

MUTATION UPDATE

Multiple Endocrine Neoplasia Type 1 (MEN1): Analysis of 1336 Mutations Reported in the First Decade Following Identification of the Gene

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by the occurrence of tumors of the parathyroids, pancreas, and anterior pituitary. The *MEN1* gene, which was identified in 1997, consists of 10 exons that encode a 610–amino acid protein referred to as menin. Menin is predominantly a nuclear protein that has roles in transcriptional regulation, genome stability, cell division, and proliferation. Germline mutations usually result in MEN1 or occasionally in an allelic variant referred to as familial isolated hyperparathyroidism (FIHP). MEN1 tumors frequently have loss of heterozygosity (LOH) of the *MEN1* locus, which is consistent with a tumor suppressor role of *MEN1*. Furthermore, somatic abnormalities of *MEN1* have been reported in MEN1 and non-MEN1 endocrine tumors. The clinical aspects and molecular genetics of *MEN1* are reviewed together with the reported 1,336 mutations. The majority (>70%) of these mutations are predicted to lead to truncated forms of menin. The mutations are scattered throughout the >9-kb genomic sequence of the *MEN1* gene. Four, which consist of c.249_252delGTCT (deletion at codons 83–84), c.1546_1547insC (insertion at codon 516), c.1378C>T (Arg460Ter), and c.628_631delACAG (deletion at codons 210–211) have been reported to occur frequently in 4.5%, 2.7%, 2.6%, and 2.5% of families, respectively. However, a comparison of the clinical features in patients and their families with the same mutations reveals an absence of phenotype–genotype correlations. The majority of *MEN1* mutations are likely to disrupt the interactions of menin with other proteins and thereby alter critical events in cell cycle regulation and proliferation. *Hum Mutat* 29(1), 22–32, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: multiple endocrine neoplasia type 1; MEN1; FIHP; tumor; somatic mutation; germline

INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1; MIM# 131100) is characterized by the combined occurrence of tumors of the parathyroid glands, pancreatic islet cells, and anterior pituitary gland [Wermer, 1954; Thakker, 2006]. Some patients may also develop adrenal cortical tumors, carcinoid tumors, facial angiofibromas, collagenomas, and lipomas. MEN1 is inherited as an autosomal dominant disorder with a high degree of penetrance, such that >95% of patients develop clinical manifestations of the disorder by the fifth decade [Benson et al., 1987; Calender et al., 1995; Trump et al., 1996; Marx et al., 1998]. The earliest age at which manifestations of MEN1 may occur has been reported to be five years [Stratakis et al., 2000]. Parathyroid tumors, which lead to hypercalcemia, are the most common feature of MEN1 and occur in about 95% of patients [Benson et al., 1987; Calender et al., 1995; Trump et al., 1996; Marx et al., 1998]. Pancreatic islet cell tumors, which consist of gastrinomas, insulinomas, pancreatic polypeptidomas (PPomas), glucagonomas, and vasoactive intestinal polypeptidomas (VIPomas) occur in about 40% of patients; and anterior pituitary tumors, which consist of prolactinomas, somatotrophinomas, corticotrophinomas, or nonfunctioning adenomas, occur in about 30% of patients [Benson et al., 1987;

Calender et al., 1995; Trump et al., 1996; Marx et al., 1998]. The clinical manifestations of MEN1 are generally related to their products of secretion and less frequently to their primary sites or metastasis. In the absence of treatment, MEN1 tumors result in an earlier mortality in patients [Wilkinson et al., 1993; Burgess et al., 1998; Doherty et al., 1998].

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Genetic mapping studies that investigated pancreatic and parathyroid tumor DNA for loss of heterozygosity (LOH) and segregation studies of families with MEN1 indicated that the *MEN1* gene was likely to represent a tumor-suppressor gene located on chromosome 11q13 [Larsson et al., 1988; Friedman et al., 1989; Thakker et al., 1989]. The *MEN1* gene, which was identified in 1997 [Chandrasekharappa et al., 1997; European Consortium on MEN1, 1997], consists of 10 exons (Fig. 1) that span approximately >9 kb of genomic DNA and encodes a 610-amino acid protein referred to as menin. The main transcript of the *MEN1* gene is a 2.8-kb mRNA. However, at least six alternative transcripts have been reported with variations in their

content of the 5'-untranslated region; none of these affect the coding region [Khodaei-O'Brien et al., 2000]. One additional very rare variant that would result in an elongation of the reading frame by 15 bases at the exon 2–intron 2 junction has also been reported [Chandrasekharappa and Teh, 2003]. Menin, which is ubiquitously expressed, is predominantly a nuclear protein in nondividing cells [Guru et al., 1998], but in dividing cells it is found mainly in the cytoplasm [Huang et al., 1999]. Menin has at least three nuclear localization signals (NLSs) [La et al., 2006]. The truncated *MEN1* proteins that would result from the nonsense and frameshift mutations, if expressed, would lack at least one of these nuclear localization signals (Fig. 1). Menin has been shown

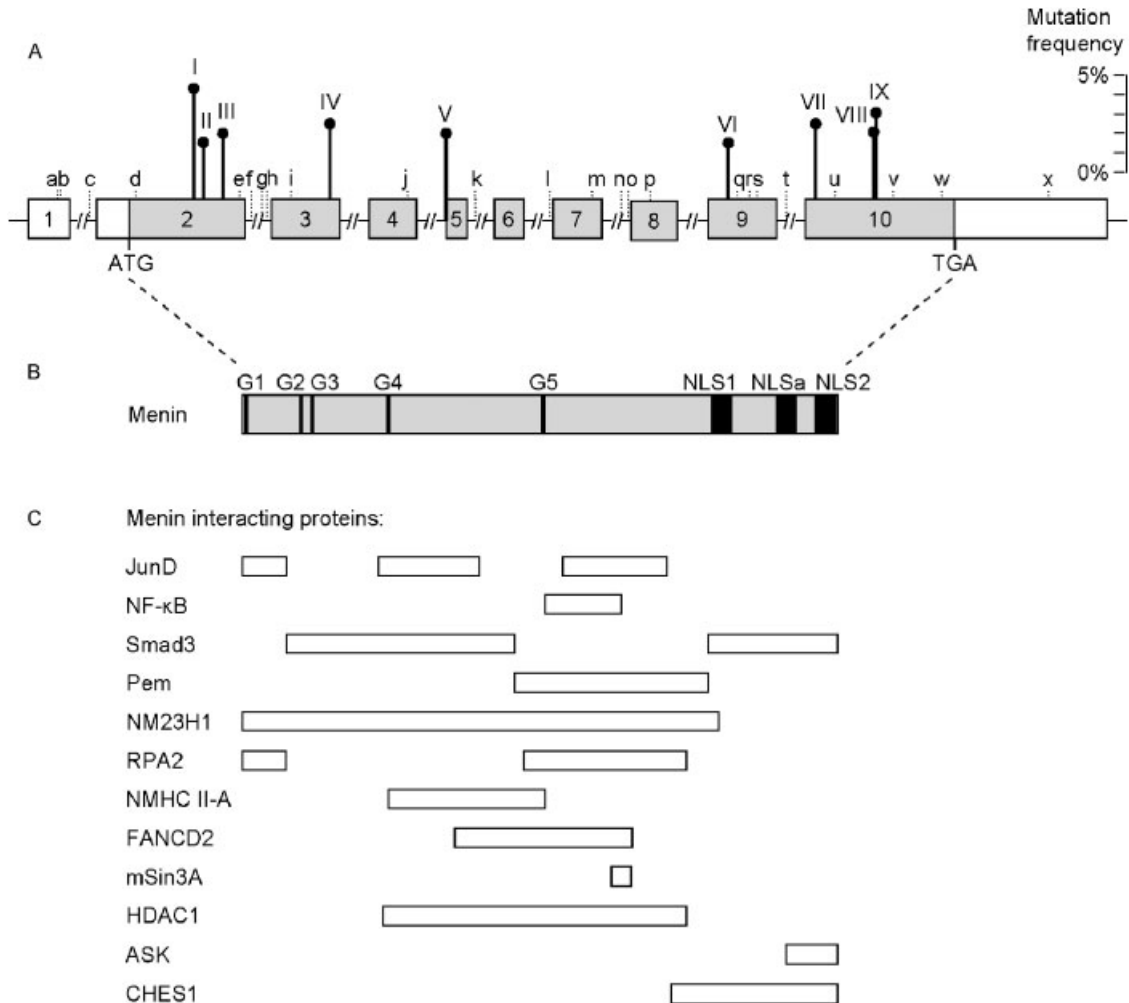


FIGURE 1. Schematic representation of the genomic organization of the *MEN1* gene, its encoded protein (menin) and regions that interact with other proteins. **A:** The human *MEN1* gene consists of 10 exons that span more than 9 kb of genomic DNA and encodes a 610-amino acid protein. The 1.83-kb coding region (indicated by shaded region) is organized into nine exons (exons 2–10) and eight introns (indicated by a line but not to scale). The sizes of the exons (boxes) range from 41–1,297 bp, and that of the introns range from 80–1,564 bp. The start (ATG) and stop (TGA) codons in exons 2 and 10, respectively, are indicated. Exon 1, the 5' part of exon 2, and the 3' part of exon 10 are untranslated (indicated by open boxes). The promoter region is located within a few 100 bp upstream of exon 2. The sites of the nine germline mutations (I–IX) that occur with a frequency >1.5% (Table 2) are shown and their respective frequencies (scale shown on the right) are indicated by the vertical lines above the gene. These germline mutations, which collectively represent 20.6% of all reported germline mutations, are: I, c.249_252delGTCT; II, c.292C > T; III, c.358_360delAAG; IV, c.628_631delACAG; V, c.784–9G > A; VI, c.1243C > T; VII, c.1378C > T; VIII, c.1546delC; and IX, c.1546_1547insC. The locations of the 24 polymorphisms (a–x; Table 3) are illustrated. **B:** Menin has three NLSs at codons 479–497 (NLS1), 546–572 (NLSa), and 588–608 (NLS2), indicated by closed boxes, and five putative guanosine triphosphatase (GTPase) sites (G1–G5) indicated by closed bars. **C:** Menin regions that have been implicated in the binding to different interacting proteins are indicated by open boxes. These are JunD (codons 1–40, 139–242, and 323–428); nuclear factor-kappa B (NF-κB) (codons 305–381); Smad3 (codons 40–278, and 477–610); placenta and embryonic expression, Pem (codons 278–476); NM23H1 (codons 1–486); a subunit of replication protein A (RPA2) (codons 1–40, and 286–448); NMHC II-A (codons 154–306); FANCD2 (codons 219–395); mSin3A (codons 371–387); HDAC1 (codons 145–450); ASK (codons 558–610); and CHES1 (codons 428–610). The regions of menin that interact with GFAP, vimentin, Smad 1/5, Runx2, MLL-histone methyltransferase complex, and estrogen receptor-alpha remain to be determined.

to interact with a number of proteins [Agarwal et al., 2005; Balogh et al., 2006] that are involved in transcriptional regulation, genome stability, cell division and proliferation (Fig. 1; Table 1). This review will focus on the germline *MEN1* mutations that have been reported in patients with MEN1 and other hereditary endocrine disorders, together with the somatic abnormalities and mutations reported in MEN1 tumors and sporadic non-MEN1 tumors.

MUTATIONS AND POLYMORPHISMS

A database of the published *MEN1* mutations, since the identification of the gene in 1997, was constructed by searching the NCBI PubMed literature database for articles in English, using the keywords *MEN1* and *Mutation*. A total of 159 articles reporting 1,336 *MEN1* sequence abnormalities (1,133 germline and 203 somatic mutations) were identified and evaluated. Sequence changes described as polymorphisms occurring in over 1% of control alleles were also assessed. By using the standard recommended nomenclature [den Dunnen and Antonarakis, 2001], we observed that some mutations that had been described as novel, were instead previously reported sequence changes and this was particularly the case for those occurring at repetitive

sequences. In addition, for some other abnormalities, it was not possible to determine the exact nucleotide change, due to insufficient data in the report. Examples of this included insertions in which the exact nucleotide sequences were not specified and nonsense mutations such as Trp198Ter (Supplementary Table S1; available online at <http://www.interscience.wiley.com/jpages/1059-7794/suppmat>), which could either result from a change of the triplet TGG to TGA, or to TAG [Agarwal et al., 1997; Hamaguchi et al., 1999]. The proportion of such undetermined nucleotide changes, due to insufficient data, was 6%. However, these were included in our analysis of the total of 1,133 independent *MEN1* germline mutations, which consisted of 459 different germline mutations (Supplementary Table S1), and 203 somatic mutations, which consisted of 167 different mutations. A total of 61 of the germline mutations were also found to occur as somatic mutations, thus yielding 565 different *MEN1* mutations.

The 1,133 germline mutations (Supplementary Table S1) were scattered throughout the entire 1,830-bp coding region and splice-sites of the *MEN1* gene, and consisted of 23% nonsense mutations, 9% splice site mutations, 41% frameshift deletions or insertions, 6% in-frame deletions or insertions, 20% missense mutations, and 1% whole or partial gene deletions. Several mutations were found to recur in apparently unrelated kindreds, thereby indicating potential mutational hot spots (Fig. 1; Table 2). Mutations at four sites accounted for 12.3% of all mutations (c.249_252delGTCT, deletion at codons 83–84; c.1546_1547insC, insertion at codon 516; c.1378C>T (Arg460Ter); and c.628_631delACAG, deletion at codons 210–211). The three deletional or insertional frameshift mutations occurred in repetitive DNA sequences and this is consistent with a replication-slippage model of mutagenesis. A total of 24 different polymorphisms (12 in the coding region [10 synonymous and two nonsynonymous], nine in the introns, and three in the untranslated regions) of the *MEN1* gene have also been reported (Fig. 1; Table 3). It is important to recognize the occurrence of these polymorphisms as they need to be distinguished from mutations when performing analysis for genetic diagnosis, and also because they may occasionally help in segregation analysis in families in whom a *MEN1* mutation has not been identified. The proportion of MEN1 patients without mutations could not be determined in this database analysis because of the underlying bias to report chiefly on identified mutations. However, some studies have reported that between 5% and 10% of MEN1 patients will not harbor mutations in the coding region or adjacent splice sites [Agarwal et al., 1997; Chandrasekharappa et al., 1997; European Consortium on MEN1, 1997; Bassett et al., 1998; Giraud et al., 1998; Teh et al., 1998; Pannett and Thakker, 1999]. Such MEN1

TABLE 1. Functions of Menin Indicated by Direct Interactions With Proteins and Other Molecules

Function	Interacting partner	Reference
Transcription regulation	JunD	Agarwal et al. [1999]
	NFκB (p50, p52, p65)	Heppner et al. [2001]
	Pem	Lemmens et al. [2001]
	Sin3A	Kim et al. [2003]
	HDAC	Kim et al. [2003]
	Smad1	Sowa et al. [2004]
	Smad3	Kaji et al. [2001]
	Smad5	Sowa et al. [2004]
	Runx2	Sowa et al. [2004]
	MLL histone methyl-transferase complex	Hughes et al. [2004]; Yokoyama et al. [2004]
	ER-alpha	Dreijerink et al. [2006]
	CHES1	Busygina et al. [2006]
Genome stability	Double-stranded DNA	La et al. [2004]
	RPA2	Sukhodolets et al. [2003]
Cell division	FANCD2	Jin et al. [2003]
	NMMHC II-A	Obungu et al. [2003]
	GFAP	Lopez-Egido et al. [2002]
Cell cycle control	Vimentin	Lopez-Egido et al. [2002]
	nm23 ^(a)	Ohkura et al. [2001]
	ASK	Schnepp et al. [2004]

^aFunctions reported include involvement in cell cycle withdrawal, decrease of cell motility, cell differentiation, apoptosis, and DNA repair.

TABLE 2. *MEN1* Germline Mutations Occurring in Over 1.5% of Affected Families

Mutation ^a	DNA sequence change ^b	Exon	Codon	Predicted effect	Frequency (%) ^c
I	c.249_252delGTCT	2	83–84	fs	4.5
II	c.292C>T	2	98	ns, Arg98Ter	1.5
III	c.358_360delAAG	2	120	if	1.7
IV	c.628_631delACAG	3	210–211	fs	2.5
V	c.784–9G>A	Intron 4	–	sp	1.9
VI	c.1243C>T	9	415	ns, Arg415Ter	1.5
VII	c.1378C>T	10	460	ns, Arg460Ter	2.6
VIII	c.1546delC	10	516	fs	1.8
IX	c.1546_1547insC	10	516	fs	2.7

^aMutation number as referred to in Fig. 1.

^bMutations are numbered in relation to the *MEN1* cDNA reference sequence (GenBank accession number NM_130799.1), whereby nucleotide +1 corresponds to the A of the ATG-translation initiation codon.

^cFrequencies based on 1,133 reported *MEN1* independent germline mutations.

fs, frameshift mutation; ns, nonsense mutation; sp, splice-site mutation; if, in-frame mutation.

TABLE 3. Polymorphisms of the *MEN1* Gene

Polymorphism ^a	DNA sequence change ^b	Exon	Codon change	Reference ^c	Allele frequency ^d
a	c.-533T>A	1	-	Giraud et al. [1998]	0.32
b	c.-533T>C	1	-	Giraud et al. [1998]	0.12
c	c.-39C>G	Intron 1	-	Giraud et al. [1998]	0.20
d	n/a	2	Leu10Leu	Nord et al. [2000]	n/a
e	c.435C>T	2	Ser145Ser	Agarwal et al. [1997]	0.01
f	c.445+183G>A	Intron 2	-	Sato et al. [2000]	0.05
g	c.446-127A>T	Intron 2	-	Fujii et al. [1999]	n/a
h	c.446-58C>T	Intron 2	-	Agarwal et al. [1997]	0.01
i	c.512G>A	3	Arg171Gln	Chandrasekharappa et al. [1997]	0.01
j	c.768T>C	4	Leu256Leu	Agarwal et al. [1997]	0.01
k	c.824+31T>C	Intron 5	-	Villablanca et al. [2002]	n/a
l	c.913-3C>G	Intron 6	-	Cebrian et al. [2003]	0.02
m	c.1026G>A	7	Ala342Ala	Bergman et al. [2000]	0.01
n	c.1050-92C>T	Intron 7	-	Jager et al. [2006]	0.03
o	c.1050-3C>G	Intron 7	-	Bassett et al. [1998]	0.02
p	c.1101A>C	8	Val367Val	Shan et al. [1998]	n/a
q	c.1254C>T	9	Asp418Asp	Chandrasekharappa et al. [1997]	0.42
r	c.1296G>A	9	Leu432Leu	Chandrasekharappa et al. [1997]	0.01
s	c.1299T>C	9	His433His	Schmidt et al. [1999]	0.01
t	c.1350+103G>C	Intron 9	-	Jager et al. [2006]	0.42
u	c.1434C>T	10	Gly478Gly	Fujii et al. [1999]	n/a
v	c.1621G>A	10	Ala541Thr	Chandrasekharappa et al. [1997]	0.04
w	c.1764G>A	10	Lys588Lys	Miedlich et al. [2000]	n/a
x	c.1833*305_1833*307 delCTC	10	-	Sato et al. [2000]	0.05

^aPolymorphism letter as referred to in Fig. 1.

^bPolymorphisms are numbered in relation to the *MEN1* cDNA reference sequence (GenBank accession number NM_130799.1), whereby nucleotide +1 corresponds to the A of the ATG-translation initiation codon.

^cFirst literature report.

^dFrequency presented in first literature report.

n/a, data not available.

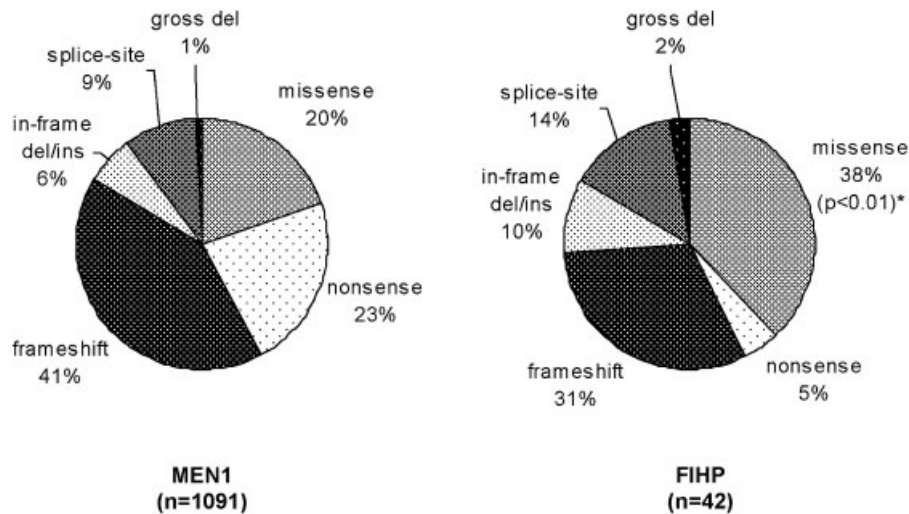


FIGURE 2. Frequencies of the types of *MEN1* mutations reported in 1,091 *MEN1* kindreds and 42 FIHP kindreds. Missense mutations were more frequent in FIHP than in *MEN1* kindreds (chi-squared test, $P < 0.01^*$). del, deletion; ins, insertion.

patients may harbor mutations in the promoter or untranslated regions, or introns, or they may represent phenocopies.

BIOLOGICAL RELEVANCE

MEN1 Mutations in Hereditary Endocrine Disorders

The role of the *MEN1* gene in the etiology of other inherited endocrine disorders, in which either parathyroid or pituitary tumors occur as isolated endocrinopathies, has been investigated by mutational analysis. *MEN1* mutations have been reported in 42 families with isolated hyperparathyroidism (FIHP) and 38% of these are missense mutations that are less likely to result in an

inactivated protein. This contrasts significantly ($P < 0.01$) with the situation in *MEN1* patients in whom 20% are missense mutations (Fig. 2). These observations are consistent with a more likely association between missense mutations and the milder FIHP variant, but it is important to note that the mutations associated with FIHP are also scattered throughout the coding region and not clustered, a situation that is similar to that found for germline *MEN1* mutations. Furthermore, the occurrence of protein-truncating mutations in FIHP patients and particularly deletions, such as the 4 bp deletion involving codons 83–84 (Table 2), which are identical to those observed in *MEN1* patients, makes it difficult to establish an unequivocal phenotype–genotype correlation.

However, the sole occurrence of parathyroid tumors is remarkable in FIHP families that harbor similar *MEN1* mutations as *MEN1* families, and the mechanisms that determine the altered phenotypic expressions of these mutations remain to be elucidated. In addition, nonsense mutations (Tyr312Ter and Arg460-Ter) have been detected in *MEN1* families with the Burin or prolactinoma variant, which is characterized by a high occurrence of prolactinomas and a low occurrence of gastrinomas [Agarwal et al., 1997; Olufemi et al., 1998; Hao et al., 2004]. Furthermore, a splice-site mutation (c.446–3C>G) has been detected in an *MEN1* kindred from Tasmania, in whom there is an absence of somatotrophinomas [Burgess et al., 1996, 2000]. However, some other families with isolated acromegaly do not have abnormalities of the *MEN1* gene [Soares and Frohman, 2004], even though segregation analysis and tumor deletion mapping have indicated that the gene is likely to be located on chromosome 11q13 [Soares and Frohman, 2004; Soares et al., 2005]. Interestingly, mutations of the aryl hydrocarbon receptor interacting protein (*AIP*) gene, which is also located on chromosome 11q13 and 2.7 Mb telomeric to the *MEN1* gene, have been identified in some families with isolated acromegaly [Vierimaa et al., 2006].

***MEN1* Mutations in Sporadic Non-*MEN1* Endocrine Tumors**

Parathyroid, pancreatic islet cell, and anterior pituitary tumors may occur either as part of *MEN1* or more commonly as sporadic, nonfamilial tumors. Tumors from patients with *MEN1* have been observed to harbor the germline mutation together with a somatic LOH involving chromosome 11q13 or point mutations [Pannett and Thakker, 2001], as expected from Knudson's model and the proposed role of the *MEN1* gene as a tumor suppressor. However, LOH involving chromosome 11q13, which is the location of *MEN1*, has also been observed in 5% to 50% of sporadic endocrine tumors, thus implicating the *MEN1* gene in the etiology of these tumors [Bystrom et al., 1990; Thakker et al., 1993]. Somatic *MEN1* mutations have been detected in ~20% of sporadic parathyroid tumors [Heppner et al., 1997; Carling et al., 1998; Farnebo et al., 1998; Shan et al., 1998; Ludwig et al., 1999; Pannett and Thakker, 2001], ~40% of gastrinomas [Zhuang et al., 1997a; Wang et al., 1998; Mailman et al., 1999], ~15% of insulinomas [Zhuang et al., 1997a; Shan et al., 1998; Cupisti et al., 2000], ~60% of VIPomas [Shan et al., 1998; Wang et al., 1998], ~15% of nonfunctioning pancreatic tumors [Hessman et al., 1998], ~60% of glucagonomas [Hessman et al., 1998], <5% of adrenal cortical tumors [Gortz et al., 1999a], ~35% of bronchial carcinoid tumors [Debelenko et al., 1997; Fujii et al., 1999; Gortz et al., 1999b], <5% of anterior pituitary adenomas [Zhuang et al., 1997b; Prezant et al., 1998; Tanaka et al., 1998], ~10% of angiofibromas [Boni et al., 1998], and ~30% of lipomas [Vortmeyer et al., 1998]. These 203 somatic mutations are scattered throughout the 1,830-bp coding region, and 18% are nonsense mutations, 40% are frameshift deletions or insertions, 6% are in-frame deletions or insertions, 7% are splice-site mutations, and 29% are missense mutations. A comparison of the locations of the somatic and germline mutations revealed a higher frequency of somatic mutations in exon 2 (39% (somatic) vs. 23% (germline); $P < 0.001$), but the significance of this observation in the context of the Knudson two-hit hypothesis remains to be elucidated. The tumors harboring a somatic *MEN1* mutation had chromosome 11q13 LOH as the other genetic abnormality, or "hit", consistent with Knudson's hypothesis. These studies indicate that although inactivation of the *MEN1* gene may

have a role in the etiology of some sporadic endocrine tumors, the involvement of other genes, for example, the *GNAS1* gene encoding the G protein-stimulatory alpha subunit [Landis et al., 1989; Lyons et al., 1990; Thakker et al., 1993], with major roles in the etiology of such sporadic endocrine tumors, is highly likely.

Function of the *MEN1* Protein (Menin)

Menin has no homology to any known proteins or sequence motifs that would provide clues as to its function other than the presence of three NLSs in its C-terminal segment. Subcellular localization studies have shown that menin is predominantly a nuclear protein [Guru et al., 1998; La et al., 2006] and studies of protein-protein interactions have revealed that menin interacts with several proteins involved in transcriptional regulation, genome stability, cell division, and proliferation (Fig. 1; Table 1). Thus, in transcriptional regulation, menin has been shown to interact with: the activating protein-1 (AP-1) transcription factor JunD [Agarwal et al., 1999] and to suppress Jun-mediated transcriptional activation [Hirai et al., 1989; Ryder et al., 1989; Pfarr et al., 1994]; members (e.g., p50, p52, and p65) of the nuclear factor-kappa B (NF- κ B) family of transcriptional regulators to repress NF- κ B-mediated transcriptional activation [Heppner et al., 2001]; members of the Smad family, Smad3 and the Smad 1/5 complex, that are involved in the transforming growth factor- β (TGF- β) [Kaji et al., 2001], and the bone morphogenetic protein-2 (BMP-2) signaling pathways [Sowa et al., 2003], respectively; Runx2, also called *cbfa1*, which is a common target of TGF- β and BMP-2 in differentiating osteoblasts [Sowa et al., 2004]; and the mouse placental embryonic (*Pem*) expression gene, which encodes a homeobox-containing protein [Lemmens et al., 2001]. Additional studies have shown that the interaction of menin with JunD may be mediated by a histone deacetylase-dependent mechanism, via recruitment of an mSin3A-histone deacetylase (HDAC) complex to repress JunD transcriptional activity [Kim et al., 2003]. Recently, the forkhead transcription factor CHES1 has been shown to be a component of this transcriptional repressor complex and to interact with menin in an S-phase checkpoint pathway related to DNA damage response [Busygina et al., 2006]. Menin uncouples ELK-1, JunD, and c-Jun phosphorylation from mitogen-activated protein kinase (MAPK) activation [Gallo et al., 2002] and suppresses insulin-induced c-Jun-mediated transactivation in CHO-1R cells [Yumita et al., 2003].

A wider role in transcription regulation has also been suggested, as menin has been shown to be an integral component of histone methyltransferase complexes that contain members from the mixed-lineage leukemia (MLL) and trithorax protein family [Hughes et al., 2004]. These can methylate the lysine 4 residue of histone H3 (H3K4) and H3K4 trimethylation is linked to activation of transcription. Menin, as a component of this MLL complex, regulates the expression of genes such as the *Hox* homeobox genes [Yokoyama et al., 2004] and the genes for cyclin-dependent kinase inhibitors, p27 and p18 [Karnik et al., 2005; Milne et al., 2005]. Menin has been shown to directly interact with the nuclear receptor for estrogen (ER α) and to act as a coactivator for ER α -mediated transcription, linking the activated estrogen receptor to histone H3K4 trimethylation [Dreijerink et al., 2006]. Menin has also been shown to bind to a broad range of gene promoters, independently of the histone methyltransferase complex, suggesting that menin functions as a general transcriptional regulator that helps maintain stable gene expression, perhaps by cooperating with other, currently unknown proteins [Scacheri et al., 2006]. Menin also directly binds to doubled-

stranded DNA and this is mediated by the positively-charged residues in the NLSs in the carboxyl terminus of menin [La et al., 2004]. The NLSs appear to be necessary for menin to repress the expression of the insulin-like growth factor binding protein-2 (IGFBP-2) gene by binding to the IGFBP-2 promoter. In addition, each of the NLSs has also been reported to be involved in menin-mediated induction of caspase 8 expression [La et al., 2006]. The NLSs may therefore have roles in controlling gene transcription as well as targeting menin into the nucleus.

A role for menin in controlling genome stability has been proposed because of its interactions with: a subunit of replication protein (RPA2), which is a heterotrimeric protein required for DNA replication, recombination, and repair [Sukhodolets et al., 2003]; and the FANCD2 protein, which is involved in DNA repair and mutations of which result in the inherited cancer-prone syndrome of Fanconi's anemia [Jin et al., 2003]. Menin also has a role in regulating cell division as it interacts with: the nonmuscle myosin II-A heavy chain (NMHC II-A), which participates in mediating alterations in cytokinesis and cell shape during cell division [Obungu et al., 2003]; and the glial fibrillary acidic protein (GFAP) and vimentin, which are involved in the intermediate filament network [Lopez-Egido et al., 2002]. Menin also has a role in cell cycle control as it interacts with: the tumor metastases suppressor NM23H1/nucleoside diphosphate kinase, which induces guanosine triphosphate (GTP)ase activity [Ohkura et al., 2001; Yaguchi et al., 2002] and the activator of S-phase kinase (ASK), which is a component of the Cdc7/ASK kinase complex that is crucial for cell proliferation. Indeed, menin has been shown to completely repress ASK-induced cell proliferation [Schnepp et al., 2004].

The functional role of menin as a tumor suppressor also has been investigated, and studies in human fibroblasts have revealed that menin acts as a repressor of telomerase activity via hTERT (a protein component of telomerase) [Lin and Elledge, 2003]. Furthermore, overexpression of menin in the human endocrine pancreatic tumor cell line (BON1) resulted in an inhibition of cell growth [Stalberg et al., 2004] that was accompanied by upregulation of JunD expression but downregulation of delta-like protein 1/preadipocyte factor-1, proliferating cell nuclear antigen, and QM/Jif-1, which is a negative regulator of c-Jun [Stalberg et al., 2004]. These findings of growth suppression by menin were observed in other cell types. Thus, expression of menin in the RAS-transformed NIH3T3 cells partially suppressed the RAS-mediated tumor phenotype *in vitro* and *in vivo* [Kim et al., 1999]. Overexpression of menin in CHO-IR cells also suppressed insulin-induced AP-1 transactivation, and this was accompanied by an inhibition of c-Fos induction at the transcriptional level [Yumita et al., 2003]. Furthermore, menin reexpression in *Men1*-deficient mouse Leydig tumor cell lines induced cell cycle arrest and apoptosis [Hussein et al., 2007]. In contrast, depletion of menin in human fibroblasts resulted in their immortalization [Lin and Elledge, 2003]. Thus, menin appears to have a large number of functions through interactions with proteins, and these mediate alterations in cell proliferation.

Functional Effects of *MEN1* Mutations

The majority of reported *MEN1* mutations are frameshift and nonsense mutations (Fig. 2) that are predicted to result either in a truncated protein, with the consequent loss of functional domains including the NLSs located in the C-terminal segment, or in loss of the translated protein because of nonsense-mediated mRNA decay (NMD) [Holbrook et al., 2004; Khajavi et al., 2006; Kuzmiak and Maquat, 2006]. The splice-site mutations (Fig. 2) are

predicted to lead to an accumulation of unspliced precursor mRNA, retention of incompletely spliced precursors, complete absence of transcripts, or the appearance of aberrantly processed mRNA from the use of alternative normally occurring splice sites or novel, or cryptic splice sites [Pagani and Baralle, 2004; Baralle and Baralle, 2005]. Such abnormalities of mRNA have only been investigated in 13 (19%) of the reported 69 different splice-site mutations (Supplementary Table S1). For example, a mutation that accounts for 1.9% of all *MEN1* mutations and involves a $g \rightarrow a$ transition in intron 4 (Fig. 1; Table 2) has been shown to lead to a novel acceptor splice site [Turner et al., 2002]. Utilization of this novel splice site has been shown to be associated with an abnormal mRNA transcript that if translated would result in a frameshift in which nine missense amino acids are incorporated prior to a premature termination codon [Turner et al., 2002]. Such intronic mutations resulting in aberrant splicing are increasingly recognized as disease-causing abnormalities [Pagani and Baralle, 2004; Baralle and Baralle, 2005] and indeed another *MEN1* mutation in intron 9 that also results in a novel acceptor splice site and is predicted to lead to a frameshift with a premature termination codon has been recently reported [Lemos et al., 2007a]. All these splice-site mutations are predicted to result in truncated and hence inactivated forms of menin. Missense mutations may lead to inactivation of menin by affecting functionally critical amino acid residues involved in protein interactions and in the tumor suppressor activity. For example, some menin missense mutants have been shown to affect the binding capacity of menin to other proteins that include JunD [Agarwal et al., 1999], nm23 [Ohkura et al., 2001], RPA2 [Sukhodolets et al., 2003], Sin3A [Kim et al., 2003], histone methyltransferase complex [Hughes et al., 2004], and ER α [Dreijerink et al., 2006], while other missense mutants have been reported to alter the capacity of menin to regulate the target promoters [La et al., 2006]. Several other *MEN1* missense mutations have also been shown to result in a reduction of protein stability and enhanced proteolytic degradation [Yaguchi et al., 2004], thereby suggesting another mechanism for the pathogenesis associated with missense mutations.

Mouse Models for *MEN1*

Mouse models for *MEN1* have been generated through homologous recombination (i.e., knockout) of the mouse *Men1* gene. The mouse *Men1* gene consists of a 1,833-bp open reading frame that encodes a 611-amino acid protein [Stewart et al., 1998; Bassett et al., 1999]. Thus, the mouse menin protein contains one more amino acid residue than the human menin, and this is a glycine at codon 528. However, the mouse and human coding regions have 89% and 96% identities of the nucleotide and amino acid sequences, respectively, indicating a high degree of evolutionary conservation. One mouse knockout model for *MEN1* was generated by introducing a floxed phosphoglycerate kinase (PGK)-neomycin cassette into intron 2 and a third loxP site into intron 8, thereby deleting exons 3–8 in one allele [Crabtree et al., 2001]. Adult heterozygous *Men1*^{+/-} mice, (9–16 months old), developed parathyroid dysplasia, adenomas, and carcinomas; pancreatic islet cell tumors that contained insulin; anterior pituitary tumors that contained prolactin; and adrenal cortical carcinomas. The tumors, which had LOH at the *Men1* locus, were not associated with any serum biochemical abnormalities, such as hypercalcemia or hypoglycemia, but those *Men1*^{+/-} mice developing pancreatic islet cell tumors or hyperplasia were found to have elevated serum insulin concentrations [Crabtree et al., 2001]. Another mouse knockout model has been generated by deleting exon 3 [Bertolino

et al., 2003a], and adult heterozygous *Men1*^{+/-} mice were found to develop parathyroid adenomas and carcinomas; pancreatic islet cell tumors that consisted of insulinomas, gastrinomas, or glucagonomas; and anterior pituitary tumors that consisted of prolactinomas or somatotrophinomas. These *Men1*^{+/-} mice also developed tumors of the thyroid, Leydig cells, ovarian stroma, and mammary glands [Bertolino et al., 2003a]. Two other mouse knockout models have also been generated by deleting either exon 2, which contains the translation start site [Loffler et al., 2007] or exons 1 and 2 [Lemos et al., 2007b]. In both of these studies, *Men1*^{+/-} mice were found to develop parathyroid, pancreatic islet, and anterior pituitary tumors. In addition, a proportion of these *Men1*^{+/-} mice also developed tumors of the thyroid, adrenals, and gonads. Thus, heterozygous *Men1*^{+/-} mice from these four different types of knockouts provide a model for the human MEN1 disease. However, in another study, heterozygous mice *Men1*^{+/-} surprisingly died as embryos in late gestation, with some embryos developing omphalocele [Scacheri et al., 2001]. Homozygous *Men1*^{-/-} mice from three studies [Crabtree et al., 2001; Bertolino et al., 2003b; Lemos et al., 2007c] have been reported to die in utero at embryonic days 11.5–13.5. In one study these *Men1*^{-/-} mice were developmentally delayed and significantly smaller, and 20% of them developed craniofacial abnormalities [Crabtree et al., 2001]. The craniofacial abnormalities have been shown to be due to dysplasia of the membranous skull bones, and this developmental pathway involves the BMP-2 signaling pathway [Sowa et al., 2003]. In another study, *Men1*^{-/-} mice were smaller and developed extensive hemorrhage and edema [Bertolino et al., 2003b]. In addition, many of these *Men1*^{-/-} mice had abnormalities of the neural tube, heart, and liver [Bertolino et al., 2003b]. Thus, many *Men1*^{-/-} mice had a failure of the closure of the neural tube, myocardial hypotrophy with a thin interventricular septum, and decreased hepatic cellularity, which was associated with an altered organization and enhanced apoptosis [Bertolino et al., 2003b]. These results from the *Men1*^{-/-} mice reveal an important role for the *MEN1* gene in the embryonic development of multiple organs.

Model of Tumorigenesis

More than 90% of tumors from MEN1 patients have LOH, and this has generally been taken as evidence that the *MEN1* gene acts as a tumor-suppressor gene, consistent with Knudson's two-hit hypothesis [Knudson, 1971; Knudson et al., 1973; Larsson et al., 1988; Friedman et al., 1989; Thakker et al., 1989]. However, this LOH represents only one mechanism by which the second hit may occur, with the other mechanisms being intragenic deletions and point mutations. MEN1 tumors (e.g., parathyroids, insulinoma, and lipoma) that do not have LOH have been shown to harbor different somatic and germline mutations of the *MEN1* gene [Pannett and Thakker, 2001], consistent with the Knudson two-hit hypothesis.

CLINICAL AND DIAGNOSTIC RELEVANCE

MEN1 is inherited as an autosomal dominant disorder in most patients [Benson et al., 1987; Calender et al., 1995; Trump et al., 1996; Marx et al., 1998]. Occasionally, MEN1 may arise sporadically (i.e., without a family history), although it may be difficult to make the distinction between sporadic and familial forms [Thakker, 2006]. In some cases, a family history may be absent because the parent with MEN1 is not available and may have already died before any manifestations developed, and other cases may be due to *de novo* mutations, which will be transmitted in an autosomal dominant manner in future generations [Agarwal

et al., 1997; Bassett et al., 1998; Teh et al., 1998]. MEN1 is an uncommon disorder, but because of its autosomal dominant inheritance, the finding of MEN1 in a patient has important implications for other family members; first-degree relatives have about a 50% risk of development of the disease [Benson et al., 1987; Calender et al., 1995; Trump et al., 1996; Marx et al., 1998]. Screening for MEN1 in patients involves the detection of tumors and ascertainment of the germline genetic state, that is, normal or mutant gene carrier [Brandt et al., 2001]. Detection of tumors entails clinical, biochemical, and radiologic investigations for MEN1-associated tumors in patients [Trump et al., 1996; Brandt et al., 2001]. The characterization of the *MEN1* gene [Chandrasekharappa et al., 1997; European Consortium on MEN1, 1997] has facilitated identification of individuals who have mutations and hence a high risk of acquiring the disease.

Molecular genetic analysis for MEN1 either by detecting mutations or by performing segregation studies using linked markers is useful in identifying individuals who are mutant carriers and thus have a high risk of tumor development [Brandt et al., 2001; Thakker, 2006]. The advantages of DNA analysis are that it requires a single blood sample and does not in theory need to be repeated because the analysis is independent of the age of the individual and provides an objective result. Such mutational analysis may be undertaken in children within the first decade because tumors have developed in some children by the age of 5 years [Stratakis et al., 2000], and appropriate intervention in the form of biochemical testing, or treatment, or both, has been considered [Lips et al., 1984; Brandt et al., 2001]. However, the great diversity together with widely scattered locations of the *MEN1* mutations [Pannett and Thakker, 1999] and a lack of genotype–phenotype correlation, make such mutational screening time consuming, arduous, and expensive [Thakker, 1998]. Nevertheless, an integrated program of both mutational analysis, to identify mutant gene carriers, and biochemical screening, to detect the development of tumors, is of advantage and used by many centers [Brandt et al., 2001; Thakker, 2006]. Thus, a DNA test identifying an individual as a mutant gene carrier is likely to lead not to immediate medical or surgical treatment but to earlier and more frequent biochemical and radiologic screening, whereas a DNA result indicating that an individual is not at risk will lead to a decision for no further clinical investigations. Thus, the identification of *MEN1* mutations may be of help in the clinical management of patients and their families with this disorder.

FUTURE PROSPECTS

The identification of the *MEN1* gene and the possibility of performing genetic testing have facilitated the clinical management of MEN1 patients and family members and increased our knowledge of this disorder. In addition, the finding of a tumor suppressor role for *MEN1* and the identification of interacting proteins involved in regulation of transcription, genome stability, cell division, and proliferation have provided some clues to the function of menin, but the pathways of menin action in normal tissues and in tumors still need to be established. The availability of MEN1 mouse models offers new approaches that may help in understanding these roles of menin and provide insights into the process of endocrine tumorigenesis. However, much still remains to be elucidated about the role of *MEN1* and its encoded protein in the development of endocrine tumors. Despite the widespread tissue expression of menin, tumors in MEN1 are mostly confined to a limited set of tissues and the basis for this specificity still remains unknown. In addition, the frequent occurrence of

nonendocrine tumors such as lipomas, collagenomas, and angiofibromas in MEN1 patients needs to be explored and the roles of menin in non-endocrine cells needs to be elucidated.

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