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EXPERT REVIEWS

Recent results of basic and clinical research in MEN1: opportunities to improve early detection and treatment

Cornelis JM Lips*[1], Koen MA Dreijerink[1], Thera P Links[2] and Jo WM Höppener[3]

[1]Department of Internal Medicine and Endocrinology, University Medical Center, Utrecht & The Hague, The Netherlands
[2]Department of Endocrinology, University Medical Center Groningen, The Netherlands
[3]Department of Metabolic Diseases, University Medical Center, Utrecht, The Netherlands
*Author for correspondence:
Tel.: +31 70 324 0428
Fax: +31 84 726 0305
lips05@zonnet.nl

Due to the variable expression of multiple endocrine neoplasia type 1 (MEN1), it is difficult to predict the course of the disease. However, knowledge about the normal function of the *MEN1* gene product, together with the effects of cellular derangement by subsequent genetic events, has increased considerably. At first, the possible existence of a genotype–phenotype correlation is discussed. Thus, mild- and late-onset phenotypes may be distinguished from more malignant phenotypes depending on the character of the primary *MEN1* disease gene mutation. Subsequently, tumor-promoting factors such as gender, additional genetic mutations and ecogenetic factors may contribute to the course of the disease. New developments in management are based on the knowledge and experience of the multidisciplinary teams involved. Finally, the metabolic effects of *MEN1* mutations in aged patients are discussed. Early identification of predisposition to the disease, together with knowledge about the natural history of specific mutations, risks of additional mutations and periodic clinical monitoring, allow early treatment and may improve life expectancy and quality of life.

Multiple endocrine neoplasia type 1 (MEN1) is an inherited endocrine tumor syndrome, predominantly characterized by tumors of the parathyroid glands, gastroenteropancreatic tumors, pituitary adenomas, adrenal adenomas and neuroendocrine tumors (NETs) of the thymus, lungs and/or stomach. MEN1 is caused by germline mutations of the *MEN1* tumor suppressor gene.

A clinical and practical definition of a patient with MEN1 is a case with at least two out of the three main MEN1-related endocrine tumors (parathyroid hyperplasia/adenomas, entero-pancreatic endocrine tumors and pituitary tumors). Currently, genetic predisposition to MEN1 may be established by demonstrating a germline mutation in the *MEN1* gene.

Thanks to the collaboration of the MEN1 families involved, positional cloning allowed mapping of the gene to a specific chromosomal region by linkage analysis. Eventually, the correct gene among all possible candidate genes within that particular region was identified.

After the gene was identified, a study of its physiological function was performed. In numerous tissues, the *MEN1* gene is expressed; however, an intact function is only needed in a restricted number of organs in order to suppress cell division. It appeared that the gene product called menin has a dual function in modification of histone proteins and regulation of DNA transcription, according to a coactivator and/or corepressor function [1,2].

In MEN1, most of the mutations are clearly inactivating, in accordance with the notion that the *MEN1* gene is a tumor suppressor gene. A clear correlation between genetic events and the variable clinical expression of MEN1 has not yet been established [3,4]. Apparently, there are no mutational hotspots in the *MEN1* gene. However, it appears that in the MEN1 syndrome, clinical expression differs between families. This

may be the result of the specific *MEN1* gene mutation in a family (genotype–phenotype correlation). Moreover, among individual members within the same family, clinical expression can also vary, possibly due to additional genetic or epigenetic factors.

As a rule, the development of a tumor depends on a series of genetic events, in other words, multistep tumorigenesis. Thus, additional cosegregating modifying factors such as germline gene mutations in other genes are likely to play a role in the interfamilial variability of MEN1. Further understanding of the genetic aspects of MEN1 and the pathogenesis of MEN1-related tumors could enable more tailored clinical screening and treatment strategies.

In this review, recent reports on aberrant clinical expression of MEN1 have been discussed, which may allow a glimpse into the pathogenesis of this intriguing disorder. In addition, new developments in clinical management are summarized. Finally, the consequences for future care are considered.

Function of the intact *MEN1* gene product

The intact *MEN1* gene (wild-type) product, menin, functions as an adaptor protein that is involved in interactions with multiple protein partners (Figure 1).

As a transcriptional coregulator, menin may function as a coactivator or corepressor by recruiting histone-modifying enzymatic activity [1]. As a coactivator, menin is involved in the regulation of histone methylation, by recruiting the mixed-lineage leukemia (MLL) proteins MLL1 and MLL2. In this way, menin can bind to nuclear receptors and activate nuclear receptor-mediated gene transcription. By tethering histone deacetylase activity to genes, menin can serve as a repressor of transcription.

As menin shows no clear homology to other known proteins, structural information on menin is critical for understanding its role as a tumor suppressor protein and as a cofactor of MLL fusion proteins. Knowledge about the 3D structure may elucidate the interactions essential to the function of menin.
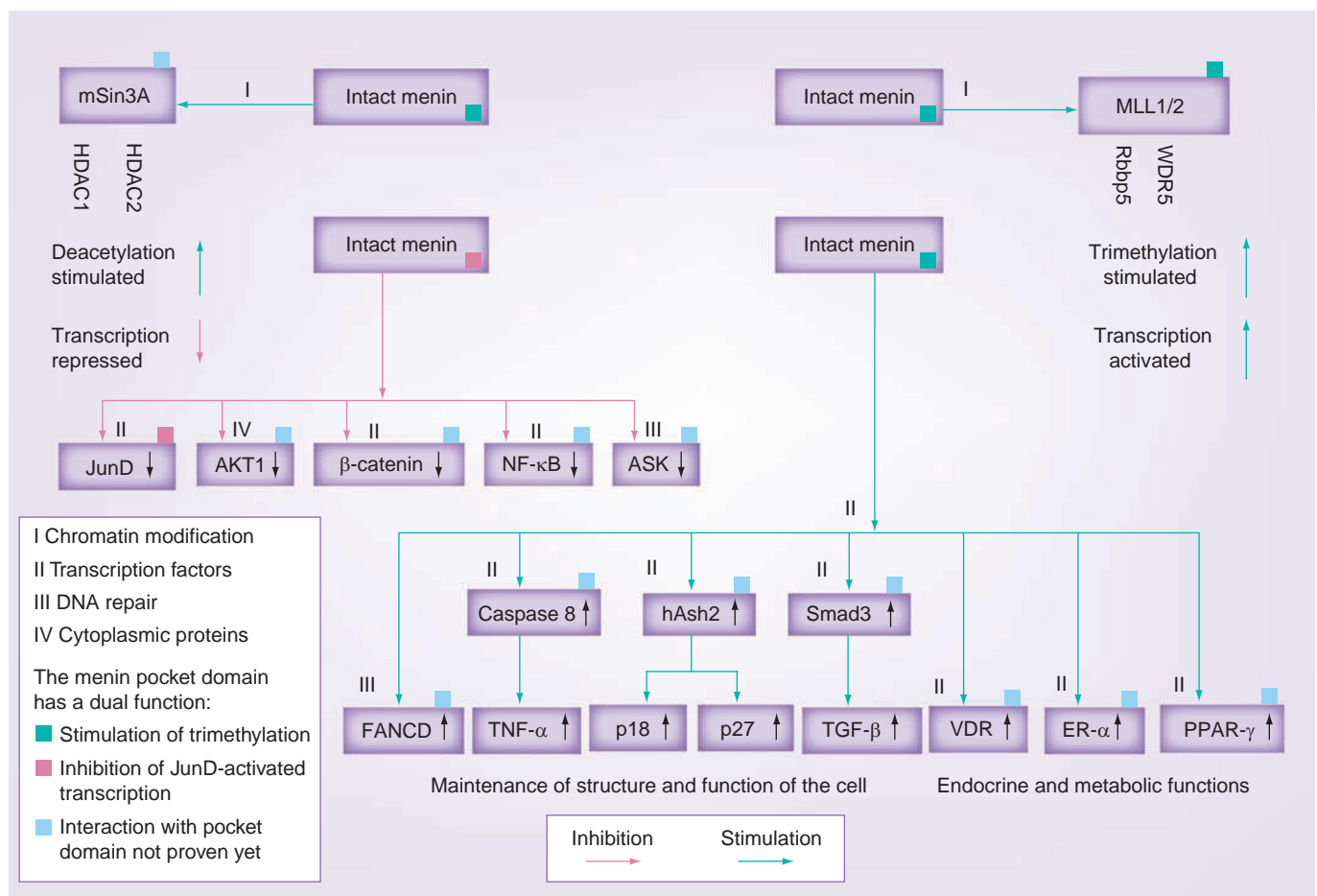
Figure 1. The normal function of menin. The normal function of menin is preservation of differentiation of the cell by modification of histone proteins and transcription of genes responsible for inhibition of cell division. Green arrows indicate stimulation of apoptosis, cell differentiation, DNA repair and endocrine metabolic functions, and inhibition of cell division is indicated in pink. In multiple endocrine neoplasia type 1 tumors, inactivating mutations in the *MEN1* gene result in alterations of histone protein modifications: both deacetylation (left) and trimethylation (right) are repressed. Consequently, the normal function of menin acting as corepressor and coactivator of gene transcription is disabled.
AKT: Serine/threonine protein kinase (also known as PKB); ASK: Activator of S-phase kinase; E2: Estradiol; ER: Estrogen receptor; FANCD: Fanconi anemia group D2 protein; GSK3: Glycogen synthase kinase; hAsh2: Human absent, small or homeotic discs; HDAC: Histone deacetylase; VDR: Vitamin D receptor.

Recently, the crystal structure of menin in *Nematostella vectensis* was reported [5]. It appears that the consensus interaction motif LXXLL of menin is well conserved and is located in an α-helix.

Huang *et al.* determined the crystal structure of human menin in its free form and presented complexes with MLL1 or JunD peptides. They showed that the same pocket domain in menin binds both MLL and JunD but has opposite effects on transcription. So, as expected, in the interaction with MLL and JunD the menin molecule appears to have a dual function instead of acting as a bridging factor [6]. On the other hand, the structural basis for the molecular control mechanism of cofactor binding to homo- and heterodimer nuclear receptors was recently published [7]. In general, modeling gene mutations into this structure will be helpful in determining the effects on protein function.

Menin is active in many functions, both in early development, as well as in repression of cell proliferation. *MEN1*-null mutant mice have indicated that menin is essential for viability [8]. Menin is involved in pancreatic islet cell development and function. Later on, it is active in many cellular processes, including gene transcription regulation, DNA replication, DNA repair and signal transduction.

Menin target genes that are important for development and proliferation include homeobox domain (*HOX*) genes, the *CDKN2C* and *CDKN1B* cyclin-dependent kinase-inhibiting genes, the human telomerase (*hTERT*) gene and nuclear receptor target genes [1,9–11]. The MEN1-related genes *MLL*, *p18* and *p27* are predominantly expressed in endocrine organs like pituitary and pancreatic islets [12].

Loss of menin function & tumor growth

Homeobox genes are potential targets for menin during embryonic development. Disruption of the *MEN1* gene in mice results in defects in development of multiple organs, including pancreatic islets. Homozygous disruption of the *MEN1* gene causes embryonic lethality [8]. A *MEN1* gene mutation may be completely detrimental to the gene's function. It may also result in a protein product with some residual function. An aberrant menin protein may be impaired in its function by several mechanisms, as menin can interact with many different proteins. Possibly, germline mutations in the *MEN1* gene selectively affect the binding of menin to its partners, leading to distinct phenotypes.

The type of missense mutation (e.g., replacement of arginine with glycine) may have a differential effect on the function of menin [13]: in-frame or missense mutations differ from frameshift/nonsense mutations [14]. Missense and in-frame mutations may affect the interactions of a menin domain with transcription factors such as JunD, Smad3 and NF-κB and nuclear receptors [1], or impair sensitization to apoptosis from caspase-3, p53 or p21 [15]. A *MEN1* gene missense mutation may result in protein instability and enhanced and early proteolytic degradation via the ubiquitin–proteasome pathway [16–18].

It was generally assumed that, in contrast to MEN2, in MEN1 there is no clear genotype–phenotype correlation [3,4]. However, several reports challenge this assumption [19].

Familial aberrant expression

Mild- versus late-onset phenotypes

To date, several disease-related *MEN1* gene intron mutations have been reported [3]. These intron mutations may affect mRNA splicing and cause mild phenotypes, with late and relatively low penetrance of the disease [20]. However, clinical expression at a young age may occur. This may be explained by interpatient variations in gene transcription and translation of the *MEN1* gene [21].

MEN1 Burin

Four large kindreds from the Burin peninsula/Fortune Bay area of Newfoundland were investigated [22–24]. They showed prominent features of prolactinomas, in addition to carcinoids and parathyroid tumors. A nonsense mutation in the *MEN1* gene has been found to be responsible for the disease in the affected members in all four of the MEN1 Burin families. This suggests that either a common ancestral mutation in the *MEN1* Burin gene or a modifying gene on 11q13 is responsible for this prolactinoma variant of MEN1 [21]. It would appear that they do not represent an early stage of MEN1.

Familial isolated hyperparathyroidism

Familial isolated primary hyperparathyroidism (pHPT) is an autosomal dominant disorder that can represent an early stage of either MEN1 caused by an allelic variant of the *MEN1* gene, or of hyperparathyroidism (HPT)-jaw tumor syndrome. Alternatively, the condition can be caused by an allelic variant of the *HRPT2* (hyperparathyroidism 2) gene, or caused by another mutant locus. Interestingly, the major proportion of *MEN1* gene germline mutations that have been found in familial isolated pHPT are seemingly mild missense mutations or in-frame deletions [25].

Useful information about the stability of specific menin mutants was obtained by investigation of proteasomal degradation. It appeared that certain protein mutants caused by missense and in-frame mutations are fairly stable and retain intrinsic biological activity. They may be associated with incomplete clinical phenotypes [18].

More malignant phenotypes: predominant mutations in MEN1 pancreatic NETs

Schaaf performed mutation analysis of the *MEN1* gene in tumors from 306 patients with MEN1, and found that patients with gastroenteropancreatic tumors more often had truncating mutations, very likely leading to completely inactivated menin [26].

Two recent case reports showed families with a high penetrance of malignant NETs of the pancreas [27,28]. Both these families carried germline mutations that completely abolish menin function.

Functional analysis of the mutant protein revealed severely reduced protein stability [16], reduced binding to JunD [21], reduced binding to the estrogen receptor-α [29] and loss of histone methylation recruiting capacity. Thus, functional analysis of this

potentially mild missense *MEN1* gene mutation shows that the protein product is in fact completely inactivated.

Tumor-promoting factors
Effect of gender
The prevalence and probability of pancreatic tumors among patients with MEN1 were higher in males than in females. This difference was due to the differential occurrence of gastrinomas [30]. The prevalence and probability of developing pituitary adenomas were significantly greater in females. Thymic tumors are found nearly exclusively in males with MEN1 [30]. However, a different distribution was found in Japan [31].

The difference in clinical expression between the genders may be explained by difference in transcription regulation of estrogen and androgen receptors. Menin can act as a coactivator of nuclear hormone receptors, including estrogen and possibly androgen receptors. A defect in the *MEN1* gene together with gender-specific differences in concentrations of the hormones involved and tissue-specific distribution of their receptors may contribute to the observed gender-specific differences in the prevalence of prolactinoma and gastrinoma.

Additional genetic effects
Loss of heterozygosity: the *AIP* gene
In agreement with the tumor suppressor function of the menin protein, MEN1-associated tumors reveal loss of the nonmutated (wild-type) allele of the *MEN1* gene. This second genetic defect is not present in the germline but is a somatic mutation, and often involves the deletion of multiple genes in the same chromosomal region (loss of heterozygosity).

The chromosomal locus containing the gene encoding the aryl hydrocarbon receptor-interacting protein (AIP) is in the same region on 11q13 that also contains the *MEN1* gene. Recently, it has been demonstrated that inactivating mutations in this *AIP* gene are associated with predisposition to pituitary adenoma [32], indicating that *AIP* can also act as a tumor suppressor [33,34]. The tumor suppressor genes *AIP* and *MEN1* are located 3 Mb apart.

Concomitant deletions of these genes may underlie predisposition to MEN1 and pituitary adenoma. Co-occurrence of mutations in the *AIP* gene and the *MEN1* gene is under investigation and may be studied in double-heterozygote animal models [35,36].

Genetic predisposition for other diseases
Genetic predisposition for other diseases may contribute to the enhancement of tumor formation in patients with MEN1 [37]. For example, normally the vitamin D receptor (VDR) on parathyroid cells inhibits the production and release of parathyroid hormone. In families with inactivating mutations in the gene encoding VDR, this is associated with end-organ resistance to calcitriol.

In the parathyroid glands of patients with MEN1, a decreased activation of the VDR exists. An additional defect in the VDR or calcium receptor may contribute to hyperactivity, hyperplasia and adenoma [38].

Additional somatic mutations involved in the acceleration of tumor growth
Data from other familial NET syndromes
– Phenocopies disclose similarities in pathways
How can we identify additional acquired mutations that are responsible for acceleration of tumor growth in MEN1? Clues for modifier genes may be found in other familial NET syndromes like MEN2 and MEN4; the latter is also referred to as MENX. Which are their traditional pathogenetic pathways, and are these involved in aberrant clinical MEN1 expression? Overlap between MEN1 and MEN2 and additional genetic events has to be explored (e.g., *p18/p27*-knockout mice develop both MEN1- and MEN2-associated tumors) [39,40].

Phenotypic overlap between MEN1- and MEN2-like syndromes was identified in the rat and named MENX. The syndrome is caused by a germline inactivating mutation in the *CDKN1B* gene, encoding p27Kip1 [41]. The protein p27Kip1 has a key role in cell cycle regulation, and is involved in differentiation, apoptosis and angiogenesis.

Subsequently, germline mutations in the *CDKN1B* gene were identified in the germline of a MEN1-like phenotype family. In these patients with pHPT and a growth hormone (GH)-producing pituitary tumor, germline mutations of the *MEN1* gene could not be detected [41]. The patients were also negative for *RET* gene mutations (MEN2). Mutations in the *CDKN1B* and related genes, but without *MEN1* or *MEN2* gene mutations, are a rare cause of this MEN1-like phenotype [42–44]. As a consequence of mutations in the *p27* gene, a novel human MEN syndrome was recognized and named MEN4. The phenotypic features associated with MEN4 are still poorly defined due to the small number of patients reported.

In mice, another cyclin-dependent kinase inhibitor p18Ink4c encoded by the *CDKN2C* gene was shown to inhibit tumorigenesis in endocrine tissues by collaboration with menin [45]. In transgenic MEN2 mice and humans with MEN2, inactivating mutations in *p18* will promote tumor growth [39,40,46,47].

Reduced expression of *CDKN1B*, but not *CDKN2C*, has been observed in parathyroid adenomas from patients with MEN1, suggesting that p27 may collaborate with menin in the suppression of MEN1 tumorigenesis in humans [38].

The association of gastric carcinoids (GCs) and HPT in families is not always related to MEN1. This appears to constitute a distinct syndrome that can be encountered in genetically predisposed individuals, and should not be regarded as 'atypical' or 'incomplete' expression of MEN1. Its prevalence and etiology should be the subject of future studies. Screening for HPT seems to be justified in patients with GC of any type [48].

Clues from sporadic parathyroid adenomas, pituitary tumors & pancreatic NETs: the NET pathway
– Parathyroid adenoma
A high rate of somatic *MEN1* gene mutations is seen in sporadic parathyroid adenomas. An interaction exists between menin and the TGF-β/Smad signaling pathway. *In vitro* experimentation has demonstrated that the presence of menin is required for TGF-β to effectively inhibit parathyroid cell proliferation and parathyroid hormone production [49].

In sporadic parathyroid adenomas, somatic mutation and germline sequence abnormalities in *CDKN1B*, encoding p27Kip1, were detected in 4.6% [50]. MEN2A is caused by activating mutations of the *RET* proto-oncogene encoding a receptor tyrosine kinase. Both reduced menin activity and receptor tyrosine kinase activation can cause downregulation of p18 and p27, indicating a common pathway in these two MEN syndromes (FIGURE 2).

In MEN1, inactivating mutations of the *MEN1* gene affect the function of the VDR. Normally, vitamin D inhibits parathyroid cells from producing and releasing parathyroid hormone. In patients with MEN1, impaired action of vitamin D may contribute to hyperplasia of the parathyroid glands. Cell division of parathyroid cells may make them more sensitive to additional mutations [38].

Pituitary adenoma

The pituitary tumor-transforming gene (*PTTG*; securin) was the first transforming gene found to be highly expressed in pituitary tumor cells, and seems to play an important role in the process of oncogenesis. Cell signaling abnormalities have been identified in pituitary tumors, but their genetic basis is unknown. Both the Raf/MEK/ERK and PI3K/AKT/mTOR pathways are overexpressed and/or overactivated in pituitary tumors [51]. These pathways share a common root, including initial activation by a tyrosine kinase receptor.

Pit-1 is a direct transcriptional target of VDR. Recruitment of histone deacetylase 1 is involved in the repressive effect of VDR on *Pit-1* expression [52]. There is a critical role for the growth factor activin in regulating the inhibition of pituitary cell growth and Pit-1/PRL expression through the Smads and menin [53]. Alterations in the activin/TGF-β downstream signaling pathways may be critical steps toward tumor formation and progression [54]. Until now, the occurrence of additional TGF-β, Pit-1, PTTG or VDR mutations in MEN1-associated tumors has not been not published.

Pancreatic NETs

In nonfamilial pancreatic NETs (pNETs), the most commonly mutated genes specify proteins implicated in chromatin remodeling: 44% of the tumors had somatic inactivating mutations in *MEN1*. Clinically, mutations in the *MEN1* gene were associated with better prognosis. Furthermore, mutations in genes in the

mTOR pathway were found in 14% of the tumors, a finding that could potentially be used to stratify patients for treatment with mTOR inhibitors [55].

Loss of menin expression is associated with overexpression of the Raf/MEK/ERK and P13K/AKT/mTOR pathways in pancreatic tumors in a mouse model [56]. Intact menin has an essential role in Wnt/β-catenin signaling and inhibits mouse pancreatic islet tumor proliferation [57]. Menin regulates subcellular localization of β-catenin via nuclear–cytoplasmic shuttling. Loss of menin leads to Wnt/β-catenin signaling activation. Expression of p27 was found to be repressed in pancreatic islet cell tumors [58].

Pathways in multistep carcinogenesis

It appears that interaction of components of the PI3K/AKT pathway is involved in NET formation [59,60]. Deregulation of the PI3K/AKT pathway in NETs can occur through a range of processes – for example, see FIGURE 2.
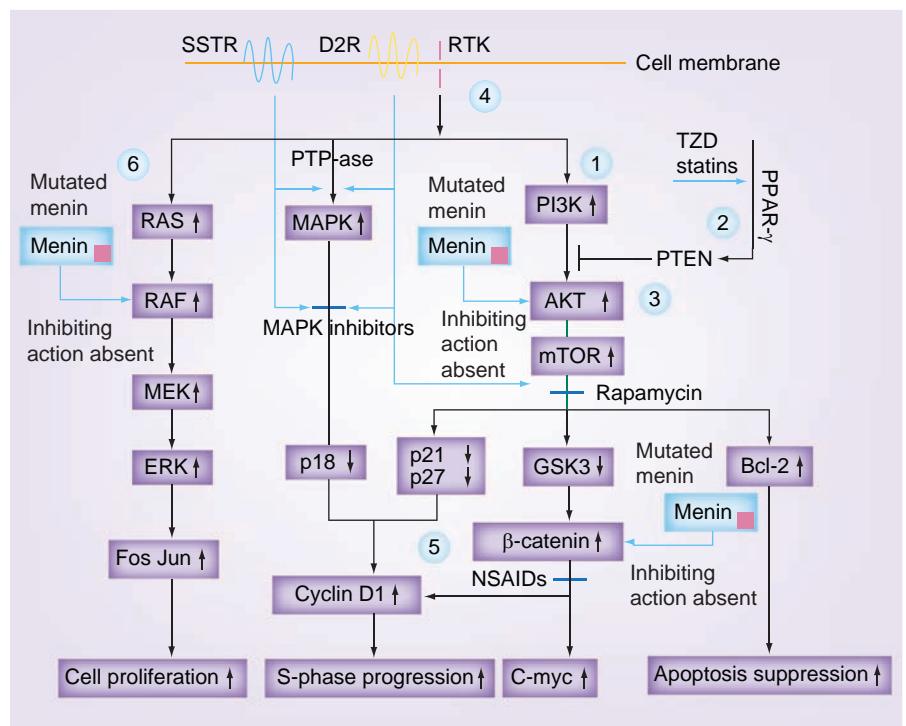
Figure 2. Additional mutations in MEN1 neuroendocrine tumors. Beyond inactivation of the *MEN1* gene, additional mutations in other genes may be responsible for acceleration of tumor growth, thus involving a process of multistep tumorigenesis. Deregulation of normal neuroendocrine development/differentiation can occur through a range of processes – for example, activation of the PI3K pathway leads to AKT phosphorylation, triggering a downstream cascade of events. Deregulation of this pathway can occur through several mechanisms: **(1)** Gain of function by oncogenic mutations of PIK3; **(2)** Loss of function of the tumor suppressor PTEN through gene deletion, mutation, miRNA expression, or epigenetic silencing; **(3)** Amplification or mutation of AKT isoforms; **(4)** Upstream activation through RTK signaling; **(5)** Downstream, loss of the tumor suppressors p18 and p27; **(6)** Increased RAS expression or activating mutations of RAF, MEK or ERK also will accelerate cell proliferation. Opportunities for treatment are represented in blue.
AKT: Serine/threonine protein kinase (also known as PKB); Bcl-2: B-cell lymphoma 2; D2R: Dopamine-2 receptor; GSK3: Glycogen synthase kinase; JNK: c-Jun-*N*-terminal kinase; PTEN: Phosphate and tensin homolog; PTP-ase: Phosphotyrosine phosphatase; RTK: Receptor tyrosine kinase; SSTR: Somatostatin receptor; TZD: Thiazolidinedione.

Gain of function occurs by oncogenic mutations of PI3K signaling, while loss of function of the tumor suppressor PTEN occurs through gene deletion, mutation, miRNA expression or epigenetic silencing, upstream activation through receptor tyrosine kinase signaling and/or downstream loss of the tumor suppressors p18 and p27.

A combination of a mutation in the *MEN1* disease gene with other specific mutations of genes in the PI3K/AKT pathway may be associated with acceleration of tumor growth. In addition, inactivation or absence of menin promotes RAS expression, while activating mutations of RAF, MEK or ERK may accelerate cell proliferation [61].

Ecogenetic factors
Common environmental factors may interact with genetic predisposition to MEN1 and contribute to enhancement of tumor formation [37]. For example, in patients with MEN1, low vitamin D or calcium levels may promote parathyroid gland hyperplasia and adenoma formation, whereas estrogenic or neuroleptic drugs may promote prolactinoma formation in the pituitary. Similarly, use of histamine-H2 receptor or proton-pump blockers may stimulate cell proliferation and hence tumorigenesis in the stomach (gastrinoma) [62]. Of course, as with many other types of tumors, the very general environmental factors smoking and exposure to radiation may also promote MEN1 tumorigenesis.

Clinical presentation, diagnosis & treatment
Parathyroid adenomas
Approximately 75–95% of patients with MEN1 develop parathyroid hyperplasia [63]. In MEN1, HPT has a milder presentation, but is associated with lower bone mineral density compared with sporadic HPT [24].

In MEN1, pHPT may be asymptomatic and therefore in a proband is often unrecognized [64]. Subtotal parathyroidectomy is the best surgical therapy for pHPT in MEN1. In addition, a thymectomy should routinely be performed in these patients [65]. Patients suffering from MEN1 with pHPT should not be treated with less than subtotal parathyroidectomy because of the unacceptably high rate of recurrent and persistent pHPT. In general, even subtotal parathyroidectomy results in a recurrence rate of >10%. Recurrence may occur in an initially considered normal gland, in a remnant of a partially excised abnormal gland, and due to a supernumerary gland [66].

Cryopreservation and autotransplantation of parathyroid glands should be performed only in experienced centers, and used only for patients with hyperplastic parathyroid tissue. Tissue samples should be systematically destroyed when patients do not have hypoparathyroidism or after 1 year of storage, to avoid accumulation of ungrafted samples with all risks involved [67].

In MEN1, HPT is associated with a high recurrence rate and high surgical morbidity due to multiple neck explorations. Cinacalcet, a calcimimetic agent, is an effective and well-tolerated medical treatment for the management of complex pHPT [68].

Endocrine duodenum & pancreas
Approximately 55% of patients with MEN1 develop pNETs [69]. Multicentric microadenomas are present in 90% of these patients [70]. Hormonal syndromes often occur late and indicate metastases in 50% of patients with MEN1 [71]. Therefore, prospective screening with biochemical markers and endoscopic ultrasound (EUS) is indicated. EUS evaluation reveals that the frequency of nonfunctioning pNETs is as high as 55% [72]. They predominantly occur in the pancreas. Negative prognostic factors include the presence of metastases, invasion of adjacent organs and perineural or angioinvasion.

Diagnosis
Monitoring of pancreatic peptides and use of diagnostic imaging allow an early pancreatic resection, in conjunction with prevention of metastatic pNETs and improvement of long-term survival.

Visualization
EUS has a prominent role in the screening of asymptomatic mutation carriers with PET-hormone hypersecretion, and in the search for a pancreatic primary in case of a suspected lesion in the liver with unknown primary [73]. EUS and MRI are complementary [74].

pNETs can be visualized by ultrasound, MRI, somatostatin receptor scintigraphy, computed tomography and ^{18}F-dihydroxy-phenylalanine-PET. ^{11}C-5-hydroxytryptophane-PET seems to be more sensitive in imaging [75]. There is limited value of ^{18}F-dihydroxy-phenylalanine-PET in pancreatic insulinoma [76].

Treatment
The rationale for surgical considerations for pNETs is to curtail the malignant progression of the disease and to cure or aid in the management of the associated biochemical syndromes.

However, a conservative approach of treatment in nonfunctioning pNETs <20 mm in diameter is indicated. If a tumor is >3 cm in diameter and/or expanding, the tumor and the peripancreatic lymph nodes should be resected, together with duodenotomy to assess the presence of gastrinomas. Enucleation of tumors in the head of the pancreas, excision of duodenal gastrinomas, clearance of lymph-node metastases and distal pancreatectomy may be performed to prevent recurrence [71]. Insulinomas may also be candidates for enucleation.

Most patients with functional syndromes eventually develop malignant growth. Early aggressive surgery (diameter and >1 cm) may prevent liver metastases [77,78]. Prevention of spread should be balanced against potential operative morbidity and mortality. Hepatic metastatic lesions can be successfully treated by surgical resection [79].

Gastrinomas & Zollinger–Ellinger syndrome
In patients with MEN1 with Zollinger–Ellison syndrome (ZES), for localization of gastrinoma, selective arterial secretagogue injection test with secretin or calcium solution may be performed as well as somatostatin receptor scintigraphy. Optimal surgery may be performed if it is guided by localization with the selective arterial secretagogue injection test. Depending on extension, local resection

of duodenal gastrinomas with dissection of regional lymph nodes and for multiple duodenal gastrinomas pancreas-preserving total duodenectomy, may be performed. pNETs >1 cm in diameter are resected either by distal pancreatectomy or enucleations.

Complete resection of gastrinomas is based on accurate localization with the selective arterial secretagogue injection test and this may be useful for biochemical cure of gastrinoma. With this strategy, no hepatic metastases developed postoperatively [80].

Recurrence

Initial surgery of pancreaticoduodenal endocrine neoplasms may provide tumor control and prevent metastases, but recurrent tumors may be multifocal and progressive. In a large study, recurrence after initial surgery was observed in 16% of the patients. Cytoreductive surgery is recommended for patients with locoregional recurrences or hepatic metastases. Completion by pancreatectomy and duodenectomy seems arduous, but outcomes are acceptable. Considering the radical nature of this treatment, individual consideration should be given to patients with MEN1 amenable to initial alternative pancreatic resections that preserve pancreatic mass and allow future pancreas-preserving reoperations [81].

Vascular involvement

In 17% of patients with MEN1 with pNETs, computed tomography provided suspicion of major vascular involvement. Extensive resection with vascular reconstruction was associated with a better overall survival. These findings suggest that surgical resection of pNETs with vascular abutment/invasion and nodal or distant metastases is indicated [82].

Recently, it has been suggested that regardless of the stage of disease at presentation, the first-line therapeutic option for such patients is surgical resection [83]. For most MEN1 patients with pNETs, pancreatectomy with lymph-node sampling is indicated, either through open or laparoscopic techniques.

For patients with locoregional recurrences or hepatic metastases, regional adjuvant therapy, such as radiofrequency ablation or transarterial chemoembolization, are often used to reduce tumor load and symptoms and prolong survival. Unfortunately, chemotherapy is not usually in patients with extensive tumor load, although the somatostatin analog octreotide has shown some beneficial effects. Other systemic agents targeting specific signaling pathways, such as receptor tyrosine kinase inhibitors (sunitinib) and protein kinase inhibitors (everolimus), have also shown promising results [84].

Ectopic GH-releasing hormone production by a pNET was published recently and found in a woman with acromegaly. Secondary somatotroph hyperplasia in the pituitary gland was present [85,86].

Pituitary adenomas

In MEN1, pituitary tumors differ from common tumors: they may be larger, show multiplicity and occur at a young age. In a large group of patients with MEN1, pituitary adenomas occur in 42% of cases and are characterized by a larger size and a more aggressive presentation than without MEN1. MEN1 pituitary adenomas were significantly more frequent in women than in men (50 vs 31%; p < 0.001). Among the 136 pituitary adenomas, there were 85 prolactinomas and 12 GH-secreting, six adrenocorticotropic hormone (ACTH)-secreting, 13 cosecreting and 20 nonsecreting tumors. Overall, 85% of MEN1-related pituitary lesions were macroadenomas (vs 42% in patients without MEN1; p < 0.001), including 32% of invasive cases. No correlation was found between the type of MEN1 germline mutation and the presence/absence of pituitary adenoma [87].

MEN1-associated adenoma may show a similar expression profile to that seen in the sporadic mammosomatotropinoma (producing both GH and prolactin). In one individual with MEN1, a morbid prolactinoma occurred at the age of 5 years. Hence, this is the earliest age at which to recommend tumor surveillance in carriers [21].

In patients with MEN1, Cushing's syndrome (CS) from endogenous hypercortisolism can result from pituitary, adrenal or other endocrine tumors. In a total of 14 patients with CS, 11 had Cushing's disease (CD) and three had ACTH-independent CS due to adrenal tumors, frequencies indistinguishable from sporadic CS [88]. CD, which is caused by an ACTH-secreting pituitary adenoma, is associated with increased morbidity and mortality.

The majority of ACTH-secreting adenomas simultaneously express somatostatin-receptor subtype 5 and dopamine-receptor subtype 2. Pasireotide is a new somatostatin analog that binds with high affinity to somatostatin receptor subtypes 1, 2 and 3, and it has especially high-affinity binding to somatostatin receptor subtype 5. Pasireotide and the dopamine-receptor subtype 2 agonist cabergoline appear to have synergistic effects in the treatment of CD [89]. Combination of pasireotide with ketokonazol, which inhibits steroidogenic enzymes in the adrenal cortex, resulted in biochemical control in nearly 90% of patients.

Adrenals

MEN1-associated ACTH-independent CS may be associated with aggressive adrenocortical disease, and an etiology for CS in MEN1 may be elusive in a substantial minority of patients [88].

In a cohort of 715 patients with MEN1, adrenal enlargement was seen in 20%. Adrenal tumors (>10 mm in diameter) were detected in 58% of these patients. Tumors were bilateral and >40 mm in size in 12 and 19% of cases, respectively [89]. Hormonal hypersecretion was restricted to patients with tumors and occurred in 15.3% of them. Compared with incidentalomas, MEN1-related tumors exhibited more cases of primary hyperaldosteronism, fewer pheochromocytomas and more adrenocortical carcinomas (13.8 vs 1.3%) [90]. MEN1 is a high-risk condition for the occurrence of adrenocortical carcinomas. They should be considered regardless of the size of the tumor, they may be bilateral [91] and genotype–phenotype correlation has been suggested [92].

Carcinoid tumors

Since currently, in MEN1, parathyroid and pituitary disease can be diagnosed and treated effectively, foregut carcinoids deserve more attention, because they are becoming important determinants of survival.

Gastric carcinoids or enterochromaffin-like-cell tumors

The terms 'GC' or 'gastric NET' may also relate to tumors in the stomach, which originate from cells secreting gastrin or sero-tonin. Therefore, MEN1-related gastric tumors should prefer-ably be termed enterochromaffin-like (ECL) cell tumors. Gastrin stimulates ECL cells in histamine production and cell division. Free serum gastrin levels are strongly correlated with the α-hCG-immunoreactivity (IR), which is a marker for ECL cells. Thus, all patients with MEN1/ZES have some degree of ECL cell changes; in 53%, these changes are advanced (at least mild hyperplasia), and 23% have ECL cell tumors.

Since patients with MEN1/ZES are at risk for developing ECL cell tumors, they require regular gastroscopy with multiple biopsies of all parts of the stomach and systematic biopsies of all mucosal lesions to detect these carcinoids [93].

Clinical and laboratory data as well as biopsy results can be used to identify a subgroup of patients with MEN1/ZES with a significantly increased risk for developing GCs, allowing the development of better surveillance strategies.

The α-hCG-IR correlated significantly with the most advanced ECL cell change. A total of 63% of routine biopsies showed dif-fuse hyperplasia, 17% linear hyperplasia and 5.5% micronodular hyperplasia [93]. Treatment involves endoscopic polypectomy, sur-gical excision, associated or not with surgical antrectomy, or total gastrectomy. Moreover, the recent introduction of somatostatin analog represents a therapeutic option.

Thymus carcinoid

MEN1-related thymic carcinoid is often insidious with a poor prognosis. In a French MEN1 group, thymic tumors were exclu-sively found in males [30], whereas in Japan, 37% of MEN1 thymus carcinoid was found in females [31,94].

We suggest chest MRI for patients with MEN1, and serological or genetic screening for sporadic patients with thymic carcinoid to screen for MEN1 (Box 1).

Most thymic NETs in MEN1 are nonfunctional; however, ectopic ACTH can be produced by these tumors in the context of MEN1 [95]. In addition, thymus carcinoid may also produce ectopic GH-releasing hormone [96].

Bronchus carcinoid

Carcinoid tumors of bronchial origin are rare in MEN1. The prev-alence of histologically confirmed cases is approximately 5–8%, although in more recent studies it is estimated that it could be much higher and a possible relationship with the presence of hypergastrinemia is suggested [30,97].

Other tumors

Prostate carcinoma

Among male mice with a heterozygous knockout of the *MEN1* gene, an increased prevalence of prostate cancer has been detected [81]. Expression of the p27-encoding *CDKN1B* gene, a transcrip-tional target of menin, which is known to be inactivated in prostate tumorigenesis, was downregulated in the prostate cancers of these mice. In addition, expression of the androgen receptor (*AR*) gene,

another putative target of menin, was altered in these tumors as compared with *AR* gene expression in prostate glands of wild-type control mice [98].

Breast cancer

Among female mice with a heterozygous knockout of the *MEN1* gene, 8% had breast cancer [98]. Perhaps p27 may be downregulated in some of these tumors. Intact menin binds to the estrogen receptor and may enhance its activity in breast cancer cells. In patients with MEN1, however, this promoting factor is not available and this may attenuate cell division and predict tamoxifen resistance [99].

The simultaneous occurrence of mutations in two different tumor suppressor genes in the same individual is a very rare event. However, a germline mutation in the *BRCA1* gene in a woman with familial MEN1 was observed, and resulted in a combination of MEN1 and an inherited breast–ovarian cancer syndrome [100]. The severity of MEN1-related biochemical and clinical findings did not significantly differ from that of other affected family mem-bers lacking the *BRCA1* mutation, except for the development of an extremely large visceral lipoma.

Lipoma & hybernoma

Menin is an important factor in PPAR-γ-mediated adipogenesis, and loss of PPAR-γ function may contribute to lipoma develop-ment in patients with MEN1 [101]. A balanced translocation deleting the wild-type *MEN1* allele primed the lipoma develop-ment [102]. Hibernomas are benign tumors with morphological features resembling brown fat. They consistently display cytoge-netic rearrangements, typically translocations, involving chromo-some band 11q13. Hibernomas are associated with concomitant deletions of *AIP* and *MEN1*, located 3 Mb apart. MEN1 and AIP displayed a low expression in hibernomas [103]. Compared with lipoma, in hibernoma there are low transcript levels of *MEN1* and *AIP*.

Metabolic effects of aberrant expression of the *MEN1* gene

In *MEN1* disease gene carriers, all VDRs and PPAR-γs are expressed, but are probably less activated owing to impaired menin function.

PPAR-γ-2 is a transcription factor that has a key role in adipo-cyte differentiation. Polymorphisms in this gene may contribute to the variability in BMI and insulin sensitivity in the general population. PPAR-γ is the receptor for the thiazolidinediones, which act as PPAR-γ agonists and lower the blood glucose levels in patients with Type 2 diabetes mellitus by increasing insulin sensitivity.

Individuals with dominant-negative *PPAR-γ* gene mutations manifest a syndrome that combines lipodystrophy with features of the metabolic syndrome, including insulin resistance, Type 2 diabetes, hepatic steatosis, dyslipidemia, hypertension and polycystic ovary syndrome in women. In patients with MEN1, decreased activation of PPAR-γ may result in insulin resistance and weight gain [104].

Box 1. Periodic clinical screening.

Eligible for periodic clinical monitoring are:

- MEN1 patients
- *MEN1* gene germline mutation carriers
- MEN1-suspected patients without a confirmed mutation: DNA analysis has to be recommended and performed in advance

Periodic screening includes[†]:

- From the age of 10 years: biannual clinical examination:
  - Medical history: are suspected symptoms present?
  - Laboratory investigation:
    - Including measurement in blood of: total/ionized calcium, chloride, phosphate, parathyroid hormone, glucose, insulin, C-peptide, glucagon, gastrin, pancreatic polypeptide, prolactin, IGF-I, platelet serotonin and chromogranin A
- From the age of 15: once every 2 years[†]:
  - MRI of the upper abdomen
  - MRI of the pituitary with gadolinium contrast
  - MRI of the mediastinum in males and females (annual screening preferred)

[†]In families with early manifestation of the disease or if suspicion arises because of clinical symptoms, early screening is recommended; if clinical suspicion regarding insulinomas is present, a fasting test should not be postponed; use of proton-pump inhibitors or H2-receptor blockers may increase gastrin and chromogranin A levels; oral contraceptives may increase prolactin levels.
MEN1: Multiple endocrine neoplasia type 1.

VDRs are found in a large number of tissues beyond the classic target tissues gut, bone and kidney. These tissues include endocrine glands such as the pituitary and parathyroid glands, and pancreatic islets, among others.

Lourenço *et al.* discussed the increased bone loss pattern found in patients with MEN1 compared with that of patients with sporadic primary HPT [24]. Besides increased bone loss, resistance to vitamin D may be associated with insulin resistance and β-cell dysfunction, leading to increased risk for Type 2 diabetes in patients with MEN1 [105,106].

Screening

In MEN1-suspected patients, mutational analysis helps to confirm the clinical diagnosis. It may identify asymptomatic family members who have close relatives with a *MEN1* gene mutation. Otherwise, they require periodical screening from an early age. Genetic screening may also identify the 50% of family members who do not have the *MEN1* mutation and can therefore have the burden of screening and anxiety regarding potential disease removed. Moreover, MEN1 mutational analysis helps to resolve diagnostic challenges due to phenocopies, which occur in 5–10% of families with MEN1.

Active MEN1 family investigation is strongly recommended. The successful identification of patients with MEN1 among close relatives will result in an increase in the number of patients requiring clinical screening. Surveillance and treatment have implications in the planning of future service provision.

An early genetic diagnosis allows early periodical screening. It facilitates clinical management and provides benefits to patients and families concerning morbidity, quality of life and life expectancy [107,108]. In children, *MEN1* gene mutational analysis should be advised before periodical screening is started, in other words at the age of 10 years. However, parents should be free to decide to carry out DNA analysis at an earlier age. Information about screening may be provided from the age of 5 years (**Box 2**).

Owing to the variety in expression, it is not easy to establish general guidelines for periodical screening. In some families, the age at which symptoms and signs occur is quite young (**Box 1**) [21,109].

The development of a dedicated multidisciplinary MEN clinic will improve the diagnosis and treatment of MEN1-associated endocrinopathies [110]. A case manager who has an overview and primary responsibilities is needed. This clinician knows the medical history of the patient and has an affinity to this complex disease.

Box 2. Suggested criteria for *MEN1* gene mutation analysis.

***MEN1* gene mutation analysis should be offered to:**

- Patients with MEN1
- Patients with three out of five major MEN1-associated lesions: parathyroid adenomas, pancreatic islet cell tumors, pituitary adenomas, adrenal adenomas and NETs
- First-degree family members (parents, brothers, sisters and children) of patients with MEN1 or asymptomatic *MEN1* gene germline mutation carriers; in children before periodical screening is started – in other words, at the age of 10 years
- MEN1-suspected sporadic patients: with two out of the five major lesions, two MEN1-associated tumors within one organ and/or a MEN1-associated tumor at a young age (<35 years)

MEN1: Multiple endocrine neoplasia type 1; NET: Neuroendocrine tumor.

Conclusion

Observation of the expression of MEN1, and collaboration among disciplines including genetics and molecular endocrinology, opened up new directions in the management of MEN1 syndrome and promoted the development of novel target-directed therapy. Since 1980, life expectancy and quality of life have improved considerably.

MEN1 is a disease with variable expression that may occur at a young age; the symptoms may be general and the lesions small. At present, patients become aged and may show metabolic disorders like osteoporosis, obesity and diabetes mellitus as a consequence of menin inactivation.

Nucleotide sequence analysis enables the identification of *MEN1* disease gene carriers. Periodic clinical monitoring enables presymptomatic detection of expression. DNA diagnosis should be established before the age of 10 years.

Malignant thymic tumors and duodenopancreatic tumors, including nonsecreting pancreatic tumors, ECL tumors of the stomach and rare but aggressive adrenal carcinomas, increase the risk of early death. In *MEN1* disease gene carriers in MEN1 families, most deaths were related to progressive disease and probably resulted from additional mutations [111].

It is possible that consecutive and specific pathogenetic pathways are involved in MEN1 tumor formation. We presume that, through the inherited germline mutation resulting in organ-specific cell division, the patient is vulnerable to additional, somatic mutations in these organs. These mutations may occur spontaneously or may be triggered by lifestyle and/or environment. Predisposition and expression of other genetic diseases may also be involved.

A complex genotype–phenotype relationship may be present. Unfortunately, currently in the majority of patients, it is not possible to predict the course of the disease.

Germline mutations that result in complete inactivation of the gene product, apparently cause more severe disease. Consequently, extensive periodic clinical examination should be performed in all *MEN1* disease gene carriers to detect progressive disease.

Expert commentary

Family investigation and genetic linkage studies have led to identification of the gene that has germline mutations in MEN1. Periodic clinical screening of patients has improved the life expectancy and quality of life. The mechanism of disease on a cellular level was investigated and cell type-dependent and specific signaling pathways have been established.

The risk for development of malignant tumors may justify early preventive surgical treatment. Genetic testing involves loss of privacy and autonomy, stigmatization and discrimination, and psychological care in attendance of families is indispensible. Furthermore, social attitude to rare hereditary diseases has to be adapted and preventive care needs to be facilitated; a safety net must involve clinical staff, laboratory facilities, patient and family registration, and practical nurses coordinating clinical care. Currently, these facilities are not incorporated into clinical care. In the future, these should be financed by health insurance companies

and/or the government, and standardized guidelines will need to be maintained and adapted continuously.

Five-year view

Until now, collaboration among basic scientists and a network of physicians resulted in improvement in the management of patients with MEN1. In the near future, however, subspecialization in medicine and lack of knowledge on molecular endocrinology in medical specialists may slow down this progress. Who has general responsibility for patients and an overview about opportunities in treatment?

Early DNA analysis allows identification of predisposition to MEN1; considering DNA analysis as a clinical test would relieve noncarriers from the burden of periodical investigations. Moreover, the clinical tests are expensive, and discussions about reimbursement of these tests by insurance companies without identification by DNA analysis are ongoing. Thus, in children, DNA analysis is recommended before periodical screening is started – in other words, at the age of 10 years.

Functional analysis of menin protein mutants is required to study the true effect of specific *MEN1* gene mutations. In embryonic stem cells, menin-dependent development of tissue specificity will be studied. In the near future, tumor gene-expression profiles and high-throughput sequencing may permit more insight into additional genetic and epigenetic events and mechanisms in multi-step carcinogenesis that cause progression of tumor development. Target-directed treatment for tumors with additional mutations and aggressive behavior will be available.

Preimplantation genetic diagnosis enables selection of embryos without *MEN1* disease gene mutations for implantation. Preimplantation genetic diagnosis is an alternative that should be offered to prospective parents, and represents a method to decrease and potentially eliminate the disease transmission of MEN1 [112]. Increasing the awareness of clinicians to the availability of these methods is most important.

miRNAs inhibit protein translation from the target mRNA. The miRNA-expression profiles may become useful biomarkers for endocrine tumor development.

Experimental studies have shown that dysregulation of miRNAs is responsible for endocrine carcinogenesis, including pancreatic, pituitary and parathyroid tumors. Depending on the expression profile, miRNAs may become potential targets for therapeutic strategies [113].

siRNAs are a class of double-stranded RNA molecules, 20–25 nucleotides in length, which may have a silencing effect on RNAs responsible for cell division [114]. This may be a basis for the development of RNA-based therapies in *MEN1* gene mutation carriers.

The significance of MEN1 Patient Alliances or Interest Groups is as follows:

• They may support individual problems, such as loss of privacy, stigmatization, discrimination, emotional distress and managing psychological problems;

• They are promoting the maintenance of continuous clinical care, and may discuss the costs of preventive periodical investigation;

- They stand up for common interests like social, ethical, insurance and employment issues;

- At this moment and in the future, Family Interest Groups are of essential value, and they will play a prominent role and promote guidance and care;

- They are becoming a necessary supplement to modern, specialized medicine.

Key issues

- A clear definition for patients with clinically suspected multiple endocrine neoplasia type 1 (MEN1) promotes identification: a case with at least two out of the three main MEN1-related endocrine tumors (parathyroid hyperplasia/adenomas, enteropancreatic endocrine tumors and pituitary tumors).

- Malignancy: most harmful MEN1 tumors are pancreatic–duodenal gastrinomas, thymus carcinoid, enterochromaffin cell-like tumors of the stomach and adrenal carcinomas.

- MEN1 is caused by mutations in the *MEN1* tumor supressor gene; DNA analysis permits identification presymptomatically.

- Patients with MEN1, their family members, family members of *MEN1* gene mutation carriers and patients who are clinically suspected to be carriers of a *MEN1* gene mutation are eligible for *MEN1* gene mutation analysis.

- MEN1-associated tumors can be detected and treated at an early stage through periodic clinical monitoring of *MEN1* gene mutation carriers according to guidelines.

- Patients interest groups or alliances may help in medical, psychological, social and ethical problems.

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