	LSPR	4/3	No	No	Asympt	No	Negative
31	49	F	LSPR	2/8	No	No	Nk	No	Na
32	57	M	LSPR	0/23	No	No	Asympt	No	Negative
33	58	F	EN, LSPR	3/4	No	No	Ins↑	No	Negative
Patients without any hormonal syndrome									
34	25	M	TP	8/624	No	No	Asympt	No	Negative
35	30	F	AUT	0/57	No	No	Ø	Nk	Na
36	43	F	LSPR	2/128	No	No	Asympt	No	Negative
37	69	F	WH	0/8	No	No	Asympt	No	Na

LSPR, left-sided pancreas resection; EN, enucleation; TP, total pancreatectomy; AUT, autopsy; WH, Whipple resection; NET, neuroendocrine tumor; Nk, not known; Asympt, asymptomatic; Ins, insulin; Ø, autopsy findings; Na, no blood samples available for mutational analysis.

PCR mixtures were prepared in 0.2-mL thin-walled reaction tubes (GeneAmp, Perkin Elmer, Norwalk, CT). They contained 100 ng of template DNA, 0.2 mM of dATP, dTTP, dGTP, and dCTP, 50 pmol of each sense and antisense primer, 1.5 mMol $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 1 U of Ampli Taq Gold DNA polymerase (Perkin Elmer) in a final volume of 50 μL. PCR was carried out in a programmable thermal cycler (DNA thermal cycler 9600; Perkin Elmer) using the following conditions: initial denaturation for 7 minutes at 94°C, 35 to 45 cycles of each denaturation for 45 seconds at 94°C, annealing for 60 seconds at 45°C to 58°C, and extension for 60 seconds at 72°C. After a final extension for 300 seconds at 72°C, heteroduplex formation was induced for DGGE analysis by initial denaturation for 10 minutes at 98°C followed by incubation at 55°C for 30 minutes and 37°C for 30 minutes.

DGGE and single-strand conformation polymorphism analysis were performed as described previously.[30] The PCR products were then visualized by silver staining.[23,24]

Samples with an aberrant banding pattern were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and then cycle sequenced on an automated sequencer (ABI Prism, Applied Biosystems, Foster City, CA). In patients in whom screening failed to detect MEN1 mutations, direct sequencing of all exons including intron/exon boundaries was additionally performed. Sequencing results were analyzed using BLAST (www.ncbi.nlm.nih.gov/Blast/) to the *men1* gene GI 18860855, and protein NP 570715.

Quantitative Analysis of Nontumorous Tissue Specimens

The total endocrine cell volume and islet cell proliferation were analyzed in nontumorous tissue from 11 patients and compared with tissue from 18 controls.

The endocrine cell volume was calculated as the ratio of the area occupied by synaptophysin-positive cells to the remaining pancreatic parenchyma. From each case (patients and controls), three sections and in each section (three randomly chosen 1 cm^2 areas) were analyzed by the point counting method using a Netzmicron occulometer (Carl Zeiss, Jena, Germany) at a magnification of × 200, as previously described.[2]

The proliferative activity of islet cells was determined by combined enzyme and fluorochrome-enhanced immunostaining.[4] The Ki-67 antigen was stained with DAB. This was followed by immunofluorescence labeling of the various islet hormones using the fluorochromes A647, A594, A488, Cy2, or Cy3. To quantify the Ki-67 immunoreactivity, a minimum of 50 islets per section were evaluated by point counting at a magnification of × 400. The data are given as mean and standard deviation.

Ethics

The procurement of human tissue for this study was approved by the Ethics Committees of the universities in question. Patients gave written informed consent.

RESULTS

Clinical and Genetic Features of the Patients

None of the patients showed features of a VHL syndrome. Table 3 summarizes the clinicopathologic and genetic data on the patients showing features of MEN1. Five of these 28 patients (17.8%) had hyperinsulinemic hypoglycemia, 15 patients (53.5%) a Zollinger-Ellison syndrome (ZES), and 2 (7.1%) both hyperinsulinemic hypoglycemia and ZES. One patient (3.5%) exhibited symptoms of excessive secretion of growth hormone-releasing hormone and ZES. Another patient (3.5%) suffered from a glucagonoma syndrome. Four patients (14.2%) had clinically silent tumors. Almost all MEN1 patients additionally had extrapancreatic endocrine tumors and/or the respective symptoms of inappropriate hormone secretion, such as parathyroid adenomas/

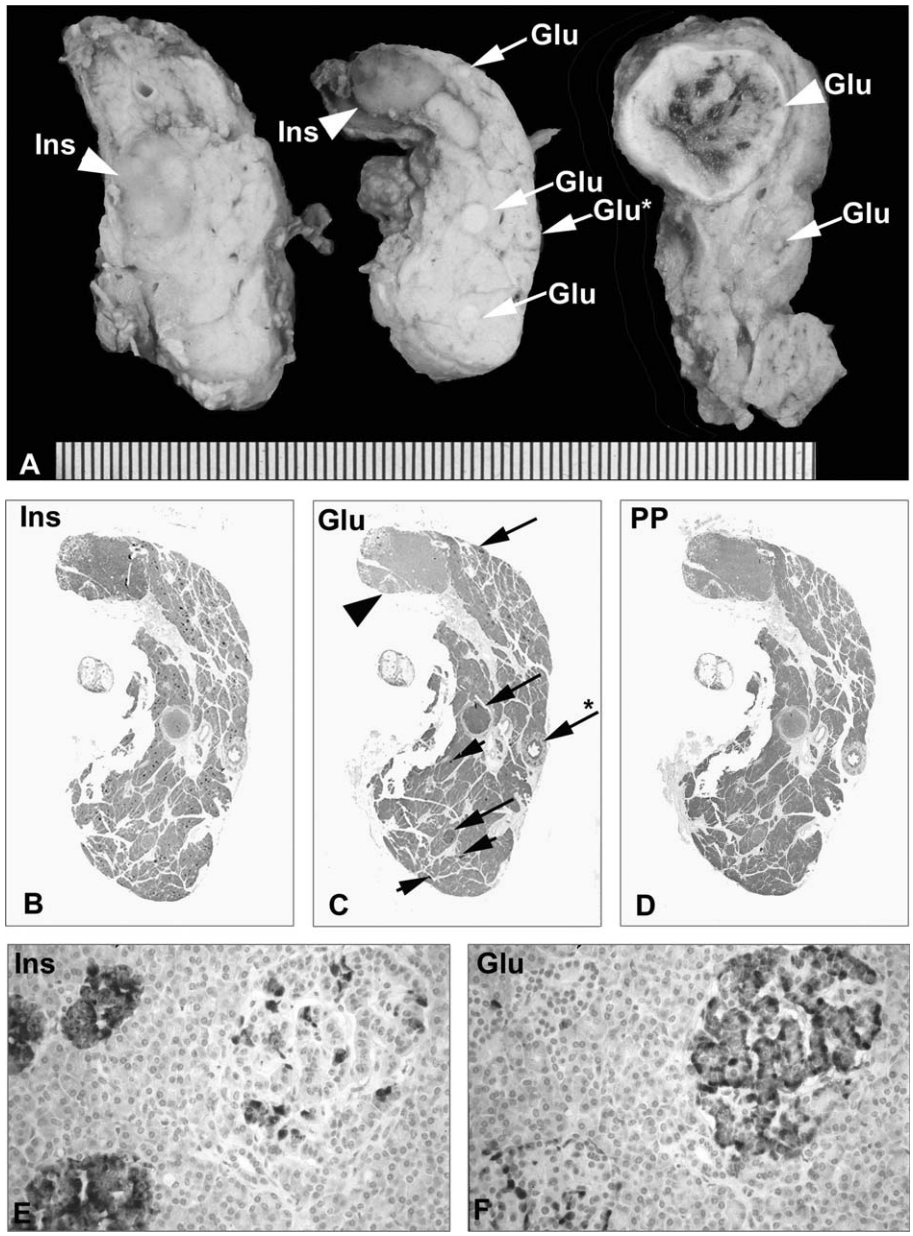

FIGURE 1. MEN1 pancreas of a patient with hyperinsulinemic hypoglycemia. A, Cut surface revealing three macrotumors (arrowheads), two producing insulin (Ins) and one glucagon (Glu), and five glucagon-positive microadenomas (arrows). B–D, Consecutive sections corresponding to the specimen in the center in A showing the hormonal phenotype of microadenomas of different sizes (large and small arrows). The insulin-expressing macrotumor is labeled by an arrowhead. E, F, Serial sections showing a microadenoma less than 300 μm in diameter and containing glucagon and a few insulin-positive cells. The neighboring islets are normal.

primary hyperparathyroidism (22; 78.4%), pituitary tumors/ACTH or prolactin hypersecretion (6; 21.4%), and/or adrenal cortex tumors (9; 32.1%). One MEN1 patient (patient no. 22) additionally exhibited a lung carcinoid and multiple small C-cell carcinomas of the thyroid. Patient no. 23, who had a ZES syndrome, additionally had an enterochromaffin-like cell tumor of the stomach.

Information on the family history was obtained for 17 patients. Eleven had a first-degree relative with an MEN1 syndrome. Germline mutations in the MEN1 gene were detected in 13 of the 15 patients (86.6%) for whom blood samples were available for analysis. The mutations were located in exons 2, 3, 8, and 10 and introns 4 and 7 and consisted of 4 frameshift mutations (deletions or insertions), 4 nonsense mutations, 2 splice-junction mutations, and 3 missense mutations. All of these mutations were heterozygous. Additionally, in 2 patients, a benign polymorphism R171Q was detected and 3 patients revealed the benign

TABLE 5. Immunohistochemical Phenotypes of Macrotumors and Microadenomas in MEN1 Patients

	Ins		Glu		Som		PP		UC		Others		Total	
Patient No.	**MT**	**MA**	**MT**	**MA**	**MT**	**MA**	**MT**	**MA**	**MT**	**MA**	**MT**	**MA**	**MT**	**MA**
Patients with hyperinsulinemic hypoglycemia														
1	2	2	—	3	—	1	—	1	—	3	—	—	2	10
2	3	—	—	26	—	—	—	5	1	—	—	—	4	31
3	1	—	—	—	—	—	1	3	—	—	—	—	2	3
4	2	1	4	6	—	—	—	—	1	1	—	—	7	8
5	1	—	4	2	—	—	3	1	—	—	—	—	8	3
Patients with Zollinger-Ellison syndrome														
6	—	2	—	6	—	—	—	—	—	2	—	—	0	10
7	—	—	—	—	—	—	—	—	1	—	—	—	1	0
8	—	—	2	—	—	1	—	—	—	—	—	—	2	1
9	—	—	2	5	—	—	—	1	—	—	—	—	2	6
10	—	—	—	11	—	—	—	—	1	—	—	—	1	11
11	—	—	—	16	—	—	—	—	—	—	—	—	0	16
12	—	6	—	3	—	—	—	3	—	1	—	—	0	13
13	—	2	2	28	—	4	—	16	2	—	—	—	4	50
14	—	3	—	14	—	1	3	12	1	—	—	—	4	30
15	—	7	—	11	—	5	2	4	—	3	—	—	2	30
16	—	—	1	12	—	—	—	—	—	—	—	—	1	12
17	—	—	—	—	—	—	1	—	—	—	—	—	1	0
18	—	—	—	2	—	—	—	9	—	6	—	—	0	17
19	—	11	—	1	2	1	3	8	2	1	—	—	7	22
20	—	—	—	—	—	—	—	—	—	2	—	—	0	2
Patients with hyperinsulinemic hypoglycemia and Zollinger-Ellison syndrome														
21	—	17	—	2	—	1	1	3	1	6	—	—	2	29
22	1	—	—	7	—	—	—	2	1	1	—	—	2	10
Patient with Acromegaly and Zollinger-Ellison syndrome														
23	—	—	—	13	—	—	—	—	—	—	1	—	1	13
Patient with glucagonoma syndrome														
24	—	—	1	2	—	—	—	—	—	—	—	—	1	2
Patients without clinical syndromes														
25	—	—	1	3	—	—	—	—	—	—	—	—	1	3
26	—	1	1	4	—	—	—	—	3	—	—	—	4	5
27	—	—	—	3	1	4	—	—	1	—	—	—	2	7
28	—	—	—	2	1	—	—	2	—	—	—	—	1	4
Total	10	52	18	182	4	18	14	70	15	26	1	0	62	348
(%)	2.4	12.7	4.4	44.4	1.0	4.4	3.4	17.1	3.7	6.3	0.3	0	15.4	84.6

Ins, insulin; Glu, glucagon; Som, somatostatin; PP, pancreatic polypeptide; Gas, gastrin; UC, unclassified; MT, macrotumor; MA, microadenoma.

polymorphism D418D. In two MEN1 patients (7.1%) who exhibited the clinical features of MEN1, no MEN1 mutation was found, despite full-length MEN1 cycle sequencing.

In total, 25 patients fulfilled the criteria for the MEN1 syndrome. The remaining 3 patients were classified as suspicious for MEN1 because they exhibited additional multifocal duodenal gastrinomas but no further endocrine tumors outside the duodenum and pancreas.

The clinicopathologic features of 9 patients with microadenomas of the pancreas without clinical features of MEN1 or VHL are shown in Table 4. Five of these 9 patients were treated surgically for hyperinsulinemic hypoglycemia. The other 4 patients had unspecific upper abdominal discomfort but did not reveal any endocrinologic symptoms. Blood samples for mutational analysis were available from 5 of these 9 patients. Full sequencing of both genes failed to reveal MEN1 or VHL mutations (Table 4).

Histopathology and Immunohistochemistry of Pancreatic Tumors

Figure 1 illustrates the macroscopic and microscopic features of pancreatic endocrine tumors in an MEN1 patient suffering from hyperinsulinemic hypoglycemia. Microadenomas were found in 26 and macrotumors in 23 of the 28 MEN1 patients. The number of microadenomas per specimen varied from 1 to 50 and that of macrotumors from 1 to 8. A total of 348 microadenomas and 62 macrotumors were identified (Tables 3 and 5).

All microadenomas displayed a trabecular or mixed solid-trabecular growth pattern. They were often surrounded by or interspersed with dense connective tissue. Their diameter ranged from 100 μm to 5 mm; the majority (219 of 348, 62.9%) had a diameter of less than 1 mm. The macrotumors were classified according to the WHO criteria as wdPETs (n = 53; 85.5%) and wdPETubs (n = 6; 9.7%). A few macrotumors (n = 3; 4.8%) were classified as wdPECs because of the presence of

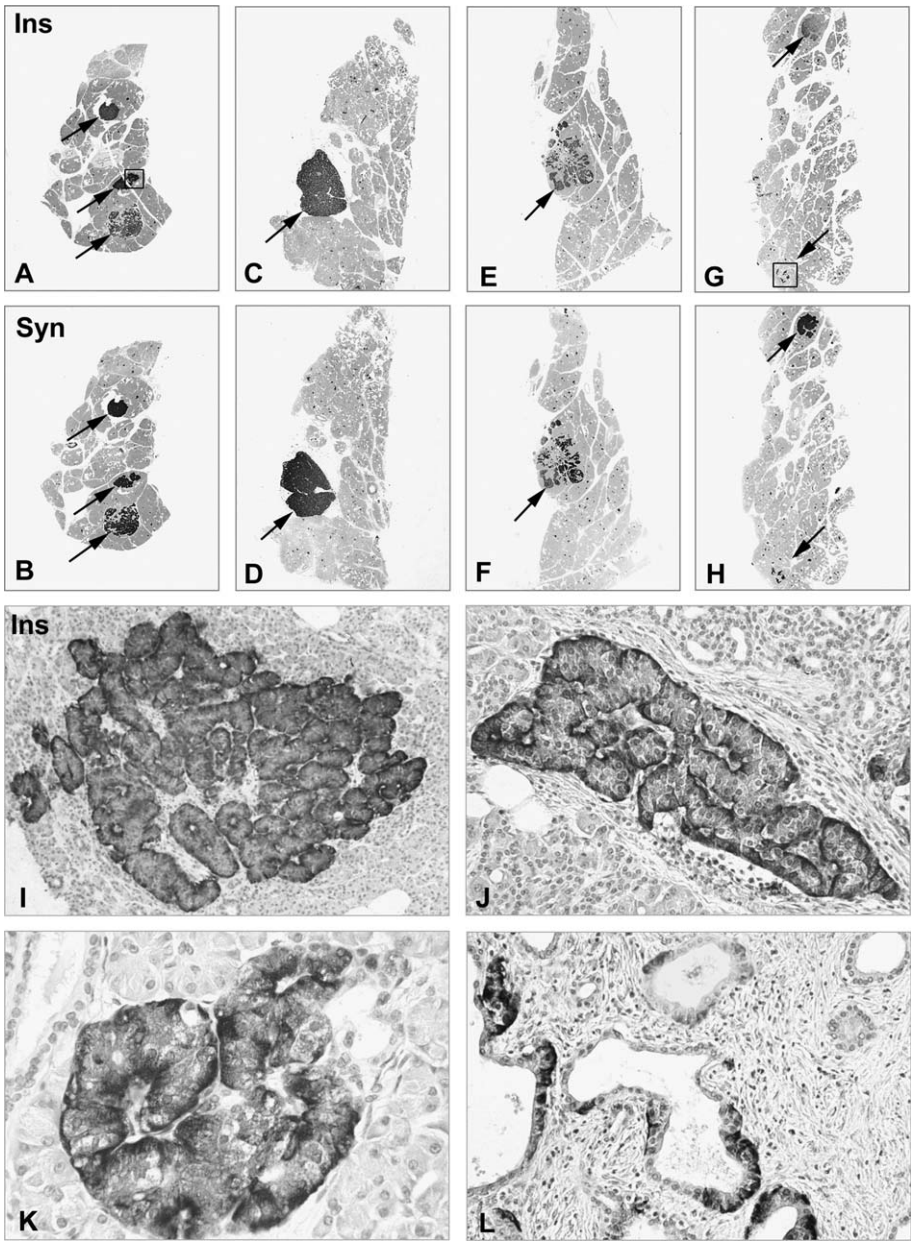

FIGURE 2. Non-MEN1, non-VHL-associated insulin-producing tumors in a patient with hyperinsulinemic hypoglycemia (arrows). A–H, Immunostaining for insulin (Ins) and synaptophysin (Syn). I, J, High power magnification of areas labeled in A and G showing the trabecular growth pattern of two microadenomas. K, L, Nontumorous pancreatic tissue showing irregularly shaped islets (K) and linear and nodular hyperplasia of insulin cells within the duct system (L).

peripancreatic lymph node metastases. Clear cell changes were only occasionally noted in the tumors.

The 62 macrotumors were immunoreactive for glucagon (18; 29.0%), PP (14; 22.6%), insulin (10; 16.1%), and somatostatin (4; 6.5%). One tumor (1.6%) was positive for growth hormone-releasing hormone. Fifteen of 62 macrotumors (24.2%) failed to express any of the hormones examined. All but 1 MEN1 patient with hyperinsulinemic hypoglycemia revealed one or more insulin-expressing macrotumors. The exception was a patient with hyperinsulinemic hypoglycemia and ZES (patient no. 21), who lacked an insulin-positive macrotumor but had 17 insulin-positive microadenomas. Insulin-positive microadenomas without hyperinsulinemic hypoglycemia were found in 6 ZES patients and 1 nonsyndromic patient.

Table 5 summarizes the results of the immunohistochemical analysis in all patients. A total of 322 of the 348 microadenomas (92.5%) expressed islet hormones. Most common was glucagon (182; 52.3%), followed

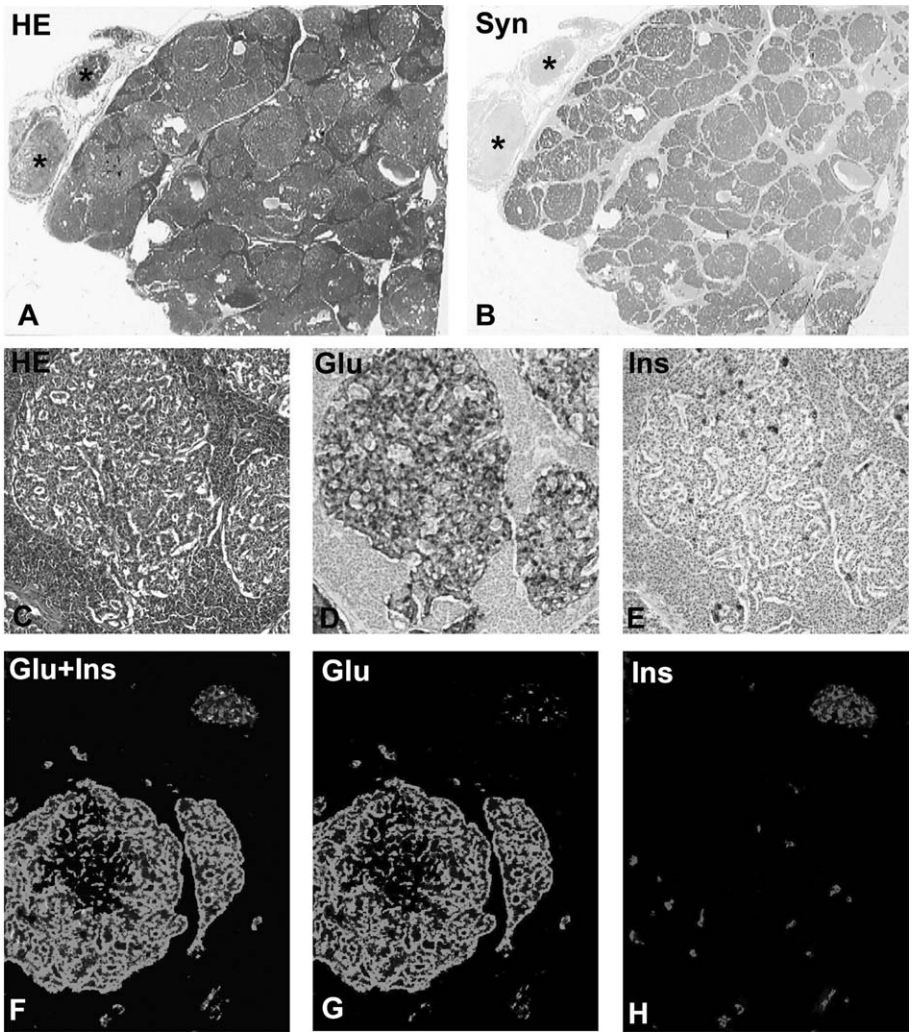

FIGURE 3. Pancreatic tissue of a non-MEN-1, non-VHL patient, showing numerous microadenomas and tumor-free peripancreatic lymph nodes (stars). A–E, Staining for hematoxylin and eosin, synaptophysin (SYN), glucagon (Glu), and insulin (Ins). F–H, Double fluorescence confocal laser microscopy for glucagon (green in composite image F and single fluorescence image G) and insulin (red in composite image F and single fluorescence image H) revealing cellular segregation of the two hormones. The adjacent islet shows a normal hormonal composition.

by pancreatic polypeptide (PP) (70; 20.1%), insulin (52; 14.9%), and somatostatin (18; 5.2%). The hormone-positive microadenomas were usually multihormonal with one predominant hormone. Double fluorescence confocal laser scanning analysis demonstrated that only one hormone was expressed per cell.

All 9 patients without the MEN1 or VHL syndrome had multiple microadenomas; in 5 patients, these were associated with a few macrotumors (Table 4). Five of these 9 patients had hyperinsulinemic hypoglycemia and had a total of 9 insulin-positive macrotumors (all wdPETs) and 42 insulin-positive microadenomas (Fig. 2; Table 4). Two of the 5 patients with hyperinsulinemic hypoglycemia had only insulin-positive microadenomas, most of which were less than 1 mm in diameter.

In 10 of the 18 patients with ZES, gastrin-positive lymph node metastases were present. In 15 of the 18 patients, duodenal gastrinomas were detected that explained the occurrence of a ZES syndrome (Table 3). In the remaining 3 patients, the source of hypergastrinemia could not be identified, but none of the patients revealed a pancreatic gastrinoma.

Four patients had multiple trabecular glucagon-expressing neoplasms, with a total of 10 macrotumors (all wdPETs) and 817 microadenomas (Table 4). One patient had more than 600 glucagon-positive microadenomas (Fig. 3). In addition to glucagon, most tumors contained single (in order of frequency) insulin, somatostatin, and/or PP cells.

Analysis of Nontumorous Tissue Specimens

Immunohistochemical analysis and confocal laser scanning double fluorescence microscopy of the normal-appearing endocrine tissue revealed the following

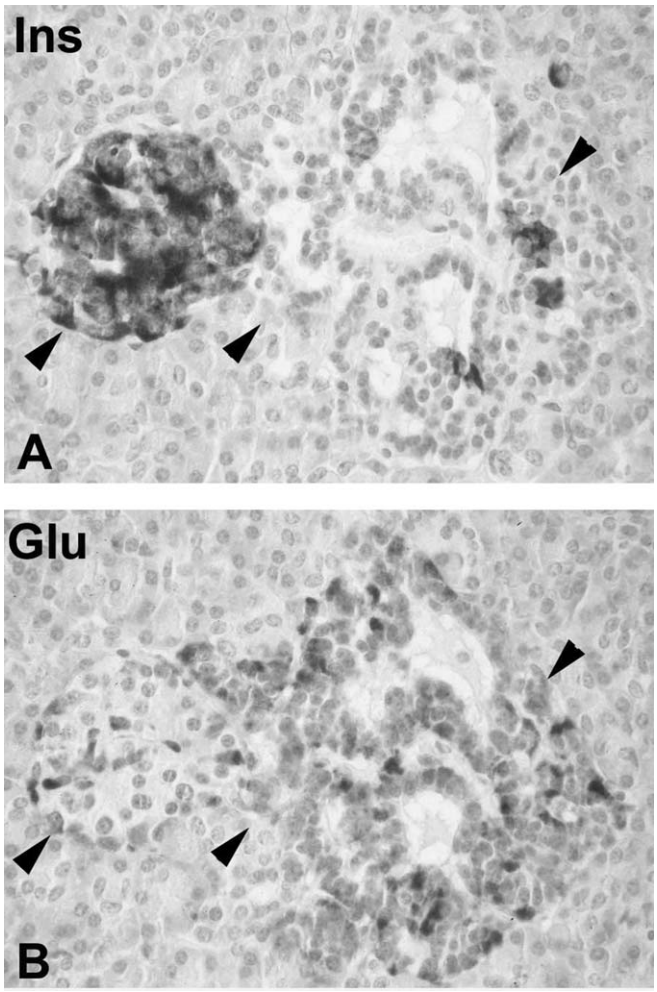

FIGURE 4. MEN1-associated microadenoma (central arrowhead) contiguous to a preexisting islet (right arrowhead). Normal islet (left arrowhead). A, B, Immunostaining for insulin (Ins) or glucagon (Glu).

abnormalities, which were not seen in the control specimens. 1) Occasionally, the duct system showed linear and nodular hyperplasia associated with the duct epithelium, in some cases forming endocrine cell buds. This focal change was seen in 2 of 28 MEN1 patients, in 2 of 5 patients with non-MEN1-associated insulin-producing tumors (Figs. 2I–L), and in all patients (4 of 4) with non-MEN1-associated glucagon-producing tumors. 2) In some islets that were enlarged or irregularly shaped, one islet cell type prevailed (Figs. 4, 5). This change was found in 13 of 28 MEN1 patients, 3 of 5 patients with non-MEN1-associated insulin-producing tumors (Figs. 2I–L), and all patients with non-MEN1-associated glucagon-producing tumors.

Analysis of the Ki-67 positivity of islet cell nuclei revealed a rate of 0.18% (± 0.11%) per islet in MEN1, 0.02% (± 0.01%) in non-MEN1-associated insulin-producing tumors, and 0.17% (± 0.08%) in non-MEN1-associated glucagon-producing tumors. One MEN1 patient showed numerous enlarged islets and an islet proliferation rate of up to 8% (Figs. 6A, B). In contrast, the islet cell proliferation rate in controls was less than 0.001%.The Ki-67-positive cells proved to be negative for all islet hormones when examined by fluorescence confocal laser scanning microscopy (Figs. 6C–F).

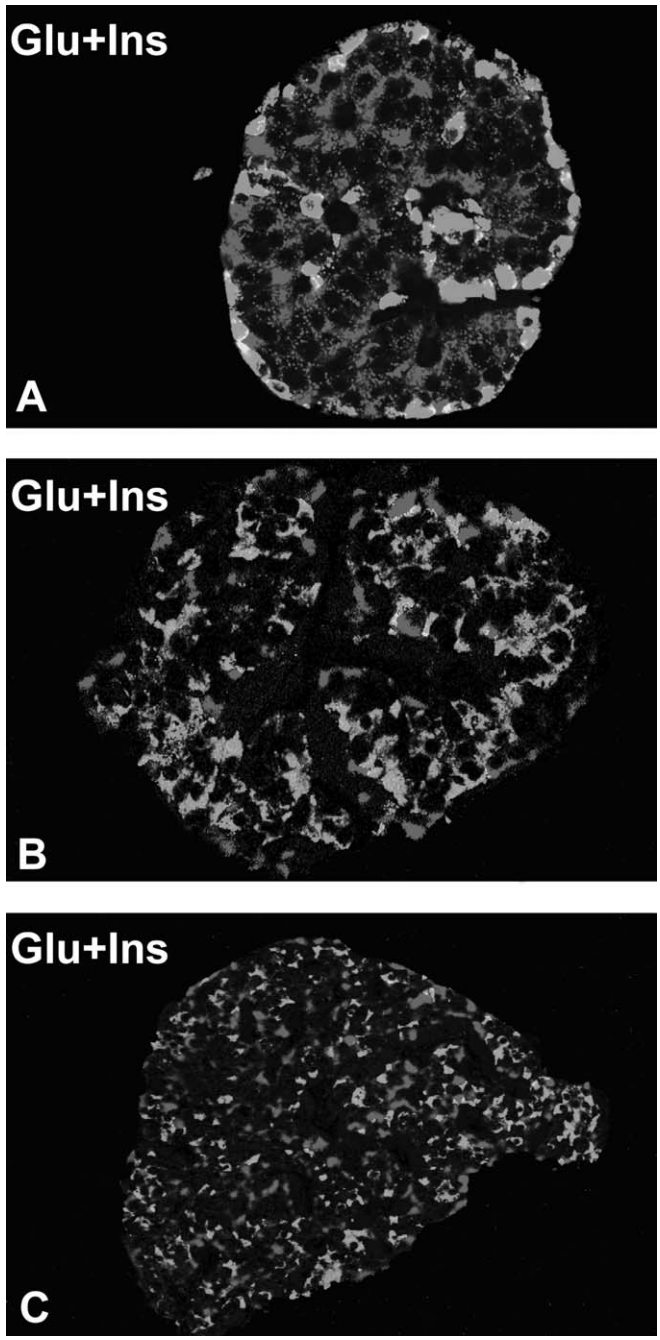

FIGURE 5. Analysis of hormones in islets of MEN1 patients. Confocal laser double fluorescence analysis of glucagon (Glu; green in A–C) and insulin (Ins; red in A–C). A, Most islets reveal a normal hormonal composition and architecture. Some scattered islets show an increase in glucagon cells (B, C) and appear to be enlarged (C).

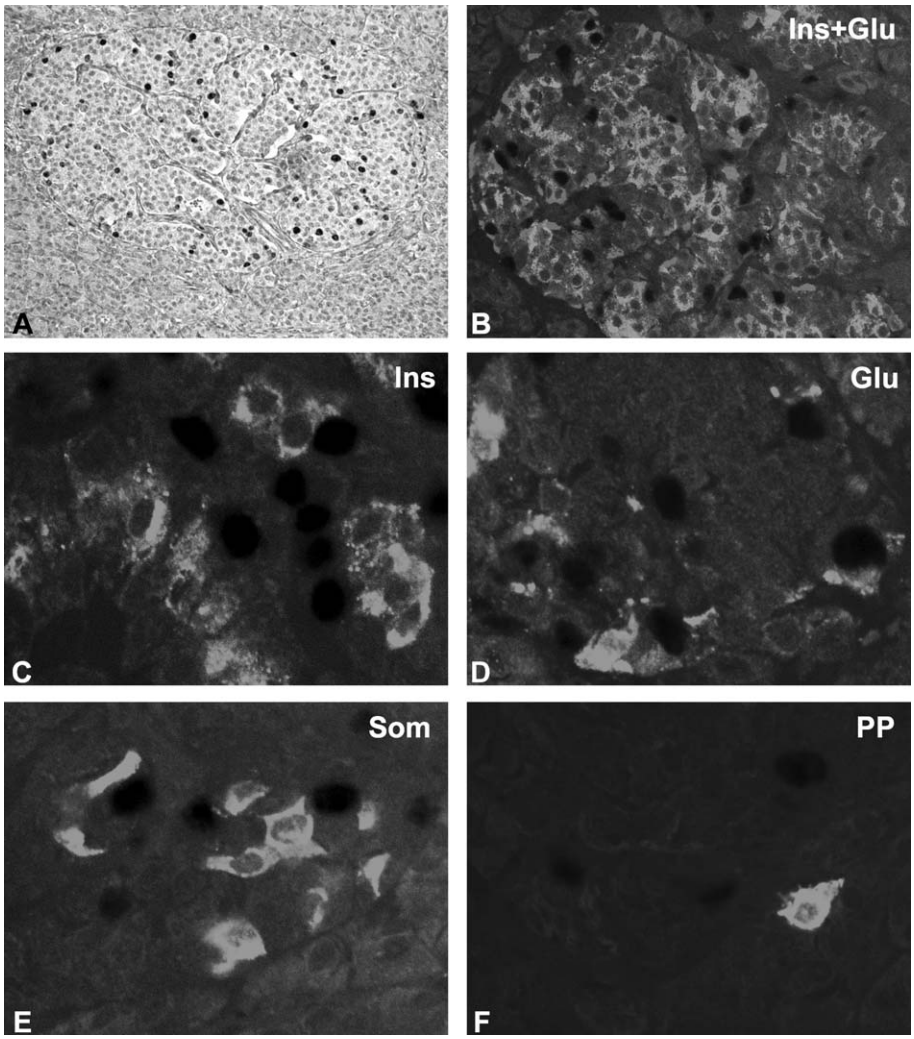

FIGURE 6. Ki-67 expression and hormone expression in an islet of an MEN1 patient. A, Enlarged islet with many Ki-67-positive endocrine cells. B, Immunostaining for Ki-67 followed by fluorochrome enhanced staining for insulin (Ins; green) and glucagon (Glu; red) reveals a normal proportion and distribution of both hormones. C–F, The high power magnification shows that Ki-67-positive cells are negative for islet hormones (green).

As shown in Fig. 7, the total volume of synaptophysin-positive cells was increased by more than 1 SD of the mean of controls in all patients with non-MEN1-associated glucagon-producing tumors (3 of 3), in all patients with non-MEN1-associated insulin-producing tumors (3 of 3), and in 3 of 5 patients with MEN1.

DISCUSSION

This study revealed that microadenomatosis of the pancreas is not restricted to genetic syndromes such as MEN1 or VHL. We found at least two types of microadenomatosis of the endocrine pancreas: one associated with the MEN1 syndrome and a second morphologically and immunohistochemically distinct type apparently associated with neither MEN1 nor VHL.

Pancreatic microadenomatosis has been reported to be a characteristic hallmark of MEN1.[21,35] This notion is confirmed and extended by our study. Twenty-eight patients in our study fulfilled the WHO criteria for an MEN1 syndrome, ie, pancreatic endocrine tumors associated with MEN1-related extrapancreatic endocrine tumors.[11] An MEN1 germline mutation was found in 13 of the 15 patients available for genetic testing. The failure to detect an MEN1 germline mutation in patients 6 and 7 is intriguing, but it is known that in 5% to 10% of MEN1 families germline mutations remain unidentified despite a full-length analysis of the MEN1 gene.[22] In three further patients (patient nos. 12, 13, and 18), pancreatic microadenomatosis and multiple duodenal gastrinomas were found, but there was no evidence of the presence of extraduodenopancreatic endocrine tumors. These patients do not fully meet the diagnostic

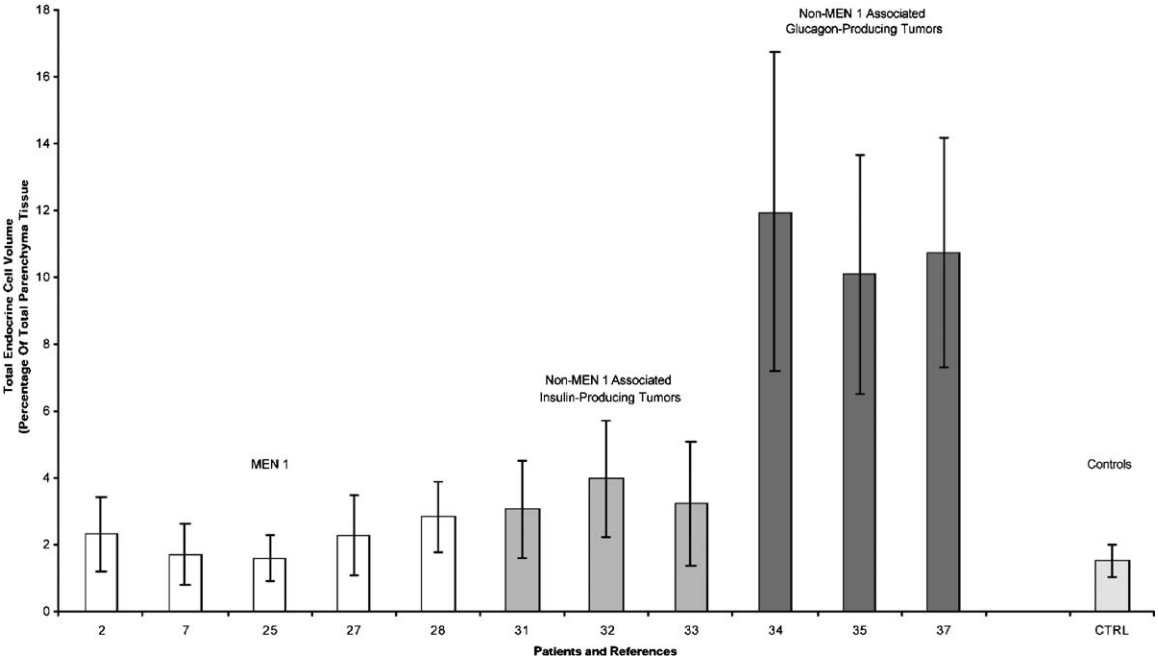

FIGURE 7. Total volume of synaptophysin-positive cells in nontumorous pancreatic specimens of patients with MEN1 and non-MEN1-VHL associated microadenomatosis.

criteria for MEN1; however, an MEN1 syndrome seems likely, as the multifocal appearance of duodenal gastrinomas is a hallmark of MEN1.[32] Unfortunately, no blood samples were available from these patients for a mutational analysis.

All but 2 MEN1 patients revealed pancreatic microadenomatosis. From this detailed analysis, we conclude that MEN1 patients have a high probability of developing microadenomatosis of the endocrine pancreas. The absence of microadenomatosis in patient nos. 7 and 17 indicates either that the penetrance of microadenomatosis in MEN1 is not 100% or that there were too few microadenomas to be detected by our sampling method.

Unexpectedly, 9 of 37 patients with microadenomatosis of the endocrine pancreas did not display any additional tumors associated with the MEN1 or the VHL syndrome, and 2 of them presented with the most severe form encountered in this study. All of the patients who were genetically analyzed (5 of 9 cases) failed to reveal mutations characterizing the MEN1 or VHL syndrome. A comparison of the hormonal profile of their tumors with those of the MEN1 patients revealed subtle differences. Whereas most MEN1 patients had multihormonal tumors, the 9 non-MEN1, non-VHL patients had tumors that were largely monohormonal. Five of these patients had insulin-expressing tumors and, regardless of the size or number of their tumors, suffered from a hypoglycemic syndrome. Four patients had glucagon-expressing tumors and were syndrome free. It seems, therefore, that the monohormonal tumors in the special setting described here cause a hypoglycemic syndrome if they express insulin and remain hormonally silent if they express glucagon. By contrast, the multihormonal microadenomas in MEN1 remain endocrinologically silent, unless there is an additional macrotumor producing a functionally relevant hormone such as insulin.

Patients with MEN1 rarely develop malignant endocrine tumors of the pancreas.[21,26,34] Malignant endocrine tumors in MEN1 are usually metastasizing duodenal gastrinomas or thymic carcinomas.[15,29] In our series, lymph node metastases from pancreatic endocrine tumors were found in only 3 patients (10.7%), 2 of them with insulin-producing macrotumors (ie, insulinomas) and 1 with a somatostatin-expressing macrotumor. In the 9 non-MEN1, non-VHL patients, lymph node metastases were lacking, even if macrotumors were present. Although the number of patients is small, this might suggest that, in this multitumor disease of the endocrine pancreas, malignancy is even rarer than in MEN1.

The initial lesions in the human endocrine pancreas giving rise to microadenomas in MEN1 have recently been described by Vortmeyer et al[35] as "atypical structures types 1 and 2." By microdissecting these lesions and analyzing the tissue with polymorphic markers flanking the MEN1 gene locus, they demonstrated the loss of the MEN1 wild-type allele in addition to the germline mutation in 5 patients. Microdissected islets lacked LOH for the MEN1 gene locus. They concluded that microadenomas derive from duct epithelium rather than from islets. In contrast to this observation, in some MEN1 knockout mice models, it has been suggested that neuroendocrine tumors arise from hyperplastic islets.[6,7,13,14]

Analysis of the nontumorous endocrine pancreas in our cases revealed that some contained enlarged islets with a predominance of one islet hormone (usually glucagon) and increased Ki-67 labeling in hormonally undetermined cells, findings that strongly contrasted with those of control islets. These findings are similar to those in an MEN1 mouse model[13] in which increased BrdU labeling was found in a subset of hyperplastic islets. As most cells of these islets lacked menin gene expression, they are thought to be the starting point for neoplastic growth. Whether in humans the MEN1 tumors also originate from islets remains speculative. The fact that these proliferative foci were only found in single islets and are only detectable by confocal laser scanning analysis may explain why Vortmeyer et al[35] found islets to be negative for LOH on 11q13.

Another source of the endocrine microadenomas in the pancreas could be the linear and nodular hyperplasia of endocrine cells in association with ducts. This was most frequently seen in the patients with non-MEN1-associated glucagon-producing tumors but was also found in some of the MEN1 patients. We therefore think that both compartments, ie, the duct system and the islets, may harbor preneoplastic progenitor cells that may give rise to microadenomas in MEN1 and non-MEN1 patients.

In summary, this study revealed that endocrine microadenomatosis of the pancreas is an MEN1 feature with high penetrance. However, pancreatic microadenomatosis is not entirely specific to MEN1 because it may also occur independently of this inherited condition and also of VHL. This latter form of microadenomatosis is characterized by the almost exclusive expression of one hormone in the tumors (ie, insulin or glucagon), a feature contrasting with the usually multiple islet hormone expression in MEN1 tumors. The fact that non-MEN1 patients were clearly separated into two groups with either insulin- or glucagon-producing tumors suggests that they could belong to two disease entities rather than to a single one.

ACKNOWLEDGMENTS

The authors thank Maike Pacena, Anja Bredtmann, Marion Zibuschka, Elke Rodenberg-Frank, Sonja Schmid, Tamara Locher, and Dieter Zimmermann for their excellent technical assistance; C. Wittekind and A. Tannapfel for providing tumor blocks; Cora Lu Meyer and Daniel Wieben for their help; Waldemar Strauss for the photo documentation; Katherine Dege and Gerhard E. Feurle for carefully reading the manuscript; all colleagues who provided clinical follow-up data; and all patients and their families for their contributions to this study.

REFERENCES

1. Agarwal SK, Lee BA, Sukhodolets KE, et al. Molecular pathology of the MEN1 gene. *Ann NY Acad Sci*. 2004;1014:189–198.
2. Albers N, Löhr M, Bogner U, et al. Nesidioblastosis of the pancreas in an adult with persistent hyperinsulinemic hypoglycemia. *Am J Clin Pathol*. 1989;91:336–340.
3. Anlauf M, Perren A, Meyer CL, et al. Precursor lesions in patients with multiple endocrine neoplasia type 1-associated duodenal gastrinomas. *Gastroenterology*. 2005;128:1187–1198.
4. Anlauf M, Weihe E, Hartschuh W, et al. Localization of xenin-immunoreactive cells in the duodenal mucosa of humans and various mammals. *J Histochem Cytochem*. 2000;48:1617–1626.
5. Asgharian B, Turner ML, Gibril F, et al. Cutaneous tumors in patients with multiple endocrine neoplasm type 1 (MEN1) and gastrinomas: prospective study of frequency and development of criteria with high sensitivity and specificity for MEN1. *J Clin Endocrinol Metab*. 2004;89:5328–5336.
6. Bertolino P, Tong WM, Galendo D, et al. Heterozygous Men1 mutant mice develop a range of endocrine tumors mimicking multiple endocrine neoplasia type 1. *Mol Endocrinol*. 2003;17:1880–1892.
7. Biondi CA, Gartside MG, Waring P, et al. Conditional inactivation of the MEN1 gene leads to pancreatic and pituitary tumorigenesis but does not affect normal development of these tissues. *Mol Cell Biol*. 2004;24:3125–3131.
8. Bordi C, De Vita O, Pilato FP, et al. Multiple islet cell tumors with predominance of glucagon-producing cells and ulcer disease. *Am J Clin Pathol*. 1987;88:153–161.
9. Brandi ML, Gagel RF, Angeli A, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab*. 2001;86:5658–5671.
10. Bystrom C, Larsson C, Blomberg C, et al. Localization of the MEN1 gene to a small region within chromosome 11q13 by deletion mapping in tumors. *Proc Natl Acad Sci USA*. 1990;87:1968–1972.
11. Calender A, Morrison CD, Komminoth P, et al. Multiple endocrine neoplasia type 1. In: DeLellis RA, Lloyd RV, Heitz PU, et al, eds. *Pathology and Genetics: Tumours of Endocrine Organs. WHO Classification of Tumors*. Lyon, France: IARC Press; 2004:218–227.
12. Chandrasekharappa SC, Guru SC, Manickam P, et al. Positional cloning of the gene for multiple endocrine neoplasia- type 1. *Science*. 1997;276:404–407.
13. Crabtree JS, Scacheri PC, Ward JM, et al. A mouse model of multiple endocrine neoplasia, type 1, develops multiple endocrine tumors. *Proc Natl Acad Sci USA*. 2001;98:1118–1123.
14. Crabtree JS, Scacheri PC, Ward JM, et al. Of mice and MEN1: insulinomas in a conditional mouse knockout. *Mol Cell Biol*. 2003;23:6075–6085.
15. Ferolla P, Falchetti A, Filosso P, et al. Thymic neuroendocrine carcinoma (carcinoid) in multiple endocrine neoplasia type 1 syndrome: the Italian series. *J Clin Endocrinol Metab*. 2005;90:2603–2609.
16. Feurle GE, Anlauf M, Hamscher G, et al. Xenin-immunoreactive cells and extractable xenin in neuroendocrine tumors of duodenal origin. *Gastroenterology*. 2002;123:1616–1626.
17. Friedman E, Sakaguchi K, Bale AE, et al. Clonality of parathyroid tumors in familial multiple endocrine neoplasia type 1. *N Engl J Med*. 1989;321:213–218.
18. Giraud S, Zhang CX, Serova-Sinilnikova O, et al. Germ-line mutation analysis in patients with multiple endocrine neoplasia type 1 and related disorders. *Am J Hum Genet*. 1998;63:455–467.
19. Guo SS, Sawicki MP. Molecular and genetic mechanisms of tumorigenesis in multiple endocrine neoplasia type-1. *Mol Endocrinol*. 2001;15:1653–1664.
20. Heitz PU, Komminoth P, Perren A, et al. Pancreatic endocrine tumours: introduction. In: DeLellis RA, Lloyd RV, Heitz PU, et al, eds. *Pathology and Genetics: Tumours of Endocrine Organs. WHO Classification of Tumors*. Lyon, France: IARC Press; 2004:177–182.
21. Klöppel G, Willemer S, Stamm B, et al. Pancreatic lesions and hormonal profile of pancreatic tumors in multiple endocrine neoplasia type I: an immunocytochemical study of nine patients. *Cancer*. 1986;57:1824–1832.
22. Komminoth P, Heitz PU, Klöppel G. Pathology of MEN-1: morphology, clinicopathologic correlations and tumour development. *J Intern Med*. 1998;243:455–464.
23. Komminoth P, Kunz E, Hiort O, et al. Detection of RET proto-oncogene point mutations in paraffin-embedded pheochromocytoma specimens by nonradioactive single-strand conformation

polymorphism analysis and direct sequencing. *Am J Pathol*. 1994; 145:922–929.
24. Komminoth P, Kunz EK, Matias-Guiu X, et al. Analysis of RET protooncogene point mutations distinguishes heritable from non-heritable medullary thyroid carcinomas. *Cancer*. 1995;76:479–489.
25. Larsson C, Skogseid B, Öberg K, et al. Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. *Nature*. 1988;332:85–87.
26. Le Bodic MF, Heymann MF, Lecomte M, et al. Immunohistochemical study of 100 pancreatic tumors in 28 patients with multiple endocrine neoplasia, type I. *Am J Surg Pathol*. 1996;20:1378–1384.
27. Lemmens I, Van de Ven WJ, Kas K, et al. Identification of the multiple endocrine neoplasia type 1 (MEN1) gene: the European Consortium on MEN1. *Hum Mol Genet*. 1997;6:1177–1183.
28. Lubensky IA, Pack S, Ault D, et al. Multiple neuroendocrine tumors of the pancreas in von Hippel-Lindau disease patients: histopathological and molecular genetic analysis. *Am J Pathol*. 1998;153:223–231.
29. Norton JA, Fraker DL, Alexander HR, et al. Surgery to cure the Zollinger-Ellison syndrome. *N Engl J Med*. 1999;341:635–644.
30. Perren A, Barghorn A, Schmid S, et al. Absence of somatic SDHD mutations in sporadic neuroendocrine tumors and detection of two germline variants in paraganglioma patients. *Oncogene*. 2002;21: 7605–7608.
31. Pilato FP, D'Adda T, Banchini E, et al. Nonrandom expression of polypeptide hormones in pancreatic endocrine tumors: an immunohistochemical study in a case of multiple islet cell neoplasia. *Cancer*. 1988;61:1815–1820.
32. Pipeleers-Marichal M, Somers G, Willems G, et al. Gastrinomas in the duodenums of patients with multiple endocrine neoplasia type 1 and the Zollinger-Ellison syndrome. *N Engl J Med*. 1990;322: 723–727.
33. Sipos B, Möser S, Kalthoff H, et al. A comprehensive characterization of pancreatic ductal carcinoma cell lines: towards the establishment of an in vitro research platform. *Virchows Arch*. 2003;442: 444–452.
34. Thompson NW, Lloyd RV, Nishiyama RH, et al. MEN 1 pancreas: a histological and immunohistochemical study. *World J Surg*. 1984;8:561–574.
35. Vortmeyer AO, Huang S, Lubensky I, et al. Non-islet origin of pancreatic islet cell tumors. *J Clin Endocrinol Metab*. 2004; 89:1934–1938.
36. Vortmeyer AO, Lubensky IA, Skarulis M, et al. Multiple endocrine neoplasia type 1: atypical presentation, clinical course, and genetic analysis of multiple tumors. *Mod Pathol*. 1999;12:919–924.
37. Zhuang Z, Ezzat SZ, Vortmeyer AO, et al. Mutations of the MEN1 tumor suppressor gene in pituitary tumors. *Cancer Res*. 1997; 57:5446–5451.