

Molecular Pathology of the *MEN1* Gene

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ABSTRACT: Multiple endocrine neoplasia type 1 (MEN1), among all syndromes, causes tumors in the highest number of tissue types. Most of the tumors are hormone producing (e.g., parathyroid, enteropancreatic endocrine, anterior pituitary) but some are not (e.g., angiofibroma). MEN1 tumors are multiple for organ type, for regions of a discontinuous organ, and for sub-regions of a continuous organ. Cancer contributes to late mortality; there is no effective prevention or cure for MEN1 cancers. Morbidities are more frequent from benign than malignant tumor, and both are indicators for screening. Onset age is usually earlier in a tumor type of MEN1 than of nonhereditary cases. Broad trends contrast with those in nonneoplastic excess of hormones (e.g., persistent hyperinsulinemic hypoglycemia of infancy). Most germline or somatic mutations in the *MEN1* gene predict truncation or absence of encoded menin. Similarly, 11q13 loss of heterozygosity in tumors predicts inactivation of the other *MEN1* copy. *MEN1* somatic mutation is prevalent in nonhereditary, MEN1-like tumor types. Compiled germline and somatic mutations show almost no genotype/phenotype relation. Normal menin is 67 kDa, widespread, and mainly nuclear. It may partner with junD, NF- κ B, PEM, SMAD3, RPA2, FANCD2, NM23 β , nonmuscle myosin heavy chain II-A, GFAP, and/or vimentin. These partners have not clarified menin's pathways in normal or tumor tissues. Animal models have opened approaches to menin pathways. Local overexpression of menin in *Drosophila* reveals its interaction with the jun-

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kinase pathway. The *Men1*^{+/-} mouse has robust MEN1; its most important difference from human MEN1 is marked hyperplasia of pancreatic islets, a tumor precursor stage.

KEYWORDS: MEN1; menin; oncogene; tumor suppressor; AP1; insulinoma; gastrinoma; carcinoid; hyperparathyroidism

INTRODUCTION: A RARE SYNDROME OFFERS BROAD INSIGHTS

Familial multiple endocrine neoplasia type 1 (MEN1) was well described more than 50 years ago. During half a century, many other tumors have been tied to MEN1; in fact, among all neoplastic syndromes, MEN1 causes the largest number of tumor types (TABLE 1).¹ A consensus definition of MEN1 is used widely.² An MEN1 case has tumor in two of the three principal organs (parathyroid, enteropancreatic endocrine tissue, and anterior pituitary). Similarly, familial MEN1 is defined as one MEN1 case plus one first-degree relative with one of the three principal tumors. Although rare (approximately 1 in 30,000), it has long generated fascination for endocrinologists, gastroenterologists, oncologists, and geneticists. All have hoped that MEN1 would yield insights about many tumors. Discovery of its gene followed by proof of this gene's frequent contribution to common tumors has strengthened these persistent hopes.¹

MEN1 TRAITS INDICATE A POSSIBLE TUMOR SUPPRESSOR GENE

Multiplicity of Tumors

Four Meanings of Tumor Multiplicity. Tumor multiplicity is a striking feature in MEN1. The term multiplicity has at least four variations here.

Tumors can occur in multiple organ types. These include many hormone-producing tumor types as well as several hormone nonproducing tumor types (TABLE 1). Some hormone nonproducing tumors ("stromal"), such as angiofibroma and leiomyoma, have been recognized only recently in MEN1, because those tumors lack the high profile from hormone hypersecretion or other morbidities.^{3,4} Even so, some of the hormone nonproducing tumor types have very high penetrance in MEN1 (TABLE 1).

Tumors can occur in multiple parts of one organ type that is anatomically discontinuous, such as enteropancreatic endocrine tissue or the parathyroid. All four glands are usually tumorous simultaneously at initial parathyroidectomy. Also relevant is a drastic asymmetry of parathyroid tumor sizes in MEN1, because each gland contains independent tumor one or more clones.

Multiple tumors can occur within one continuous tissue (such as multiple leiomyomas of the esophagus or uterus⁴).

Tumor multiplicity may be a predictor of cancer. The high number and longer duration of tumors in some tissues may be a precursor for cancer in a few. This seems valid in enteropancreatic tissues but is not evident in the parathyroids of MEN1. As managements of the endocrine expressions of MEN1 have improved, the awareness of inherent and usually slowly progressing malignancies has increased. MEN1-asso-

TABLE 1. MEN1 tumors (penetrance at age 40 years)

Hormone-Producing Tumors	
Parathyroid adenoma (90%)	Anterior pituitary tumor
Enteropancreatic tumor	Prolactinoma (20%)
Gastrinoma (40%) ^a	Other-growthhormone+prolactin(5%)
Insulinoma (10%)	Growth hormone (5%), NF (5%)
NF^b also pancreatic polypeptide (20%) ^c	ACTH (2%), TSH (rare)
Glucagon, VIP, somatostatin, etc. (2%)	Adrenal cortex NF (25%)
Foregut carcinoid	Adrenal medulla (1%)
Thymic carcinoid NF (4%)	
Bronchial carcinoid NF (2%)	
Gastric enterochromaffin-like NF (10%)	
Hormone-Nonproducing Tumors ^d	
Facial angiofibroma (85%)	Leiomyoma (10%???)
Truncal collagenoma (70%)	Ependymoma (1%)
Lipoma (30%)	Meningioma (5%)

^aBold font, tumor type with substantial (above 25% of cases) malignant potential.

^bNF, nonfunctioning. Does not oversecrete enough to produce a humoral expression.

^cOmits clinically silent tumors detectable at abdominal surgery in MEN1.

^dMay produce nonclassical hormones, such as leptin or adiponectin.

ciated malignancy is a major contributor to death in one third of cases with MEN1. Among the deaths from MEN1, 60% are from enteropancreatic endocrine malignancy and 20% are from foregut carcinoid malignancy.⁵ Several treatment protocols have been explored to prevent or cure cancers in MEN1. Subtotal thymectomy is routinely done during initial parathyroidectomy in MEN1. Even so, several cases have developed malignant thymic carcinoid postoperatively.⁶ Similarly, distal pancreatectomy is done during abdominal exploration in MEN1 to decrease the mass of islet tissue. Because of likely low impact, these two procedures have been done only incidental to other operations. Surgical and medical treatments of established enteropancreatic endocrine cancer and foregut malignant carcinoid have not resulted in important successes. Somatostatin analogs for delivery of radioactivity to either of these cancers are also under evaluation.

Relation of Multiplicity to Recurrence. Multiplicity is also relevant to tumor recurrence, which is 50% for parathyroid adenoma within 8–12 years after successful subtotal parathyroidectomy in MEN1. Frequent recurrence likely relates to aspects of the above mechanisms: (1) new neoplasia arising in residual normal tissue, or (2) neoplasia progressing in the residual tissue.

Relation of Tumor Multiplicity and Penetrance. In general, strong penetrance of a tumor correlates positively with its multiplicity. Similarly, weakly penetrant tumors show less multiplicity. Thus, pheochromocytoma in MEN1 is rare and virtually always unilateral. Surprisingly, although penetrance of pituitary tumor is 40% in adults with MEN1, pituitary tumor in MEN1 seems solitary.⁸

Tumor Onset Age: Relation to Two-Hit Hypothesis of Tumorigenesis

The typical onset ages in MEN1-associated versus sporadic tumor are: parathyroid adenoma (25 vs. 55 yr); gastrinoma (35 vs. 45 yr); prolactinoma or foregut carcinoid tumor (35 yr; no age difference). Lack of age difference in prolactinoma may be because the *MEN1* gene is rarely implicated in nonhereditary prolactinoma. Consequently, prolactinomas in MEN1 versus sporadics behave as different diseases with independent onset ages in the two settings.⁵

Germline *MEN1* mutation in all cells can explain both tumor multiplicity and earlier tumor onset age in MEN1 than sporadics. The subsequent tumorigenic events are likely to "hit" in *MEN1* at more susceptible cells and at earlier age. These same two features in hereditary retinoblastoma led to Knudson's two-hit hypothesis of neoplasia, and they often have predicted tumorigenesis via an underlying tumor suppressor gene.⁸

Neoplastic versus Nonneoplastic Paradigm in Hormone Excess Syndromes

The relatively young tumor onset age in MEN1 is still strikingly older than with certain syndromes of nonneoplastic hormone excess.⁹ The latter are expressed very early, that is, already in infants or even neonates. The most frequent example is familial hypocalciuric hypercalcemia (or familial benign hypercalcemia). Less frequent but still typical examples of this nonneoplastic pathophysiology are persistent hyperinsulinemic hypoglycemia of infancy, testotoxicosis or male-limited precocious puberty, and neonatal nonimmune hyperthyroidism.⁹

Comparisons between MEN1 (and other neoplasia syndromes) versus these nonneoplastic hormone excess syndromes establish two paradigms by several broad criteria: delay of onset age after birth (beyond 10 years vs. no delay); histology (multifocal neoplasia and sometimes cancer vs. mild diffuse hyperplasia and no cancer); response to subtotal gland ablation (long-lasting remission but possible late recurrence versus no benefit).⁹ The paradigms highlight that excess of the same hormone can arise from stepwise clonal neoplasia or from a secretion regulatory defect with polyclonal haploinsufficiency.

Some Genetic Counseling Implications in MEN1

Roles of Screening. Screening has two main meanings here: (1) carrier ascertainment and (2) monitoring for tumors. Either type of screening as a guide to management has only a modest impact in MEN1. Carrier ascertainment should support provision of information for patient, family, and health care providers. Proven carriers are offered periodic tumor monitoring, stratified by organ, age, benefit, and cost.²

Carrier Ascertainment in Children. There is no effective prevention or cure for the cancers in MEN1 (above).² However, preventable or treatable morbidity from benign tumor at young age is possible but unusual. One MEN1 case had an aggressive prolactinoma at age 5 years.¹⁰ Thus, we recommend onset of carrier ascertainment after age 5 years and then periodic monitoring in known carriers.

Negative Mutation Test. *MEN1* sequencing identifies a mutation in only 70% of families with unequivocal MEN1. Most of the rest have currently undetectable mutations in the *MEN1* gene (see below). In this remainder and in cases suspected to be from this category, traditional endocrine-based carrier ascertainment must be

used, augmented by evaluation of *MEN1*-associated skin tumors³ and 11q13 haplotype within a family.

***MEN1* LOH AND MUTATION PATTERNS SUPPORT TUMOR SUPPRESSOR ROLE**

***MEN1* Gene Identification**

In 1988, a Swedish team initiated the genetic era for *MEN1*. By genetic linkage analysis in *MEN1* families, they located the gene at chromosome 11q13. At the same time they also found that two *MEN1* insulinomas had an acquired loss of all of the chromosome 11 alleles derived from the unaffected parent. This suggested that the gene's role in tumorigenesis was a two-hit loss of function, that is, tumor suppression.^{8,11}

A decade passed until the *MEN1* gene was identified by positional cloning.¹² This process required pathologic DNA at three stages: (1) narrowing the candidate interval via boundaries mapped to near *MEN1* in meiotic recombinations of gametes in *MEN1* families; (2) further narrowing with boundaries mapped from tumor DNA deletions, involving only a part of chromosome 11; and (3) ultimate identification of one gene. This last step was possible because the syndrome is so robust that a panel of germline DNA from index cases could be assembled, with virtually each contributing another in a spectrum of underlying mutations in the sought-after gene. Each remaining candidate gene was sequenced in each case of this panel; sequencing of only one candidate gene showed a different mutation in almost each index case, proof of *MEN1* discovery.

Germline MEN1 Mutations in Familial and Sporadic Index Cases

Germline *MEN1* mutation is identifiable in 70 to 90% of typical *MEN1* families and in somewhat fewer among sporadic *MEN1*.^{1,2,13} Some without identified mutation have large deletions not recognizable by polymerase chain reaction; many of the rest are likely to have *MEN1* mutation outside of the tested open reading frame and intron-exon junctions. There has been no correlation of genotype with phenotype, except perhaps in familial isolated hyperparathyroidism (FIHP). *MEN1* mutation has been found in 20 FIHP kindreds; these are only a small subset of all FIHP.¹⁴ These 20 show a larger fraction with *MEN1* missense mutation than does typical *MEN1*; furthermore, there is clustering of these missense mutations in FIHP between codons 255 and 305, that is, a approximately exons 4–7 (not shown).

Somatic MEN1 Mutations in Tumors

Nonhereditary tumors of the types seen also in *MEN1* often show *MEN1* mutation. In fact, a higher number of sporadic endocrine tumor types have mutation of *MEN1* than of any other gene. Sporadic tumors with approximately 20% *MEN1* mutation include parathyroid adenoma, gastrinoma, insulinoma, and bronchial carcinoma.^{1,15} Tumors with lower *MEN1* mutation frequencies include anterior pituitary and angiofibroma.⁵ Certain nonhereditary tumors have had no *MEN1* mutations: small cell lung cancer, melanoma, and lymphoma.¹ There has been no clear pattern of mu-

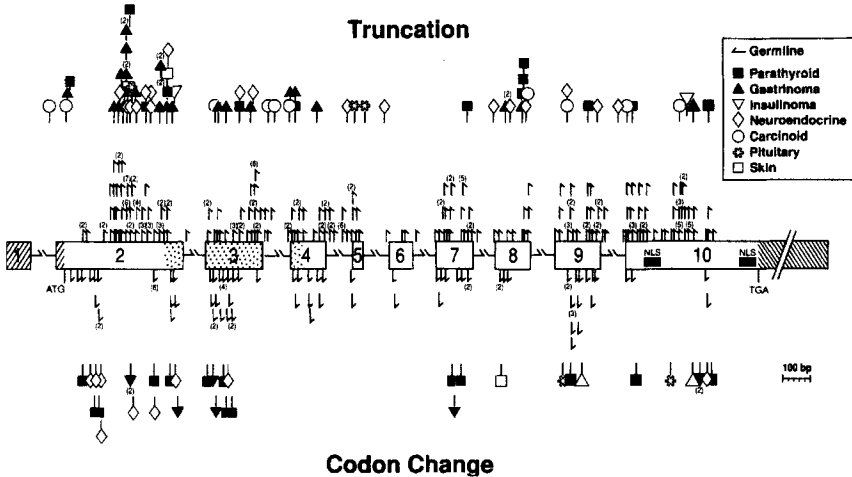


FIGURE 1. Germline and somatic mutations of *MEN1* gene. Germline *MEN1* mutations in families and sporadic cases; somatic mutations in tumors. *MEN1* messenger RNA has exons numbered and untranslated regions cross-hatched. Germline mutations, checks about the mRNA; somatic mutations, flags along borders (tumor types in insert). Repeating mutations appear once (number in *parentheses* indicates total occurrences). Truncating mutations (frameshift, splice error, and stop codons) are in upper half of panel; codon change mutations (missense mutations or small in-frame insertions or deletions) are in lower half; large deletions are omitted. *Stippling* about exon 3 is main zone of menin interaction with junD. NLS = nuclear localization signal. From prior Ref. 1.

tation specific to any one tumor type. There also has been no correlation of genotype with tumor aggressiveness.¹⁶ Thus, *MEN1* sequencing is not suggested for tumor staging.

Patterns in Germline and Somatic Mutations of the MEN1 Gene

The overall patterns of germline and somatic mutations in *MEN1* are similar (FIG. 1). Approximately 80% predict truncation or absence of the encoded menin. This reflects stop codons, frameshifts, splice errors, and large deletions. These plus loss of heterozygosity (LOH) about the other *MEN1* copy support tumorigenesis by inactivation of both *MEN1* copies. The remaining 20% of mutations predict in-frame missense or similar codon changes. The truncating mutations cluster at about exon 2. The missense mutations cluster mildly in and around exon 3. Still, there is no striking clustering of missense mutations that might point to a critical domain. There is no strong pattern of genotype/phenotype relations among germline and somatic *MEN1* mutations (see above).

Insights from Allelic Losses at the MEN1 Locus (MEN1 gene inactivation)

Biallelic MEN1 Inactivation Supports Tumor Cause by MEN1. 11q13 LOH or allelic loss by fluorescence in situ hybridization continues to be tested in certain

research settings. For example, *MEN1* cases have been recognized to have higher frequency of certain tumor types, not traditionally associated with *MEN1*. If LOH or allelic loss about 11q13 is found repeatedly in a *MEN1* tumor type, this supports dependency of that tumor type on biallelic inactivation of *MEN1*. Recent examples are angiofibroma, collagenoma, leiomyoma, and pheochromocytoma.^{4,17,18}

11q13 LOH in Tumors without MEN1 Mutation. Nonhereditary endocrine tumors typically show 11q13 LOH approximately twice as frequently as *MEN1* mutation. Thus, efforts have been made to prove inactivation of another tumor suppressor gene in the general locus of the *MEN1* gene.¹⁹ However, considering that the tumors without *MEN1* mutation have the same organ types as those with *MEN1* mutation, another speculative possibility is that the other copy of the *MEN1* gene has been inactivated by another process such as hypermethylation.

MEN1 Mutation without 11q13 LOH. In a small fraction of tumors in *MEN1* cases, no 11q13 LOH is identified, even after tumor microdissection. In this highly selected group, point mutation in the second copy of the *MEN1* gene has been frequent.²⁰ This contrasts with 11q13 LOH never being found in two common tumor types of *MEN1*, benign adrenocortical tumor and thymic carcinoid (TABLE 1).^{8,21} Unrecognized types of "second hit" are possible, at the *MEN1* locus and elsewhere. Alternately, such tumors might develop from one hit at the *MEN1* locus (haploinsufficiency). Because both of these tumor types have the capability to develop into cancers in *MEN1*, their germline *MEN1* mutation clearly can contribute to neoplasia.

Menin Reversion of Malignancy (MEN1 gene overexpression). One more support of the tumor suppressor function of menin would be for normal menin to revert a malignant menin-null cell, but such a cell has not been available. Thus, ras-transformed NIH3T3 cells have been used as surrogates for menin-null cells. When menin was overexpressed by 10–25-fold in these cells, there was a partial normalization of the transformed phenotype by many criteria.²² Overall, the tumor suppressor mechanism is supported in several settings; menin loss promotes tumorigenesis and menin excess promotes tumor reversion.

NORMAL OR PATHOLOGIC FUNCTIONS OF THE MENIN MOLECULE

Normal Menin Molecule

Protein Properties. Menin is a 67-kDa protein, widely expressed, located in the nucleus, but also detected in cytoplasm and about telomeres.²³ Sequence analysis shows no homologous proteins, but there is one ortholog in each of mice, fish, birds, flies,²⁴ frogs, and snails. There is no ortholog in *Caenorhabditis elegans* or yeast. Menin sequence has two nuclear localization signals but no other signature domain.²⁵ Intrinsic GTPase activity has been proposed.²⁶

Menin Molecular Partners. Menin may bind directly or indirectly to the following: transcription factors, junD, NF- κ B, p53, SMAD3; a DNA processing factor, RPA2; FANCD2, a DNA repair factor; nucleoside diphosphate kinase, NM23 β ; non-muscle myosin heavy chain II-A; and cytoskeleton-associated proteins, vimentin and GFAP.^{27–29} These menin partners lack a pattern, excepting a predominance of nuclear proteins. A large menin complex has lysine-4 histone methyltransferase

activity.³⁰ No physiologic role for menin's interaction with any of these partners has been proved.

JunD belongs to the AP1 family of DNA-binding transcription factors that includes subfamilies of jun and fos/fra. They bind, via their basic/leucine zipper domain, as homodimers or heterodimers upon DNA at a small seven base recognition site (TPA-response element [TRE] site). When overexpressed, menin inhibits transcription (based on reporter assays in transfected cells) that is stimulated by junD; in contrast, menin does not bind cjun but actually augments transcription stimulated by cjun.²⁷ If junD is engineered to have a missense mutation that prevents its binding to menin, then the missense mutant junD remains able to activate transcription, but the menin effect on it is reversed to resemble that with cjun.³¹ JunD is unique in the AP1 family as the only member (1) that binds menin and (2) that does not promote cell proliferation. Proliferation indices in stable cell lines showed that junD, deprived of menin, switched from a growth suppressor to a growth promoter.³² This led to speculation that menin inactivation switches junD into an oncogene, and this could account for MEN1-related tumors.

Pathways Downstream of Menin. Neither the finding of a tumor suppressor mechanism nor the identification of binding partners has established the ultimate pathways of menin action in normal tissues or in tumors. There have been suggestions of increased DNA damage in MEN1 germline and in MEN1 tumors.³³ Several animal models offer new approaches to menin's downstream pathways (see below).

Animal Models: Some Confirmations and Some Surprises

Drosophila Melanogaster. *Drosophila* has a *Mnn1* gene encoding menin with 46% identity to human menin.²⁵ When *Drosophila* menin is driven to a localized overexpression in the dorsal ectoderm, the resulting defect in thoracic closure can be traced to interactions of menin with the jun-kinase signaling pathway.^{34,35}

Cyclin-Dependent Kinase Inhibitor Knockout Mouse. Various combinations of knockouts in the *p18*, *p21*, and *p27* genes were engineered in the mouse.³⁶ These genes encode cyclin-dependent kinase inhibitors; knockout of each alone rarely leads to any endocrine tumor. Surprisingly, combined knockouts of *p18* and *p27* caused expression of all the major tumors of MEN1 and MEN2. This raised the possibility that MEN1 and MEN2 develop, in part, through common downstream pathways involving the cyclin-dependent kinase inhibitors.

The Men1 Knockout Mouse. Heterozygous knockout of *Men1* provides an excellent model of MEN1.³⁷ Mice gestate and develop normally, but by 16 months they show some major tumors of MEN1: frequent parathyroid tumors, insulinomas, and prolactinomas. Their differences from human MEN1 are also important.

The *Men1*^{+/-} mice do not develop gastrinoma; this is reminiscent of unusual but large MEN1 families with high penetrance for hyperparathyroidism and prolactinoma but low penetrance for gastrinoma.^{1,13}

Seven percent of mice show pheochromocytoma, sometimes bilaterally. This supports pheochromocytoma as inherent, albeit rare, in human MEN1 (TABLE 1).

Marked hyperplasia of pancreatic islets occurs after 9 months. LOH testing therein indicated still one normal *Men1* allele. Similarly, histology showed that menin protein was present in beta cells during their hyperplastic stage.³⁸ This is the stron-

gest evidence for a tumor precursor stage in MEN1, thus a preneoplastic target for intervention. It could lead to tools to evaluate tumors and precursor stages in many tissues, including in tissues of man.

FUTURE DIRECTIONS

MEN1 gene identification opened a period of rapid progress. This predicts understandings of MEN1 and of many common tumors and advances in patient care.

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