Am. J. Hum. Genet. 63:455–467, 1998

Germ-Line Mutation Analysis in Patients with Multiple Endocrine Neoplasia Type 1 and Related Disorders

Sophie Giraud,[1,3] Chang X. Zhang,[1,3] Olga Serova-Sinilnikova,[1,3] Virginie Wautot,[1,3] Janine Salandre,[1] Nathalie Buisson,[1] Christine Waterlot,[6] Catherine Bauters,[6,10] Nicole Porchet,[7] Jean-Pierre Aubert,[7] Philippe Emy,[11] Guillaume Cadiot,[12] Brigitte Delemer,[13] Olivier Chabre,[14] Patricia Niccoli,[15] Frédéric Leprat,[16] Francoise Duron,[17] Brigitte Emperauger,[17] Patrick Cougard,[20] Pierre Goudet,[20] Emile Sarfati,[18] Jean-Paul Riou,[2] Sylvie Guichard,[2] Michel Rodier,[21] Alain Meyrier,[22] Philippe Caron,[23] Marie-Christine Vantyghem,[8] Michel Assayag,[19] Jean-Louis Peix,[4] Michel Pugeat,[4] Vincent Rohmer,[24] Michel Vallotton,[25] Gilbert Lenoir,[1,3] Patrick Gaudray,[26] Charles Proye,[9] Bernard Conte-Devolx,[15] Philippe Chanson,[27] Yin Y. Shugart,[28] David Goldgar,[5] Arnaud Murat,[29] and Alain Calender[1,3]

[1]Service de Génétique and[2]Service d'Endocrinologie, Hôpital Edouard Herriot, [3]Laboratoire de Génétique et Cancer, CNRS UMR 5641, Faculté de Médecine Rockefeller, [4]Services d'Endocrinologie et de Chirurgie, Hôpital de l'Antiquaille, and [5]Unité de Génétique et Epidémiologie, Centre International de Recherches sur le Cancer, Lyon; [6]Service d'Endocrinologie, [7]Service de Biochimie, INSERM U 377, [8]Service d'Endocrinologie, and [9]Service de Chirurgie Endocrine, Hôpital Claude Huriez, Lille; [10]Service de Médecine et d'Endocrinologie, Centre Hospitalier Germon et Gauthier, Bethune, France; [11]Service d'Endocrinologie, Centre Hospitalier, Orleans; [12]Service d'Hépato-Gastro-Entérologie and [13]Service d'Endocrinologie, Centre Hospitalier Universitaire, Reims; [14]Service d'Endocrinologie, Hôpital Nord, Grenoble; [15]Services d'Endocrinologie et de Chirurgie Endocrine, Hôpital La Timone, Marseille; [16]Service d'Endocrinologie, Hôpital Haut Lévêque, Bordeaux; [17]Service d'Endocrinologie, Hôpital St. Antoine, [18]Service de Chirurgie Endocrine, Hôpital St. Louis, and [19]Département de Médecine Interne et d'Endocrinologie, Hôpital Lariboisière, Paris; [20]Département de Chirurgie Générale, Urgences et Endocrinienne, Hôpital Général, Dijon; [21]Service d'Endocrinologie et de Médecine Interne, Centre Hospitalier, Nîmes; [22]Service de Néphrologie, Hôpital Avicenne, Bobigny; [23]Service d'Endocrinologie, Centre Hospitalier Universitaire, Hôpital Purpan, Toulouse; [24]Département d'Endocrinologie et de Diabétologie, Hôpital Universitaire, Canton de Genéve; [25]Département d'Endocrinologie et de Diabétologie, Hôpital Universitaire, Geneva; [26]Laboratoire "Instabilité et Altérations des Génomes," CNRS UNSA UMR 6549, Nice; [27]Service d'Endocrinologie, Hôpital Bicêtre, Le Kremlin Bicêtre, France; [28]Department of Clinical Epidemiology, School of Medicine, University of Pittsburgh, Pittsburgh; and [29]Clinique Endocrinologique, Centre Hospitalier Universitaire, Hôtel Dieu, Nantes

Summary

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant syndrome predisposing to tumors of the parathyroid, endocrine pancreas, anterior pituitary, adrenal glands, and diffuse neuroendocrine tissues. The *MEN1* gene has been assigned, by linkage analysis and loss of heterozygosity, to chromosome 11q13 and recently has been identified by positional cloning. In this study, a total of 84 families and/or isolated patients with either MEN1 or MEN1-related inherited endocrine tumors were screened for *MEN1* germ-line mutations, by heteroduplex and sequence analysis of the *MEN1* gene–coding region and untranslated exon 1. Germ-line *MEN1* alterations were identified in 47/54 (87%) MEN1 families, in 9/11 (82%) isolated MEN1 patients, and in only 6/19 (31.5%) atypical MEN1-related inherited cases. We characterized 52 distinct mutations in a total of 62 *MEN1* germ-line alterations. Thirty-five of the 52 mutations were frameshifts and nonsense mutations predicted to encode for a truncated MEN1 protein. We identified eight missense mutations and five in-frame deletions over the entire coding sequence. Six mutations were observed more than once in familial MEN1. Haplotype analysis in families with identical mutations indicate that these occurrences reflected mainly independent mutational events. No *MEN1* germ-line mutations were found in 7/54 (13%) MEN1 families, in 2/11 (18%) isolated MEN1 cases, in 13/19 (68.5%) MEN1-related cases, and in a kindred with familial isolated hyperparathyroidism. Two hundred twenty gene carriers (167 affected and 53 unaffected) were identified. No evidence of genotype-phenotype correlation was found. Age-related penetrance was estimated to be >95% at age >30 years. Our results add to the diversity of *MEN1* germ-line mutations and provide new tools in genetic screening of MEN1 and clinically related cases.

Received December 1, 1997; accepted for publication June 11, 1998; electronically published July 17, 1998.

Address for correspondence and reprints: Dr. Alain Calender, Laboratoire de Génétique et Cancer, CNRS UMR 5641, Faculté de Médecine Rockefeller, Lyon, France. E-mail: calender@cismsun.univ-lyonl.fr

0002-9297/98/6302-0021$02.00

Introduction

Multiple endocrine neoplasia (MEN) syndromes are familial disorders characterized by the combined occur-

rence of tumors in several endocrine glands. In MEN type 1 (MEN1; Wermer syndrome [MIM 131100]), the disease phenotypes include hyperplasia and/or tumors of the parathyroid, pancreatic islets, anterior pituitary, endocrine cells in the gastro-duodenal and thymic-bronchic tracts, and adrenocortical glands and lipoma (Wermer 1954; Sheperd 1991; Metz et al. 1994). Beyond the typical clinical manifestations, recent studies have suggested that cutaneous lesions and nervous-tissue tumors also are observed in MEN1 patients (Kato et al. 1996; Trump et al. 1996; Darling et al. 1997; Giraud et al. 1997). MEN1 is transmitted as an autosomal dominant trait with high penetrance. Prognosis in MEN1 patients is related to hormonal hypersecretion by tumors, such as hypergastrinemia causing severe peptic ulcer disease (Zollinger-Ellison syndrome [ZES]), primary hyperparathyroidism, and acute forms of hyperinsulinemia. Some tumors, primarily in pancreas, thymus, and bronchi, undergo malignant progression. The prevalence of MEN1 has been estimated as 1/30,000–1/50,000, on the basis of autopsy and epidemiological studies (Metz et al. 1994).

The *MEN1* gene has been localized to chromosome 11q13, by both linkage analysis and deletion mapping in tumor genomic DNA (Larsson et al. 1988; Friedman et al. 1989; Byström et al. 1990). Loss of heterozygosity (LOH) at 11q13 in MEN1-related endocrine tumors has suggested that the *MEN1* gene–encoded protein could be a tumor-suppressor gene, consistent with the two-hit model according to Knudson (1971; also see Friedman et al. 1989; Byström et al. 1990; Radford et al. 1990). The *MEN1* gene recently has been cloned (Chandrasekharappa et al. 1997; Lemmens et al. 1997) and spans ≈9 kb of genomic DNA containing 10 exons. Exon 1 and the 3′ 832 bp of exon 10 are untranslated. A major, 2.9-kb *MEN1* transcript has been detected in a variety of human tissues, and an additional, 4-kb transcript has been identified in pancreas and thymus (Lemmens et al. 1997). Germ-line mutations have been identified in most MEN1 families tested. The observed mutations include nonsense, missense, and frameshift mutations scattered throughout the entire coding region (Argawal et al. 1997; Chandrasekharappa et al. 1997; Lemmens et al. 1997; Mayr et al. 1997; Shimizu et al. 1997; Bassett et al. 1998). Results published so far suggest an absence of correlation between genotype (i.e., mutation location and type) and the clinical phenotype. A common feature among the mutations described to date is a likely loss of function of the resulting MEN1 protein.

Here we report the results of mutation analysis in 54 independent MEN1 families, 11 isolated MEN1 cases, and 19 isolated and/or familial cases with uncommon associations of MEN1-related tumors, defined as atypical situations. We also screened one familial isolated hyperparathyroidism (FIHPT). All these patients and families have been collected since 1993, within the framework of the French Groupe d'Etude des Néoplasies Endocriniennes Multiples (GENEM) clinical network.

Subjects and Methods

Patients and Families

Patients and families were identified through the GENEM network (Calender et al. 1995), including 84 probands and related subjects from 54 unrelated MEN1 families, 11 isolated MEN1 patients, and 19 individuals with uncommon associations of MEN1-related tumors. Patients were classified as either typical MEN1 and atypical MEN1-related cases. Typical MEN1 in both familial and isolated contexts was defined according to the criteria established at the 5th International MEN Workshop (Skogseid et al. 1995*a,* 1995*b*). In brief, MEN1 was diagnosed in patients with at least three of the five major lesions described in the syndrome, including tumors of parathyroid glands (HPT), endocrine pancreas (PET), anterior pituitary (PIT), thymic/bronchic neuroendocrine tissues (NET), and adrenal glands (ADR). Familial MEN1 (denoted by an arabic numeral with a prefix "F") was characterized by the presence of both a proband affected by at least two MEN1 major lesions and a first-degree relative with one major lesion (HPT and/or PET and/or PIT and/or NET and/or NET and/or ADR). Patients with unknown family history at the time of the study were defined as isolated MEN1 cases (denoted by an arabic numeral with a prefix "I"). Patients and families with uncommon inherited/isolated associations of no more than of the major lesions occurring in MEN1 were classified as atypical MEN1-related cases (denoted by an arabic numeral with a prefix "A"). One family was identified as having FIHPT, on the basis of a long-term ($\geqslant$10 years) follow-up. Differential diagnosis of FIHPT led us to exclude familial hypocalciuric hypercalcemia (FHH) related to the calcium sensor–receptor gene isolated on chromosome 3 (Pollak et al. 1993). In the family that we studied, FHH was excluded on the basis of both clinical evidence of hypercalciuria and histopathological observations of multiglandular parathyroid adenomas and hyperplasia in two probands.

For each patient and family member, clinical information included tests of total and/or free calcemia, parathyroid hormone 1-84, 24-h urinary calcium, glycemia, peptide C and insulin, basal gastrin, prolactin, growth hormone, and dynamic endocrine, related to each clinical situation, when available. Radiological studies (abdominal echography and/or echoendoscopy, thoracic and abdominal computed-tomography scan, pituitary magnetic-resonance imaging, and standard thoracic X-ray) were performed on all patients included in this re-

port, according to GENEM recommendations (Chanson et al. 1997). Histopathological reports were examined carefully for each patient treated by surgery. In this study, 71.8% (120/167) of the subjects classified as affected by MEN1 were diagnosed clinically, whereas 28.2% (47/167) were detected through biochemical screening. In the latter group, most ($\geqslant$90%) were diagnosed as having primary HPT (pHPT). When a mutation was identified in a proband, all available related members in the family were screened for the presence of the variant. Fifty unrelated control individuals (100 chromosomes) were screened for the presence of any of the missense variants found, to exclude frequent polymorphisms.

MEN1 Gene–Mutation Analysis

DNA and RNA extraction and cDNA synthesis.—DNA was extracted from peripheral blood lymphocytes and/or Epstein-Barr–virus immortalized lymphoblastoid cell lines. A modification of the protocol described by Sambrook et al. (1989) was used for RNA extraction. In brief, the cell pellets were lysed in the RNA extraction buffer containing 4 M guanidinium thiocyanate, 25 mM sodium citrate, 1% sodium laurylsarcosine, and 0.2 M β-mercaptoethanol. The solution was extracted with a same volume of phenol saturated with water, 0.1 vol of 2 M sodium acetate (pH 4.0), and 0.2 vol of chloroform, and then it was precipitated with isopropanol and was washed with 70% ethanol. Single-strand cDNA was synthesized with 2 μg total of RNA, by use of the first-strand cDNA synthesis kit (Pharmacia) according to the manufacturer's instructions.

Reverse transcription–PCR (RT-PCR) was performed with forward and reverse primers at nucleotide positions 325 and 1340, respectively, of the MEN1 cDNA sequence, by use of *Taq* polymerase (Red Hot DNA Polymerase; Applied Biosystems) according to the manufacturer's recommendations. Primers sequences used for RT-PCR were as follows: forward, 5′-GCC GTC GAC CTG TCC CTC TAT C-3′ (in exon 2); and reverse, 5′-GAA GCA CTC AGG GTC CTG GAG-3′ (in exon 9).

Heteroduplex (HD) screening.—Exons 2–10 of the *MEN1* gene were amplified from genomic DNA by PCR using a set of specific primers of the *MEN1* coding region, as described and referenced in a previous report (Lemmens 1997). Exon 1 untranslated sequence was amplified by use of primers E1F (5′-ACG CTT CCT GCC TGG TGG TCG G-3′) and E1R (5′-CTG CTG GGA CAT GAA GTC CCG-3′), which allowed the amplification of a region from, respectively, 33 bp upstream of the 5′ end of exon 1 and 45 bp downstream of the 3′ end of exon 1. For HD analysis, the following combinations of primers were used: 1 forward and 4R (exon 2), 5F and 7R (exons 3 and 4), 8F and 9R (exons 5 and 6), 10F and 10R (exon 7), 11F and 11R (exon 8), 12F and 12R (exon 9), and 13F and 15R (exon 10). *MEN1* cDNA was available for 15 probands and was screened when no mutation was found in the genomic sequence. PCR reactions were performed in a total volume of 15 μl containing 50 ng of genomic DNA and/or cDNA, 1 μM each primer, 0.5 μCi of $\alpha[^{33}P]$-dATP (1–3 mCi/mmol) (ICN), and 0.1 U of *Taq* DNA polymerase (Eurobio). PCR was performed in a Perkin Elmer Cetus model 9600 sequencer, as follows: 96°C for 3 min; 32 cycles of 96°C for 30 s, annealing temperature for 30 s, and 72°C for 1 min; and 72° for 7 min. PCR products were denatured at 98°C for 5 min and were cooled to room temperature for $\geqslant$30 min, to induce HD formation. DNA fragments were electrophoresed through 0.75 × MDE gel (FMC BioProducts) at 600 V for 14 h in 0.6 × Tris-borate buffer, by use of a vertical gel-electrophoresis apparatus (Biorad). Gels were dried and exposed to Kodak BioMax MR film. All variant bands found were subsequently sequenced.

Sequence analysis.—The sequencing reaction was performed directly from PCR-amplified DNA or cDNA, by use of the PRISM Ready Reaction Dye Deoxyterminator cycle sequencing kit (Perkin Elmer), and were run on an automated sequencer (model ABI 377; Applied Biosystems), with the same primers as had been used for HD analysis.

Southern blot analysis.—a search for large genomic rearrangements was performed by two independent Southern blot experiments, with *Eco*RI and with *Kpn*I (New England Biolabs). In brief, 5 μg of DNA were cleaved with either *Eco*RI or *Kpn*I and were separated by 1% agarose gel electrophoresis and then were transferred to Hybond N+ (Amersham) membranes. The membranes were hybridized to a ^{32}P-labeled probe corresponding to the *MEN1* cDNA. Prehybridization, hybridization, and autoradiography were performed according to standard techniques.

Genotype-Phenotype Correlation Studies

For each phenotype of interest (e.g., HPT and/or PET and/or PIT and/or NET and/or ADR), the proportions of affecteds within each mutation class were compared, by a standard χ^2 test. For the less common phenotypes (i.e., ADR and NET), an exact hypergeometric test was used. To ensure that sample sizes were adequate, mutations were grouped into three classes: frameshifts or nonsense mutations in exons 2–5, frameshifts or nonsense mutations in exons 6–10, and missense mutations regardless of location within the gene.

Linkage and Haplotypes

Haplotype analysis using polymorphic markers was performed in every MEN1 family with a minimum of two affected members, as described elsewhere (Larsson

Table 1

Germ-Line Mutations of *MEN1* Gene in MEN1 Patients

Exon and Family/Individual	Type of Mutation[a]	Codon	Mutation	Clinical Features[b]	Associated Lesion(s)[c]
Exon 2:					
F1	FS	67	310dup5-ter120	HPT, PIT	THYR
F2	FS	76	337delC-ter118	HPT, PET/ZES	ADR
F3	FS	83	359del4-ter116	HPT, PET/ZES	
F4	FS	83	359del4-ter116	HPT, PIT	
F5	FS	83	359del4-ter116	HPT, PET/ZES	
F6	FS	84	360dupGT-ter119	HPT, PET	MEL
I1	NS	96	Gln96ter	HPT, PET, PIT	THYR
F7	NS	98	Arg98ter	HPT, PET, PIT	ADR
F8	NS	98	Arg98ter	HPT, PET/ZES, NET	ADR
F9	NS	108	Arg108ter	HPT, PET, NET	LIP, NET-meta
F10	FS	110	437ins-GG-ter119	HPT, PET, PIT	NET, ADR
A1	NS	133	Tyr133ter	HPT, PIT	
Exon 3:					
I2	MS	172	Asp172Tyr	HPT, PET/ZES, PIT	
F11	IF	197	699DELACC	HPT, PET, NET, PIT	NET-meta
I3	FS	210	738del4-ter222	HPT, PET, PIT	
F12	SP	218	764G→T	HPT, PIT	
Exon 4:					
F13	FS	222	776delC-ter223	HPT, PET, PIT	ADR
F14	FS	223	778delT-ter280	HPT, PET, PIT	ADR, LIP
A2	MS	223	Leu223Pro	HPT, PET	
F15	NS	261	Arg261ter	HPT, PET, PIT	
Intron 4:					
F16	SP		893+1G→T	HPT, PET, PIT, NET	
A3	SP		894-1G→C	HPT, NET	
Exon 5:					
I4	NS	265	Tpr265ter	HPT, PET, PIT	
F17	FS	272	924insC-ter316	HPT, PET, NET	NET-meta
Exon 7:					
F18	MS	314	Arg314Pro	HPT, PET	
I5	FS	315	1054insA-ter316	HPT, PET/ZES, PIT	
F19	MS	337	Ala337Asp	HPT, PET	
I6	MS	341	Trp314Arg	HPT, PET	ADR
Exon 8:					
F20	MS	368	Ala368Asp	HPT, PET/ZES, PIT	
Exon 9:					
F21	FS	397	1300delC-ter557	HPT, PET	ADR
F22	FS	405	1325delG-ter444	HPT, PET, PIT	THYR
F23	NS	415	Arg415ter	HPT, PET, NET, PIT	CNS
I7	FS	418	1363delAC-ter447	HPT, PET, PIT	
F24	IF	418	1362del12	HPT, PET, NET, PIT	THYR
F25	MS	418	Asp418Asn	HPT, PET, PIT	
F26	FS	422	1374delA-ter444	HPT, PET, NET, PIT	NET-meta
F27	IF	425	1384delAGG	HPT, PET/ZES, PIT	ADR, CNS
F28	FS	438	1424del4-ter443	HPT, PET, NET, PIT	ADR
F29	FS	447	1449del11-ter444	HPT, PET/ZES	
Intron 9:					
A4	SP		1460+4del2	HPT, PIT	
Exon 10:					
F30	IF	459	1487ins6	HPT, PET, PIT	
F31	FS	460	1491insCC-ter496	PET/ZES, PIT	
F32	FS	460	1499dup8-ter561	HPT, PET, PIT	PET-meta
F33	NS	460	Arg460ter	HPT, PIT	ADR
F34	NS	460	Arg460ter	HPT, PIT	
I8	FS	467	1508ins-ter564	HPT, PET/ZES, PIT	
F35	FS	499	1607delA-ter558	HPT, PET/ZES, PIT	ADR
I9	FS	507	1630insC-ter530	HPT, PIT	ADR
F36	FS	514	1650insC-ter530	HPT, ZES	

(*continued*)

Table 1 (continued)

Exon and Family/Individual	Type of Mutation[a]	Codon	Mutation	Clinical Features[b]	Associated Lesion(s)[c]
F37	FS	514	1650insC-ter530	HPT, PET, PIT	
F38	FS	514	1650insC-ter530	HPT, PIT, NET	NET-meta
F39	FS	514	1650insC-ter530	HPT, PET/ZES	
F40	FS	514	1650delC-ter558	HPT, PIT, ZES	
F41	FS	514	1650delC-ter558	HPT, PET, PIT	
F42	NS	527	Arg527ter	HPT, PET, NET	ADR
F43	NS	527	Arg527ter	HPT, PET, NET, PIT	ADR
F44	NS	536	Gln536ter	HPT, PET/ZES	THYR
F45	FS	550	1768delT-ter558	HPT, ZES, PIT	
F46	FS	550	1768delT-ter557	HPT, PET, PIT	
A5	MS	555	Ser555Asn	HPT, PET	
F47	IF	557	1780deAGA	HPT, PET, PIT	
A6	FS	558	1782delA-ter558	HPT, PIT	

[a] SP = splice junction and intronic mutations; all other abbreviations are as defined in the text.

[b] ZES = ZES individuals/families affected by duodenal gastric ulcers with or without identified tumors to date; all other abbreviations are as defined in the text.

[c] THYR = thyroid epithelial adenoma, MEL = melanoma, NET-meta = metastatic evolution of NET, CNS = meningioma, ependymoma, and/or gliobastoma of CNS, and PET-meta = metastatic evolution of NET; all other abbreviations are as defined in the text.

et al. 1995). Common microsatellite loci used were, from centromere to telomere, D11S480, D11S1883, PYGM, D11S449, D11S1889, and D11S913. Families in which no mutation was found and for which sufficient numbers of DNA samples were available were analyzed by multipoint linkage analysis with markers D11S480, PYGM, and D11S913, by means of the VITESSE program (version 1.0) (O'Connell and Weeks 1995). A dominant mode of inheritance was assumed, with a disease-allele frequency of .000025 and age-related penetrance maximizing at age 50 years, as estimated in previous studies (Sheperd 1991; Trump et al. 1996).

Results

Germ-Line Mutations

Germ-line mutations were identified in a total of 47/54 (87%) MEN1 families, 9/11 (82%) patients with isolated MEN1, and 6/19 (31.5%) probands who were atypical MEN1-related cases. We identified 52 distinct mutations in a total of 62 *MEN1* germ-line alterations, which are detailed in table 1 (also see Genome Database). The 52 mutations occurred throughout the entire coding sequence and three exon/intron boundaries (fig. 1). Most (45/62) mutations were located in exons 2, 9, and 10. Twenty-five mutations were frameshifts (deletions and insertions), 10 were nonsense mutations, 4 were splice-junction mutations, 8 were missense mutations, and 5 were in-frame deletions or insertions. HD screening of genomic DNA detected 44 (≈85%) of the 52 mutations, with high and mild sensitivity, respectively, for frameshifts (25/25 [100%]) and point mutations (12/18 [67%]). HD screening therefore was considered a powerful tool in the identification of MEN1 families with identified germ-line mutation. The majority (35/52) of these germ-line mutations were predicted to result in premature translation termination. Of the 52 independent mutations, 8 (359del4, Arg98ter, Trp265ter, Asp418Asn, Arg460ter, Arg527ter, 1650insC, and 1650delC) have been described elsewhere (Argawal et al. 1997; Mayr et al. 1997; Shimizu et al. 1997; Bassett et al. 1998). In our series, eight mutations were observed more than once: 359del4 was identified in three families; 1650insC was found in four families; and each of the mutations Arg98ter, Arg460ter, 1650delC, Arg527ter, and 1768delT were identified in two families.

Four missense variants were detected in four unrelated typical MEN1 families (F18, F19, F20, and F25) and were shown to cosegregate with the disease. Four missense mutations also were identified, in two isolated MEN1 patients (patients 12 and 16) and in two MEN1-related cases (individuals A2 and A5). Only one mutation (Asp418Asn) has been described elsewhere (Bassett et al. 1998). These missense mutations were not observed in 50 normal unrelated individuals (100 chromosomes) and thus likely represent true deleterious mutations.

One mutation (in F16), which was not detected through HD analysis of genomic DNA, was detected at the cDNA level, by RT-PCR (table 1). Further genomic DNA sequencing revealed a G→T point mutation at the first base of intron 4, in the donor-splice junction of exon 4/intron 4, at position 893+1. This mutation alters

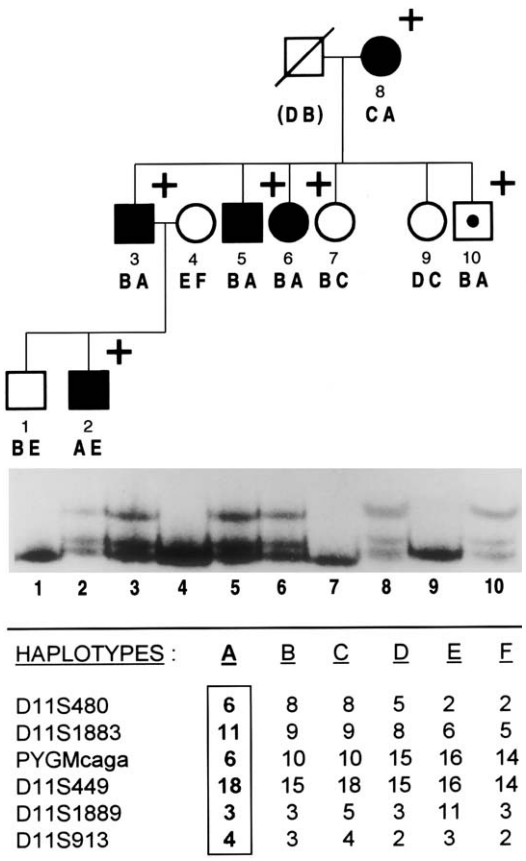

Figure 1 Cosegregation analysis of 337delC MEN1 germ-line mutation in exon 2, detected by HD screening for the disease and for 11q13 haplotypes in part of a large MEN1 family. Five distinct haplotypes (A–E) have been identified, each of which includes alleles for markers D11S480, D11S1883, PYGM(caga), D11S449, D11S1889, and D11S913, from centromere to telomere, respectively. The haplotype associated with the disease was "A" (see below). A plus sign (+) denotes mutant-gene carrier status, as defined by haplotypes and identification of 337delC by HD screening. Affected gene carriers presented the following lesions: individual 2, HPT and ADR; individual 3, HPT; individual 5, HPT; individual 6, HPT and PET; and individual 7, HPT and ADR. Individual 10 was defined as an asymptomatic gene carrier at age 38 years, during a recent ($\leqslant$6 mo prior to this study) screening excluding any endocrine biological and/or biochemical abnormalities.

an *Hph*I site and thus can be detected easily by PCR–restriction-enzyme analysis (fig. 2*A*). RT-PCR and cDNA sequence analysis (fig. 2*B*) revealed complete skipping of the in-frame exon 4 from the MEN1 transcript, which removed 43 amino acids from the *MEN1* protein. Splice-site mutations were found in three other cases, at the donor sites of exons 3 and 9 and at the acceptor sites of exon 5, at positions 764, 894−1, and 1460+4, respectively. cDNA was not available from affected individuals carrying these splice-site alterations. However, all three mutations affect highly conserved splice consensus sequences. No mutation was found in the untranslated part of the MEN1 gene, including exon 1 and the 5′ untranslated portion of exon 2.

Only six mutations were found in 19 atypical MEN1-related familial cases (patients and/or first-degree relatives with no more than two MEN1-related tumors). As shown in table 1, atypical families were characterized by single endocrine lesions occurring in first- or second-degree relatives. In six families with *MEN1* mutations, clinical lesions found in two individuals from the same family were HPT and PIT (in A1, A4, and A6), HPT and PET (in A2 and A5), and HPT and a bronchic NET (in A3).

Polymorphisms in the MEN1 *Gene*

Five polymorphisms were observed in the MEN1 gene. One of them occurred in intron 1 (position −16), at nucleotide 2249, as a C→G transition observed in 20% of the normal population; two of them had been described elsewhere; two of them, Ser145Ser (AGC→AGT) and Asp418Asp (GAC→GAT), were observed at respective frequencies of 1% and 45%, and the same polymorphisms have been reported, at similar allele frequencies, by others (Argawal et al. 1997; Bassett et al. 1998). A polymorphism in exon 3 led to an Arg→Gln substitution at codon 171. This benign polymorphism also has been observed, in the same allelic proportion (5%), in a National Institutes of Health study (Argawal et al. 1997). We identified a new polymorphism in exon 1, at nucleotide 1755, T→A (32%) and T→C (12%).

Haplotype Analysis in MEN1 Families with Identified Mutations

To check for a possible founder effect in the case of recurrent mutations, haplotype analysis were performed with 11q13 polymorphic markers surrounding the PYGM and MEN1 loci (Larsson et al. 1995; Courseaux et al. 1996). Linkage data were available for six of the eight recurrent mutations occurring in 15 families in which at least two affected individuals have been sampled (table 2). Families with recurrent mutations 359del4, Arg98ter, Arg460ter, and Arg527ter were shown to carry apparently different haplotypes; thus, a founder effect is not a likely explanation of the recurrence of these mutations. Of four families with mutation 1650insC, two of them, F36 and F37, carry a common haplotype, suggesting a common ancestor (table 2). Families F40 and F41, carrying mutation 1650delC, have both a partial haplotype match for affected individuals, in the proximal region from D11S480 to PYGM, and a divergence in the distal part, from D11S449 to D11S913. These two families were from the same geographic origin in northern France. Knowing that the MEN1 gene is located ≈55 kb distal to PYGM

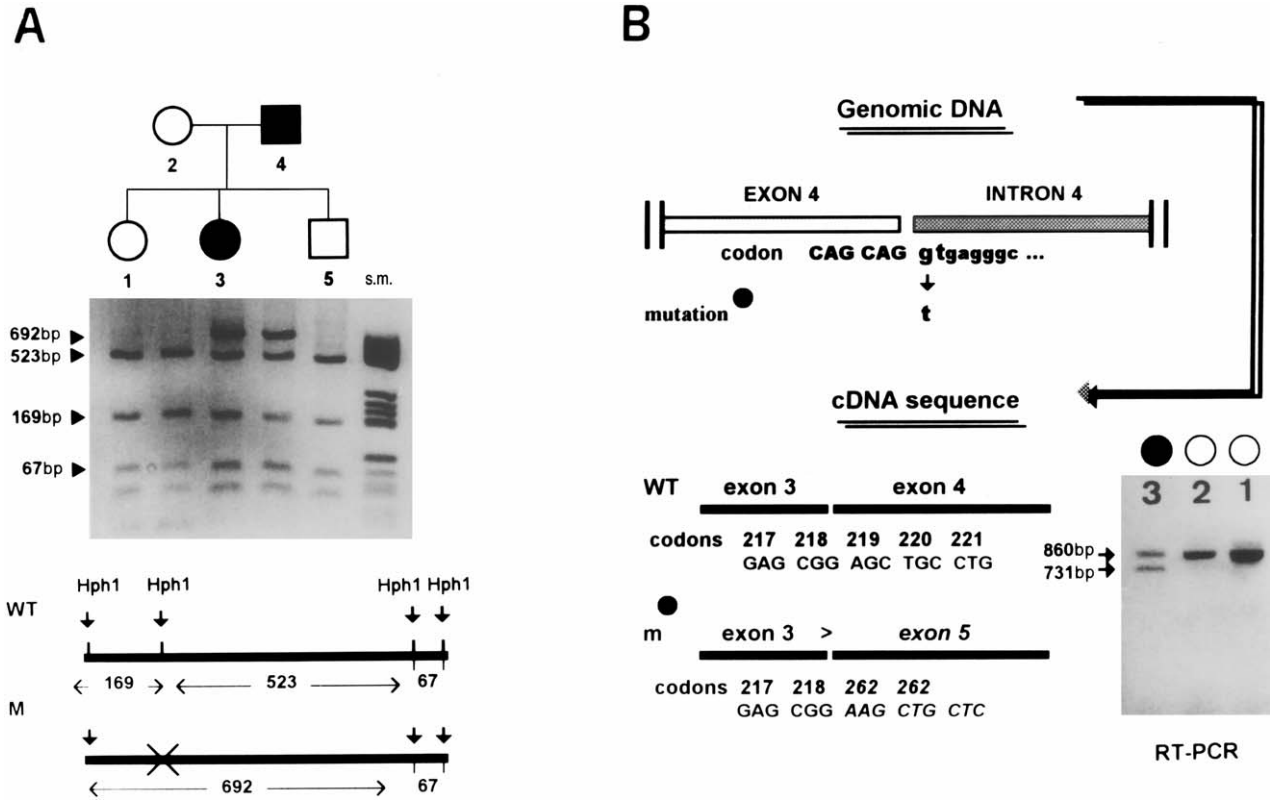

Figure 2 *A,* Detection of mutation 893+1 in intron 4 in a selected nuclear part of F16, by PCR–restriction-enzyme analysis. A point mutation has caused a G→T substitution in the donor splice site of intron 4 and results in the loss of an *HpH*I restriction-enzyme site from the normal (wild-type [WT]) sequence. This generates a large, 692-bp fragment. PCR was performed by means of primers 4F and 6R, as referenced elsewhere (Lemmens et al. 1997). Detection of mutations was thus facilitated in F16, in other affected members. Patients 3 (age 35 years) and 4 (age 65 years) were both affected by HPT, PET, and PIT. The unaffected individuals (1 and 5) were homozygous for the wild-type sequence, and the affected individuals (3 and 5) were heterozygous for the wild-type sequence. Size markers are designated as "s.m." *B,* 893+1 mutation in intron 4, which results in the in-frame skipping of exon 4 in *MEN1* cDNA. RT-PCR of the MEN1 gene was performed in unaffected and affected members of family F16. Primers used were forward 2f4 (Lemmens et al. 1997) and reverse 1121-SK21139 at position 1268 (authors' unpublished data), generating an 860-bp fragment in wild-type cDNA. Deletion of exon 4 in affected members such as individual 3 leads to an additional fragment, ≈730 bp, in concordance with the heterozygous removal of exon 4 (129 nucleotides). Individuals denoted as l–3 are the same as those shown in the pedigree in panel *A*. A schematic representation of the in-frame deletion of exon 4 as detected by cDNA sequence analysis has been provided on the left-hand side of the figure.

(Lemmens et al. 1997), we could expect that F40 and F41 are genetically related if a recombination event is assumed to have occurred, during the genetic history of these families, in the ≈500–600-kb interval between MEN1 locus and D11S449.

Clinical Expression in Mutant-Gene Carriers

The wild-type or mutant-*MEN1* carrier status was established in 356 individuals from 47 MEN1 families and in 9 isolated MEN1 patients in whom *MEN1* mutations had been identified. Two hundred twenty gene carriers were identified. Age-related penetrance was estimated to 25% at age 0–20 years, 75% at age 20–30 years, and $\geqslant$95% at age >30 years. Of the 220 gene carriers, 24.1% (53/220) were asymptomatic (mean age 19.5 years), 21.4% (47/220) were detected by biochemical screening (mean age 34.5 years), and 54.5% (120/220) had clinical symptoms (mean age 47.3 years). In the two latter groups, pHPT has been the first lesion diagnosed in 150 patients (90%) by biological screening of families, before genetic analysis. The youngest patient in the group of symptomatic gene carriers was a 6-year-old boy who was affected by insulinoma, in an MEN1 family. The oldest age among asymptomatic gene carriers was 70 years, in a large, previously documented MEN1 family showing malignant phenotypes for neuroendocrine tumors (Giraud et al. 1997). Our observations clearly indicate that *MEN1* germ-line mutations can be, in some situations, not fully penetrant. We confirm the high prevalence of pHPT as the first and most common lesion during the natural history of MEN1.

Genotype-phenotype correlation was analyzed by

Table 2

Haplotype Analysis in MEN1 Families/Patients with Recurrent Mutations

	Haplotype in[a]														
	359del4			Arg98ter		Arg460ter		1650insC				1650delC		Arg527ter	
Marker	F3	F4	F5	F7	F8	F33	F34	F36	F37	F38	F39	F40	F41	F42	F43
D11S480	8	8	4	6	3	4	8	8	8/4	8	4/5	8	8	5	8
D11S1883	…	…	…	11	6/9	…	…	…	…	…	…	…	…	8	3
PYGM(caga)	6	3	9/1	9	11	7	9	14	14/16	7	13	13	13	12	12
D11S449	15/18	15	17/18	…	…	2	17	15	15/14	18	15/19	16	12	15	16
D11S1889	…	…	…	3	2	3	3	…	…	…	…	…	…	2	3
D11S913	3	2	4	4	4	3	3/4	3	3	2	4	3	4	4	3/4

[a] Haplotypes shown refer only to the affected (or risk-associated) haplotypes in each family. In families with poor informativity or in which a unique patient was analyzed, both alleles of a marker could be linked to the disease and thus have been given (with "/" between them). Common haplotypes are underlined. For F40 and F41, the shared proximal haplotype (D11S480–PYGM) could suggest a recombination event occurring between PYGM-MEN1 and D11S449. All the families listed here were analyzed in a single experiment, to allow a uniform designation of alleles.

comparison of clinical expression in mutant gene carriers and both location and type of mutations within the coding sequence. No statistical differences in the gene carriers with specific clinical phenotypes could be identified as a function of either type of mutations (missense/in frame [MS/IF] vs. frameshift/nonsense [FS/NS]) ($\chi^2 = 0.99$; $P \geqslant .90$; 4 df), location in the gene (exons 2–6 vs. exons 7–10) ($\chi^2 = 3.47$; $P \geqslant .65$; 4 df), or type and location simultaneously ($\chi^2 = 5.4$; $P \geqslant .80$; 8 df). Although, globally, we could rule out only large effects of mutation type and position, we cannot exclude the possibility of mutation-specific effects. For example, two unrelated families with Arg98ter were found to have a similar range of MEN1-associated tumors, including HPT, PET, PIT, and ADR (table 1). The same clinical expression was observed in both families with Arg527ter. On the other hand, 359del4 (in three families) and Arg460ter (in two families) seemed not related to a homogeneous clinical expression, and we would expect that such a mutation-specific influence is not a common phenomenon in MEN1.

MEN1 Patients/Families in Which No Mutation Was Found

No *MEN1* germ-line mutations were found in ~14% of MEN1 cases (7/54 MEN1 families and 2/11 MEN1 patients without familial history). When patients and/or families with atypical MEN1-related disease were considered, the proportion of negative cases was significantly higher, 13/19 (68.5%) (table 3). Large germ-line *MEN1* rearrangements were excluded in all these families/patients, by Southern blot experiments using the *MEN1* cDNA probe and two distinct restriction enzymes (*Eco*RI and *Kpn*I). Multipoint-LOD-score analysis was performed in two families (F49 and F51) that have typical features of MEN1 and in which mutations are missing. Linkage to the *MEN1* locus was confirmed in both families, with LOD scores of 4.99 (for F49) and 2.96 (for F51). Family F52 showed evidence against linkage to 11q13 (LOD score −2.12), suggesting either genetic heterogeneity (i.e., the identification of a rare MEN1 family unlinked to 11q13) or the occurrence of phenocopies. In family F52 the proband is affected by multifocal pancreatic endocrine tumors, whereas his sister has a single parathyroid adenoma. The three sons of the proband share the same MEN1 paternal haplotype but have no biological/clinical signs of the disease at age >30 years. As shown in table 3, of 12 families with atypical clinical features and missing *MEN1* mutations, 7 (58%) showed intrafamilial associations of HPT and PIT: one of them (i.e., A14) had a related case affected by adrenal hyperplasia responsible for Cushing syndrome, two (16%) were characterized by isolated occurrence of HPT and PET, two (16%) were characterized by isolated occurrence of either HPT or ADR, and one (A19) (8%) showed two related cases of isolated PET.

No *MEN1* mutation was found in one family with FIHPT (A8) (table 3). A LOD score of 0.6 in this kindred did not provide a clear conclusion in favor of linkage to *MEN1*. This FIHPT family includes three patients with primary HPT, two of whom have been treated surgically for multiglandular disease. A long-term follow-up ($\geqslant$10 years) enables us to exclude any other endocrine abnormalities. Four children of age 4–19 years share the family MEN1 haplotype defined in the three affected patients but showed no biochemical abnormalities in a recent screening.

Discussion

Location and Diversity of MEN1 Germ-Line Mutations

In this study, we have screened 54 MEN1 families, 11 isolated MEN1 patients, and 19 atypical MEN1-related cases, for germ-line mutations in the *MEN1* gene. We

Table 3

Clinical Description of Families without Identified SCG2 Mutations

Clinical-Features Category and Family/Patient	Clinical Feature(s)	Associated Lesions	Comments	LOD Score[a]
Typical of MEN1:				
F48	HPT, PIT	ADR, breast cancer	ZES to be confirmed	ND
F49	HPT, PET, PIT	CNS	Probable consanguinity	4.99
F50	HPT, PET/ZES			ND
F51	HPT, PET, PIT			2.96
F52	HPT, PET			−2.11
F53	HPT, PIT	Thyroid cancer	Familial mental defect	ND
F54	HPT, PIT		Familial renal lithiasis	ND
I10	HPT, PET, PIT	PET-meta	Family history unknown	ND
I11	HPT, PET, PIT			ND
Atypical of MEN1:[b]				
A7	PIT	ADR		ND
A8	HPT	FIHPT		.60
A9	HPT, PIT	Parathyroid cancer	Pituitary macro-adenoma	ND
A10	PIT	ADR (Conn adenoma)		ND
A11	HPT, PIT			ND
A12	HPT, PIT	Cushing syndrome		ND
A13	HPT, PIT		Parathyroid single adenoma	ND
A14	HPT, PIT			ND
A15	HPT, PET			ND
A16	HPT, PET			ND
A17	PET		ZES, glucagonoma, and ectopic secretion of ACTH	ND
A18	HPT, PIT			ND
A19	HPT, PIT			ND

NOTE.—Linkage analysis was performed with 11q13 polymorphic markers—D11S480, D11S1883, PYGM(caga), D11S449, D11S1889, and D11S913 (from centromere to telomere).

[a] Posterior probability that family shows linkage to 11q13, as determined by the VITESSE program (version 1.0). NA = not applicable.

[b] Includes isolated lesions.

have identified 52 distinct mutations in a total of 62 *MEN1* germ-line alterations observed in this series. As with regard to the previous study of *BRCA1* and *BRCA2* in our laboratory (Serova et al. 1997), HD screening was used as a first screening method and was shown to be highly sensitive. The main characteristics of mutations were comparable to those found in previous reports (Argawal et al. 1997; Lemmens et al. 1997; Mayr et al. 1997; Shimizu et al. 1997; Bassett et al. 1998) (table 1 and fig. 3). In the present report, most (35/52) germ-line *MEN1* alterations described—including 10/52 nonsense mutations and 25/52 frameshifts, or deletions, or insertions—are likely to result in a premature translation stop and in functional loss of the menin protein. The pathogenic effect of these mutations could be related to the presence of at least two nuclear localization signals (NLS), NLS-1 (codons 479–497) and NLS-2 (codons 588–608), in the C-terminal region of the *MEN1*-encoded protein, which recently has been demonstrated to be located within the nucleus (Guru et al. 1998). Deletion constructs of an enhanced green-fluorescent protein-tagged menin-fusion protein suggest that loss of NLS and subsequent instability of menin could be one of the critical events in MEN1 pathogenesis. Of 35 nonsense and frameshift mutations identified in our study, 27 would result in a truncated menin lacking both NLS-1 and NLS-2, and 8 NS/FS occur after codon 497 and delete only the putative NLS-2.

Eight missense point mutations and five in-frame deletions were detected in exons 3, 4, and 7–10 and seemed not to be restricted to specific regions, thus confirming previous reports (Argawal et al. 1997; Bassett et al. 1998). All missense mutations, together with all in-frame deletions/insertions except one (1384delAGG), when analyzed with the programs predicting the secondary structure of the protein (Predict Protein; SOPMA), appeared to be located either inside or on the border of the predicted helix structures. This observation suggests that these seemingly dispersed structures could be of significance for the MEN1-protein function. However, none of these amino acid substitutions affect the putative NLS, suggesting that other functional domains in menin account for MEN1 pathogenesis.

Four mutations were observed at exon/intron boundaries and occurred at splice-junction sites. For one of them (893+1 G→T in intron 4), we were able to demonstrate a complete in-frame deletion of exon 4 (fig. 2). The mechanism involved in such an exon skipping cor-

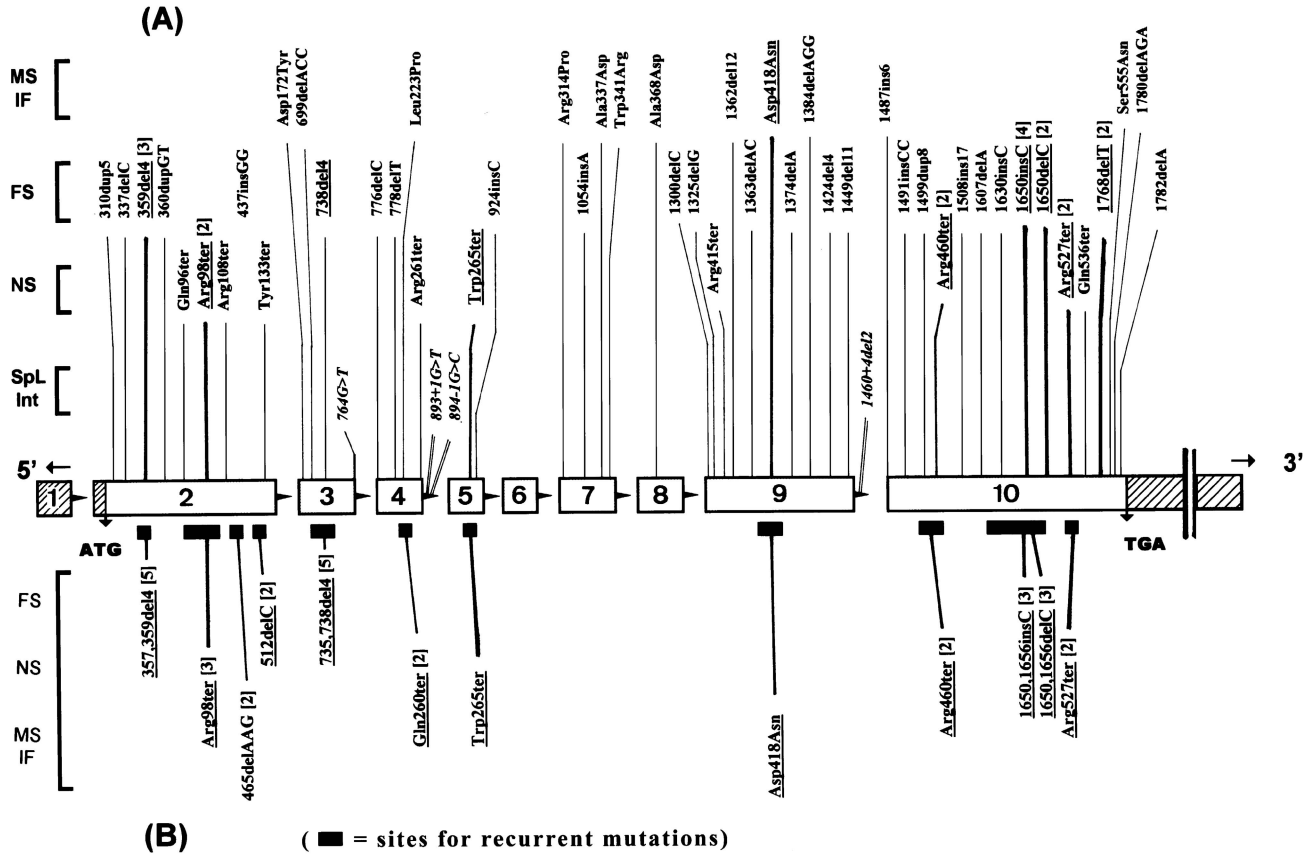

Figure 3 Localization of *MEN1* germ-line mutations, from exon 2 to exon 10. Mutation nomenclature is the same as that in table 1. Exon 1 and the distal part of exon 10 (*diagonally hatched boxes*) are untranslated regions of the gene, and the coding region is denoted by nonhatched boxes. The start (ATG) and stop (TGA) sites were localized within exons 2 and 10, respectively. Broken lines represent introns and the distal part of exon 10 and are not drawn to scale. Mutations are classified as FS, NS, and MS/IF. The splice junction and intronic mutations are in italics and are denoted as "SpL/Int." *A,* Mutations identified in the present study. *B,* Recurrent mutations identified by our own and other groups (Argawal et al. 1997; Mayr et al. 1997; Shimizu et al. 1997; Bassett et al. 1998). The sites for recurrent mutations (i.e., hot spots) in the *MEN1* coding sequence are denoted by blackened boxes under the gene structure. Numbers in square brackets after the mutations denote the number of patients reported for that mutation.

responds to a consequence frequently observed when one of the splicing sites of an internal exon is affected (Maquat 1996).

Most mutations occurred once, whereas some of them were observed in more than one family. When a common ancestor is excluded by haplotyping analysis, these recurrent mutations might be accounted for by mutational hot spots in the *MEN1* gene. Our data, added to those from other groups, show >10 recurrent mutations; 7 of them (359del4, Arg98ter, 738del4, Arg260ter, 1650insC, 1650delC, and Arg527ter) have been identified at least four (and as many as eight) times among 160 mutations described so far (fig. 3). Recurrent frameshift insertions or deletions occur in a repetitive motif (CCCCCCCCG) starting at position 1650 in exon 10. These mutations might be due to replication errors by slipped-strand mispairing (Weitzmann et al. 1997). Similar mechanism have been suggested for 359del4 and 738del4, in exons 2 and 3, respectively (Bassett et al. 1998). Taken together, our data and those from other groups indicate the occurrence of multiple and independent disease-causing mutations in the *MEN1* gene. Unlike the results with regard to MEN1 families in Newfoundland (Petty et al. 1994; Olufemi et al. 1998), no founder effect could be found in MEN1, for the majority of recurrent mutations. Similar observations has been made elsewhere, in two independent linkage-disequilibrium studies—one with 10 Japanese MEN1 families (Sakurai et al. 1996) and one with 96 European MEN1 families (The European Consortium on MEN1 1997).

Genotype-Phenotype Correlations

No genotype-phenotype correlations were found by comparison of families having the different types of mutations (table 1 and fig. 3). In our study, the four un-

related families and one isolated patient with missense mutations were characterized by the complete MEN1 phenotype, with two or more major lesions (HPT and PET, with or without PIT). Clinical analysis of five families (F11, F24, F27, F30, and F47) with in-frame deletions and of the one family (F16) with complete in-frame skipping of exon 4 revealed no particular disease expression. The occurrence of frequently associated lesions such as adrenocortical tumors was observed in families with both early- and late-truncating mutations (in exons 2, 9, and 10).

Previously we had published an extensive clinical characterization of the family with the Arg415ter mutation (in exon 9). In this family (F23), patients with highly malignant thymic carcinoids were observed, and a medullary ependymoma occurred in a 26-year-old gene carrier (Giraud et al. 1997). Of five families with mutations leading to truncations in the same region (exon 9), two showed particularly aggressive phenotypes, one with malignant neuroendocrine tumors and the second with Cushing syndrome related to malignant ADR, a compressive pituitary-tumoral process associated with a meningioma.

These results are in agreement with those from previous studies (Argawal et al. 1997; Bassett et al. 1998) and suggest the absence of a clear relationship between the predicted size of the truncated MEN1 protein and the clinical expression/severity of the disease. Nevertheless, interpretation of genotype-phenotype correlations could be biased by several factors, including (1) different clinical follow-up in independent medical centers, (2) lack of information in some families, (3) age-related penetrance of MEN1, and/or (4) environmental and socioeconomic factors that could affect the clinical expression of the disease among gene carriers.

Clinical States in the Absence of MEN1 Mutation

We failed to find *MEN1* mutations in seven familial MEN 1 patients and two isolated MEN1 patients. In cases of families F49 and F51, linked to 11q13 (with LOD scores 4.99 and 2.96, respectively), it is likely that a mutation in the *MEN1* gene has been missed. It therefore is possible that mutations responsible for the disease in these families occur outside the *MEN1* sequence screened in this study. Another possibility is a large germline deletion, either within or including the MEN1 gene, that escapes detection by PCR-based sequencing. In Southern-blot analysis of MEN1 patients without an identified *MEN1* mutation, we failed to detect any abnormalities, which suggests that such a deletion could be rare and/or infrequently detected by gene-dosage techniques such as quantitative PCR, as recently has been shown elsewhere (Kishi et al. 1998). Family F52 showed a negative LOD score with 11q13 markers. This finding might reflect either genetic heterogeneity of the MEN1 syndrome or the presence of phenocopies in this family. Phenocopies have to be considered carefully in MEN1, mainly because of the high frequency of common lesions such as pHPT and prolactinoma in the non-MEN1 population (Teh et al. 1996*b;* Stock et al. 1997). Frequently encountered MEN1 manifestations in families and patients without *MEN1* mutations were primary HPT and pituitary lesions (table 3). Elsewhere, hereditary hyperparathyroidism and prolactinoma has been described as a genetic variant of MEN1, so called $MEN1_{Burin}$ (Petty et al. 1994), but other recent studies have suggested that some families with unusual features of the syndrome and frequent expression of pituitary tumors do not shown linkage to 11q13 (Stock et al. 1997). Genetic heterogeneity in inherited endocrine tumors could be another explanation for MEN1 manifestations in families without *MEN1* germ-line mutations.

No *MEN1* germ-line mutation was found in a case of FIHPT (table 3). Similar observations in five unrelated FIHPT families have been published elsewhere (Argawal et al. 1997). The absence of *MEN1* mutations could be assessed on the basis of the fact that, in our FIHPT case, MEN1 was excluded by a careful long-term ($\geqslant$10 years) follow-up of all three probands. It cannot be ruled out that some cases of FIHPT are allelic variants of MEN1. On the other hand, FIHPT has been described elsewhere as a feature of at least two independent genetic syndromes, named "HPRT-1" (familial hyperparathyroidism) and "HPRT-2" (HPT–jaw-tumor syndromes), which are characterized by recurrent parathyroid adenoma and an increased risk of parathyroid carcinoma (Wassif et al. 1993; Teh et al. 1996*a*). The FIHPT family that we studied did not show any of the associated lesions, such as jaw tumors and/or renal cancers, that elsewhere had been described in HPRT-2. Further studies will be necessary to establish the incidence of various forms of FIHPT.

Conclusions

Germ-line mutation analysis of the *MEN1* gene is now possible in families predisposed to MEN1 and, in the majority of MEN1 families and isolated cases, will be a useful tool for both earlier detection of MEN1-related tumors and appropriate genetic counseling. Further clinical issues concern the frequency of germ-line *MEN1* mutations among patients with a single endocrine lesion, a situation that appears to be the most common in clinical practice (Sheperd 1991). *MEN1* inactivation also has been suggested as a primary pathogenic event in a significant proportion of sporadic endocrine tumors. This has been demonstrated in 20%–30% of sporadic parathyroid tumors (Heppner et al. 1997), 17%–33% of insulinomas/gastrinomas (Zhuang et al. 1997*b;* Hessmann et al. 1998), and 36% of lung carcinoids (Debelenko et al. 1997). On the other hand, inactivation of

MEN1 does not appear to play a prominent role in sporadic pituitary tumors (Zhuang et al. 1997*a;* Prezant et al. 1998). The type and location of *MEN1* mutations identified in sporadic tumors were similar to those of germ-line mutations and thus are supposed to have similar functional consequences at the somatic level. Our findings, added to those from other groups, point out the diversity of *MEN1* mutations identified to date. Further progress on the *MEN1* gene promotor(s) and regulatory sequences, as well as functional studies of the *MEN1* gene together with selected mutations, will be critical steps for a better understanding of its role in endocrine tumoral pathogenesis.

Acknowledgments

We are indebted to patients and families for their contribution to the genetic diagnosis program, through the GENEM clinical network. We thank Mrs. Corinne Montvernay and Colette Bonnardel for helpful epidemiological assistance. Clinical and genetic studies in France were supported by Institut National de la Santé et de la Recherche Médicale contract 494017 and by Ministère de la Santé et des Affaires Sociales and Hospices Civils de Lyon fund PHRC 95-030. We thank our colleagues of the GENEM clinical network who provided blood samples and clinical informations. N.B. was subsequently supported by contract PHRC 95-030 and a special training–award fellowship from the World Health Organization International Agency for Research on Cancer (Lyon). This paper is dedicated to the memory of Dr. Patrick Hamon (Unit of Endocrinology, Centre Hospitalier, Chambéry, France).

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for MEN1)

Genome Database (GDB), http://www.gdb.org/gdb-bin/genera/hgd/DBObject/GDB:1201723 (for MEN1 mutations [accession numbers 9837808, 9837809, 9837813–9837820, 9837822, 9837823, 9837825–9837831, 9837967–9837978, 9837981–9837994, 9837997, 9838200, and 9838201]).

References

Argawal SK, Kester MB, Debelenko LV, Heppner C, Emmert-Buck MR, Skarulis MC, Doppman JL, et al (1997) Germline mutations of the MEN1 gene in familial multiple endocrine neoplasia type 1 and related states. Hum Mol Genet 6: 1169–1175

Bassett JHD, Forbes SA, Pannett AAJ, Lloyd SE, Christie PT, Wooding C, Harding B, et al (1998) Characterization of mutations in patients with multiple endocrine neoplasia type 1. Am J Hum Genet 62:232–244

Byström C, Larsson C, Blomberg C, Sandelin K, Falkmer U, Skogseid B, Oberg K, et al (1990) Localization of the MEN1 gene to a small region within chromosome 11q13 by deletion mapping in tumors. Proc Natl Acad Sci USA 87: 1968–1972

Calender A, Giraud S, Cougard P, Chanson P, Lenoir GM, Murat A, Hamon P, et al (1995) Multiple endocrine neoplasia type 1 in France: clinical and genetic studies. J Intern Med 238:263–268

Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, et al (1997) Positional cloning of the gene for multiple endocrine neoplasia type 1. Science 276:404–407

Chanson P, Cadiot G, Murat A, and the GENEM 1 (1997) Management of patients and subjects at risk for multiple endocrine neoplasia type 1: MEN1. Horm Res 47:211–220

Courseaux A, Grosgeorge J, Gaudray P, Pannett AA, Forbes SA, Williamson C, Bassett D, et al (1996) Definition of the minimal MEN1 candidate area based on a 5-Mb integrated map of proximal 11q13: the European Consortium on Men1 (GENEM 1: Groupe d'Étude des Neoplasies Endocriniennes Multiples de type 1). Genomics 37:354–365

Darling TN, Skarulis MC, Steinberg SM, Marx SJ, Spiegel AM, Turner ML (1997) Multiple facial angiofibromas and collagenomas in patients with multiple endocrine neoplasia type 1. Arch Dermatol 133:853–857

Debelenko LV, Brambilla E, Argawal SK, Swalwell JI, Kester MB, Lubensky IA, Zhuang ZP, et al (1997) Identification of *MEN1* gene mutations in sporadic carcinoid of the lung. Hum Mol Genet 6:2285–2290

European Consortium on MEN1, The (1997) Linkage disequilibrium studies in multiple endocrine neoplasia type 1 (MEN1). Hum Genet 100:657–665

Friedman E, Sakaguchi K, Bale AE, Falchetti A, Streeten E, Zimering MB, Weinstein LS, et al (1989) Clonality of parathyroid tumors in familial multiple endocrine neoplasia type 1. N Engl J Med 321:213–218

Giraud S, Choplin H, Teh BT, Lespinasse J, Jouvet A, Labat-Moleur F, Lenoir GM, et al (1997) A large multiple endocrine neoplasia type 1 family with clinical expression suggestive of anticipation. J Clin Endocrinol Metab 82: 3487–3492

Guru SC, Goldsmith PK, Burns AL, Marx SJ, Spiegel AM, Collins FS, Chandrasekharappa SC (1998) Menin, the product of the MEN1 gene, is a nuclear protein. Proc Natl Acad Sci USA 95:1630–1634

Heppner C, Kester MB, Argawal SK, Debelenko LV, Emmert-Buck MR, Guru SC, Manickam P, et al (1997) Somatic mutation of the MEN1 gene in parathyroid tumors. Nat Genet 16:375–378

Hessman O, Lindberg D, Skogseid B, Carling T, Hellman P, Rastad J, Akerström G, et al (1998) Mutation of the multiple endocrine neoplasia type 1 gene in nonfamilial, malignant tumors of the endocrine pancreas. Cancer Res 58:377–379

Kato H, Uchimura I, Morohoshi M, Fujisawa K, Kobayashi Y, Numano F, Goseki N, et al (1996) Multiple endocrine neoplasia type 1 associated with spinal ependymoma. Intern Med 35:285–289

Kishi M, Tsukada T, Shimizu S, Futami H, Ito Y, Kanbe M, Obara T, et al (1998) A large germline deletion of the *MEN1*

gene in a family with multiple endocrine neoplasia type 1. Jpn J Cancer Res 89:1–5

Knudson AG Jr (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 68:820–823

Larsson C, Calender A, Grimmond S, Giraud S, Hayward NK, Teh BT, Farnebo F (1995) Molecular tools for presymptomatic testing in multiple endocrine neoplasia type 1. J Intern Med 238:239–244

Larsson C, Skogseid B, Oberg K, Nakamura Y, Nordenskjöld M (1988) Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. Nature 332: 85–87

Lemmens I, Van de Ven WJ, Kas K, Zhang CX, Giraud S, Wautot V, Buisson N, et al (1997) Identification of the multiple endocrine neoplasia type 1 (MEN1) gene: the European Consortium on MEN1. Hum Mol Genet 6:1177–1183

Maquat LE (1996) Defects in RNA splicing and the consequence of shortened translational reading frames. Am J Hum Genet 59:279–286

Mayr B, Apenberg S, Kothämel T, von zur Mühlen A, Brabant G (1997) *Menin* mutations in patients with multiple endocrine neoplasia type 1. Eur J Endocrinol 137:684–687

Metz DC, Jensen RT, Bale AE, Skarulis MC, Eastman RC, Nieman L, Norton JA, et al (1994) Multiple endocrine neoplasia type 1: clinical features and management. In: Bilezikian JP, Levine MA, Marcus R (eds) The parathyroids. Raven Press, New York, pp 591–645

O'Connell JR, Weeks DE (1995) The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recording and fuzzy inheritance. Nat Genet 11:402–408

Olufemi SE, Green JS, Manickam P, Guru SC, Agarwal SK, Kester MB, Burns AL, et al (1998) Common ancestral mutation in the *MEN1* gene is likely responsible for the prolactinoma variant of MEN1 (MEN1 Burin) in four kindreds from Newfoundland. Hum Mutat 11:264–269

Petty EM, Green JS, Marx SJ, Taggart RT, Farid N, Bale AE (1994) Mapping the gene for hereditary hyperparathyroidism and prolactinoma ($MEN1_{Burin}$) to chromosome 11q: evidence for a founder effect in patients from Newfoundland. Am J Hum Genet 54:1060–1066

Pollak MR, Brown EM, Chou YH, Hebert SC, Marx SJ, Steinmann B, Levi T, et al (1993) Mutations in the human Ca(2+)-sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Cell 75:1297–1303

Prezant TR, Levine J, Melmed S (1998) Molecular characterization of the *MEN1* tumor suppressor gene in sporadic pituitary tumors. J Clin Endocrinol Metab 83:1388–1391

Radford DM, Ashley SW, Wells SA Jr, Gerhard DS (1990) Loss of heterozygosity of markers on chromosome 11 in tumors from patients with multiple endocrine neoplasia syndrome type 1. Cancer Res 50:6529–6533

Sakurai A, Katai M, ltakura Y, Nakajima K, Baba K, Hashizume K (1996) Genetic screening in hereditary multiple endocrine neoplasia type 1: absence of a founder effect among Japanese families. Jpn J Cancer Res 87:985–994

Sambrook J, Fritsch EF, Maniatis T (1989) Extraction, purification and analysis of messenger RNA in eucaryotic cells. In: Molecular cloning, a laboratory manual, 2d. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 6–26

Serova OM, Mazoyer S, Puget N, Dubois V, Tonin P, Shugart YY, Goldgar D, et al (1997) Mutations in BRCA1 and BRCA2 in breast cancer families: are there more breast-cancer susceptibility genes? Am J Hum Genet 60:486–495

Sheperd JJ (1991) The natural history of multiple endocrine neoplasia type 1: highly uncommon or highly unrecognized? Arch Surg 126:935–952

Shimizu S, Tsukada T, Futami H, Ui K, Kameya T, Kawanaka M, Uchiyama S, et al (1997) Germline mutations of the MEN1 gene in Japanese kindred with multiple endocrine neoplasia type 1. Jpn J Cancer Res 88:1029–1032

Skogseid B, Oberg K (1995*a*) Adrenal lesion in multiple endocrine neoplasia type 1. Surgery 118:1077–1082

Skogseid B, Oberg K (1995*b*) Experience with multiple endocrine neoplasia type 1 screening. J Intern Med 238: 255–261

Stock JL, Warth MR, Teh BT, Coderre JA, Overdorf JH, Baumann G, Hintz RL, et al (1997) A kindred with a variant of multiple endocrine neoplasia type 1 demonstrating frequent expression of pituitary tumors but not linked to the multiple endocrine neoplasia type 1 locus at chromosome region 11q13. J Clin Endocrinol Metab 82:486–492

Teh BT, Farnebo F, Kristofferson U, Sundelin B, Cardinal J, Axelson R, Yap A, et al (1996*a*) Autosomal dominant primary hyperparathyroidism and jaw tumor syndrome associated with renal hamartomas and cystic kidney disease: linkage to 1q21-q32 and loss of the wild type allele in renal hamartomas. J Clin Endocrinol Metab 81:4204–4211

Teh BT, McArdle J, Parameswaran V, David R, Larsson C, Sheperd J (1996*b*) Sporadic primary hyperparathyroidism in the setting of multiple endocrine neoplasia type 1. Arch Surg 131:1230–1232

Trump D, Farren B, Wooding C, Pang JT, Besser GM, Buchanan KD, Edwards CR, et al (1996) Clinical studies of multiple endocrine neoplasia type 1. QJM 89:653–669

Wassif WS, Moniz CF, Friedman E, Wong S, Weber G, Nordenskjöld M, Peters TJ, et al (1993) Familial isolated hyperparathyroidism: a distinct genetic entity with an increased risk of parathyroid cancer. J Clin Endocrinol Metab 77:1485–1489

Weitzmann MN, Woodford KJ, Usdin K (1997) DNA secondary structures and the evolution of hypervariable tandem arrays. J Biol Chem 272:9517–9523

Wermer P (1954) Genetic aspects of adenomatosis of endocrine glands. Am J Med 16:363–375

Zhuang Z, Ezzat SZ, Vortmeyer AO, Weil R, Oldfield EH, Park WS, Pack S, et al (1997*a*) Mutations of the MEN1 tumor suppressor gene in pituitary tumors. Cancer Res 57: 5446–5451

Zhuang Z, Vortmeyer AO, Pack S, Huang S, Pham TA, Wang C, Park WS, et al (1997*b*) Somatic mutations of the MEN1 tumor suppressor gene in sporadic gastrinomas and insulinomas. Cancer Res 57:4682–4686