Thematic Review

MEN4 and CDKN1B mutations: the latest of the MEN syndromes

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Abstract

Multiple endocrine neoplasia (MEN) refers to a group of autosomal dominant disorders with generally high penetrance that lead to the development of a wide spectrum of endocrine and non-endocrine manifestations. The most frequent among these conditions is MEN type 1 (MEN1), which is caused by germline heterozygous loss-of-function mutations in the tumor suppressor gene MEN1. MEN1 is characterized by primary hyperparathyroidism (PHPT) and functional or nonfunctional pancreatic neuroendocrine tumors and pituitary adenomas. Approximately 10% of patients with familial or sporadic MEN1-like phenotype do not have MEN1 mutations or deletions. A novel MEN syndrome was discovered, initially in rats (MENX), and later in humans (MEN4), which is caused by germline mutations in the putative tumor suppressor CDKN1B. The most common phenotype of the 19 established cases of MEN4 that have been described to date is PHPT followed by pituitary adenomas. Recently, somatic or germline mutations in CDKN1B were also identified in patients with sporadic PHPT, small intestinal neuroendocrine tumors, lymphoma and breast cancer, demonstrating a novel role for CDKN1B as a tumor susceptibility gene for other neoplasms. In this review, we report on the genetic characterization and clinical features of MEN4.

Introduction

Among the multiple endocrine neoplasia (MEN) syndromes, the most frequent is type 1 or MEN1 (OMIM #131100). MEN1 is characterized by primary hyperparathyroidism (PHPT) due to parathyroid gland hyperplasia, and functional or nonfunctional pancreatic neuroendocrine tumors (pNETs) and pituitary adenomas (Thakker et al. 2012). MEN2, a less common entity, is characterized by medullary thyroid carcinoma (MTC), pheochromocytoma and PHPT. MEN2 is further divided into MEN2A (OMIM #131100) that typically manifests with MTC, pheochromocytoma, and PHPT and MEN2B (OMIM #162300) that manifests with MEN2A features, although typically lacking PHPT, ganglioneuromas of the lips, tongue and colon and a marfanoid habitus (Brandi et al. 2001). The genetic cause of MEN1 was initially localized to 11q13 through positional cloning (Larsson et al. 1988, Chandrasekharappa et al. 1997), and later identified as germline heterozygous loss-of-function mutations in the tumor suppressor gene MEN1 (Agarwal et al. 1997), which consists of 10 exons and codes for the protein menin. The genetic defect in the MEN2 syndromes is due to mutations in the RET (rearranged in transfection) proto-oncogene on chromosome 10q11.21 (Brandi et al. 2001). Mutations in MEN1 have been detected
in ~90% of familial cases (including germline deletions) and fewer among patients with sporadic MEN1 (Brandi et al. 2001, Thakker et al. 2012). The numbers are higher for patients with one of the MEN2 syndromes: almost all with MEN2A have one or the other RET mutation (Brandi et al. 2001). Overall, approximately 10% of patients with familial MEN1-like phenotype and many more with sporadic MEN1 have negative MEN1 mutational analysis (Brandi et al. 2001, Namihira et al. 1999); patients with a ‘mixed’ MEN1/MEN2 phenotype have also been described (El-Maouche et al. 2016).

A novel MEN syndrome was discovered, initially in rats where it was named ‘MENX’ and then in humans, now known as MEN4 (OMIM #610755). MEN4 is caused by germline mutations in Cdkn1b in rats and CDKN1B in humans, coding for p27<sup>Kip1</sup> (commonly referred to as p27 or KIP1, hereafter p27), a putative tumor suppressor gene regulating cell cycle progression. The most common phenotypic features of patients with MEN4 are parathyroid and pituitary neoplasias. Recently, somatic or germline mutations in CDKN1B were also identified in patients with sporadic PHPT, small intestinal neuroendocrine tumors, lymphoma and breast cancer, demonstrating a novel role for CDKN1B as a tumor susceptibility gene for endocrine and other neoplasms. In this review, we present the clinical and genetic characterization of the MEN4 syndrome and describe the role of p27 in other tumors.

**Identification of a new syndrome predisposing to multiple endocrine neoplasias: MENX**

In 2000, Franklin and coworkers tested the theory that cyclin-dependent kinase inhibitor (CDKI) genes may function as tumor suppressor genes in mouse models (Franklin et al. 2000). They showed that loss of both p18 and p27 function resulted in spontaneous development of a wide spectrum of neuroendocrine tumors (NET) affecting various organ systems, including the pituitary, adrenals, thyroid, parathyroid and the gastroduodenal tract (Franklin et al. 2000). It was noted that somatic biallelic inactivation of CDKN1B, albeit a rare event, lead to several non-endocrine human tumors, suggesting that p27 is a haploinsufficient tumor suppressor and a potential candidate gene for tumorigenesis in humans.

In 2002, an MEN-like syndrome in rats that did not involve mutations in the MEN1 or RET genes was described but the responsible genetic defect(s) remained unknown (Fritz et al. 2002). The spontaneous development of multiple endocrine neoplasia phenotypes (e.g.: bilateral pheochromocytoma, bilateral MTC, multigland parathyroid neoplasia and pancreatic islet cells hyperplasia) within the first year of life with high penetrance characterized this syndrome as intermediate or combinatorial of both MEN1 and MEN2, termed ‘MENX’ (Fritz et al. 2002).

In 2004, the MENX locus was mapped to the distal part of rat chromosome 4 by a genomewide linkage analysis that excluded RET, which is present on the same chromosomal region (Piotrowska et al. 2004). In 2006, Pellegata and coworkers fine-mapped the locus of interest to a ~3 Mb interval on the distal part of rat chromosome 4, and suitable candidate genes were identified and sequenced, including the Cdkn1b gene encoding the p27 protein (Pellegata et al. 2006). By that time, it was known that the Cdkn1b<sup>−/−</sup> mice developed features of overgrowth with multiple tissue hyperplasias and pituitary adenomas of the intermediate lobe (Fero et al. 1996, Kiyokawa et al. 1996, Nakayama et al. 1996). Indeed, Pellegata and coworkers identified in these rats an 8-bp tandem duplication on exon 2 of the Cdkn1b gene (p.G177fs) leading to a homozygous frameshift mutation encoding a protein predicted to code for an elongated mutant protein with a different C-terminus than the wild-type p27 (Pellegata et al. 2006). This protein was later found to be highly unstable and therefore absent, or present at low levels, in vivo in the mutant rat. The phenotype of this rat included increased body weight and a reduced life span of 10±2 months when compared with wild-type (healthy homozygous or heterozygous) littermates of approximately 24–30 months of age; this syndrome was described in rats as MENX (Pellegata et al. 2006).

**CDKN1B gene function and cyclins**

In 1994, p27 was identified in molecular complexes of cyclin-dependent kinases (CDK) as a member of the CDKI family that regulate cell cycle progression and arrest through their inhibitory function on several cyclin/CDKIs, particularly the transition from G1 to S phase (Hengst et al. 1994, Polyak et al. 1994). The gene encoding the protein p27, called CDKN1B (also referred to as p27), is located on chromosome 12p13.1 and has two coding exons resulting in a 2.5-kb-long coding region for a nuclear protein and one noncoding exon. The human and mouse p27 genes share similar structures in their exon–intron, with >90% sequence homology in cDNA (Philipp-Staheli et al. 2001). The U-rich element located in the 5′UTR of p27 mRNA is necessary for...
efficient translation of p27 in proliferating and quiescent cells (Millard et al. 2000, Philipp-Staheli et al. 2001).

In humans, two CDKI families were identified: the INK4a/ARF and Cip/Kip family. INK4 proteins strictly inhibit and bind to CDK monomers while Cip/Kip proteins bind to both cyclin and CDK and can be inhibitory or activating. The Cip/Kip family proteins inhibit cyclin D and CDK4 or CDK6 complexes. INK4 are inhibitors that include p15 (encoded by CDKN2B), p16/p14 (encoded by CDKN2A), p18 (encoded by CDKN2C) and p19 (encoded by CDKN2D). The kinase inhibitor proteins (Cip/Kip) include p21 (encoded by CDKN1A), p27 and p57 (encoded by CDKN1C) (Sherr & Roberts 1999).

p27 primarily inhibits cyclin E/CDK2 with high and low affinities and undergoes inactivation at the posttranslational level by active cyclin/CDK2 complexes (Fig. 1) (Sheaff et al. 1997). p27 is regulated by ubiquitin-mediated proteasomal degradation via the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol-3 kinase (PI3K) pathways (Pagano et al. 1995, Donovan et al. 2001, Andreu et al. 2005). In sporadic tumors, point mutations of the CDKN1B-coding region are not common (Kawamata et al. 1995, Pietenpol et al. 1995, Ponce-Castaneda et al. 1995), despite LOH in some tumors (Pietenpol et al. 1995, Stegmaier et al. 1995). In others, there is lower CDKN1B mRNA expression (Hengst et al. 1996), and/or increased degradation (Pagano et al. 1995, Chiappetta et al. 2007), or cytosolic mislocalization of the p27 protein (Min et al. 2004).

The CDKN1B gene is not a classic tumor suppressor gene and does not always follow Knudson’s ‘two-mutation’ criterion for a tumor suppressor gene (Fero et al. 1998). Although the loss of one allele of p27 is a frequent event in many human cancers, the remaining allele is rarely mutated or lost by LOH in human cancers (Philipp-Staheli et al. 2001). In MEN1, p27 can act as a disease modifier associated with MEN1 germline mutations (Fig. 1). The CDKN1B gene is transcriptionally regulated by menin through epigenetic mechanisms, such as promotion of histone modifications and maintenance of transcription at multiple loci encoding cell cycle regulators (Hughes et al. 2004, Karnik et al. 2005), suggesting a common pathway for tumorigenesis between MEN1 and MEN4 (Fig. 1). It is known that menin regulates the expression of p27 by forming a transcriptional activation complex with methyltransferases (MLL1 or MLL2) and the large subunit of RNA polymerase II (POL II). Menin inactivation leads to decreased p27. Mutations in CDKN1B, either solely or in combination with a mutation in MEN1, lead to a greater decrease in expression of p27 protein, triggering neoplasia (Fig. 1). In one study, Borisari and coworkers showed that MEN1 biallelic inactivation could be directly related to downregulation of p27 expression through the inhibition of CDKN1B gene transcription (Borisari et al. 2017).

CDKN1B mutations that were initially identified in mice and humans behave as loss-of-function mutations and occur in heterozygosity. To date, most of the reported mutations in humans were missense and not found in controls. They were deemed pathogenic due to their in vivo or in vitro effects on the function of p27. Thus, given the small number of cases reported to date (described later in this review) and the lack of segregation with the disease phenotype in the reported families, assigning a possible pathogenic role for these mutations required functional

![Figure 1](image-url)

**Figure 1**
A pathway depicting the alterations in p27 expression in MEN1 and/or MEN4 that lead to tumorigenesis. Menin, encoded by MEN1, regulates the expression of p27 by forming a transcriptional activation complex with methyltransferases (MLL1 or MLL2) and RNA polymerase II (POL II). Menin inactivation (MEN1 mutation) leads to decreased p27 expression. Mutations in CDKN1B, either solely or with MEN1 as a second germline hit, leads to a greater decrease in expression of p27 protein, triggering uncontrolled cell cycle progression.
Table 1  Established cases of MEN4.

<table>
<thead>
<tr>
<th>CDKN1B mutation</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>Clinical manifestations</th>
<th>Family history</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.W76X (c.692G &gt; A)</td>
<td>48</td>
<td>F</td>
<td>Caucasian</td>
<td>Acromegaly PHPT</td>
<td>3 family members with the same variant. Father had acromegaly (not tested for the variant). Sister had renal angiomyolipoma (positive for same p27 variant); her son had testicular cancer</td>
<td>Pellegata et al. (2006)</td>
</tr>
<tr>
<td>p.K25fs (c.59_77dup19)</td>
<td>47</td>
<td>F</td>
<td>Caucasian</td>
<td>Small-cell neuroendocrine cervical carcinoma ACTH-dependent Cushing syndrome (Cushing disease) PHPT Multiple sclerosis</td>
<td>Negative</td>
<td>Georgitsi et al. (2007)</td>
</tr>
<tr>
<td>ATG-7G &gt; C in the 5’-UTR</td>
<td>61</td>
<td>F</td>
<td>NA</td>
<td>Bilateral nonfunctional adrenal masses Uterine fibroids PHPT</td>
<td>Two asymptomatic daughters (ages 47 and 48), positive for same variant</td>
<td>Agarwal et al. (2009)</td>
</tr>
<tr>
<td>p.P95S (c.283C &gt; T)</td>
<td>50</td>
<td>F</td>
<td>NA</td>
<td>Zollinger–Ellison syndrome with masses in duodenum and tail of pancreas PHPT</td>
<td>NA</td>
<td>Agarwal et al. (2009)</td>
</tr>
<tr>
<td>ATG-7G &gt; C in the 5’-UTR</td>
<td>50</td>
<td>F</td>
<td>NA</td>
<td>Uterine fibroids PHPT</td>
<td>Two asymptomatic daughters (ages 47 and 48), positive for same variant</td>
<td>Agarwal et al. (2009)</td>
</tr>
<tr>
<td>Stop &gt; Q (c.595T &gt; C)</td>
<td>50</td>
<td>F</td>
<td>NA</td>
<td>PHPT</td>
<td>Monozygotic twin sister positive for same variant with PHPT (1 parathyroid tumor) at age 66. Aunt and cousin have PHPT, not tested for the variant</td>
<td>Agarwal et al. (2009)</td>
</tr>
<tr>
<td>p.P69L (c.678C &gt; T)</td>
<td>79</td>
<td>F</td>
<td>Caucasian</td>
<td>Papillary thyroid carcinoma with neck lymph node metastases Bilateral multiple lung metastatic from bronchial carcinoid PHPT Nonfunctioning pituitary microadenoma Subcutaneous epigastric lipoma Type 2 diabetes mellitus</td>
<td>Negative</td>
<td>Molatore et al. (2010)</td>
</tr>
<tr>
<td>p.G9R (c.25G &gt; A)</td>
<td>68</td>
<td>M</td>
<td>Caucasian</td>
<td>PHPT</td>
<td>NA</td>
<td>Costa-Guda et al. (2011)</td>
</tr>
<tr>
<td>p.P133T (c.397C &gt; A)</td>
<td>53</td>
<td>F</td>
<td>Caucasian</td>
<td>PHPT (1 parathyroid tumor)</td>
<td>NA</td>
<td>Costa-Guda et al. (2011)</td>
</tr>
<tr>
<td>Heterozygous GAGA deletion in the 5’-UTR (ATG-32–29del) (c.32_29delGAGA)</td>
<td>69</td>
<td>F</td>
<td>Hispanic</td>
<td>Gastric NET PHPT</td>
<td>Negative</td>
<td>Malanga et al. (2012)</td>
</tr>
<tr>
<td>p.A55T (c.163G &gt; A)</td>
<td>42</td>
<td>F</td>
<td>Hispanic</td>
<td>Zollinger–Ellison syndrome with gastrinoma and hepatic metastases PHPT</td>
<td>Negative</td>
<td>Belar et al. (2012)</td>
</tr>
<tr>
<td>p.S125X (c.374_375delCT)</td>
<td>53</td>
<td>F</td>
<td>Caucasian</td>
<td>Gastrointestinal NET PHPT</td>
<td>The patient's 35-year-old son, who had no MEN4-associated clinical features, was the first reported male mutation carrier in CDKN1B</td>
<td>Tonelli et al. (2014)</td>
</tr>
<tr>
<td>p.E126D (c.378G &gt; C)</td>
<td>15</td>
<td>F</td>
<td>Caucasian</td>
<td>Early-onset PHPT Recurrent renal calculi and hypercalcemia</td>
<td>Mother (46 years) and maternal grandfather (74 years) carried the same missense mutation but both were normocalcemic with normal PTH levels</td>
<td>Elston et al. (2015)</td>
</tr>
</tbody>
</table>
studies for confirmation. These studies demonstrated that some MEN4-associated mutations led to a truncated p27 protein that is very unstable and rapidly degraded, in part, by the proteasome (Sherr & Roberts 1999; Lee & Pellegata 2013). Missense mutations, on the other hand, led to a reduced binding to interacting partners or decreased nuclear localization (Table 1) (Lee & Pellegata 2013). Overall, CDKN1B mutations causing MEN4 affect p27’s cellular localization, stability or binding with Cdk2 or Grb2 (Agarwal et al. 2009).

Translation of CDKN1B involves regulatory elements within its 5’UTR, such as the upstream ORF (uORF) (Gopfert et al. 2003). Mutations in CDKN1B 5’UTR have been studied: the GAGA deletion encompassing nucleotides −32/−29 of 5’-UTR of CDKN1B significantly impairs transcription and, possibly, translation of p27 mRNA and its expression in tumor cells (Malangà et al. 2012), while the 4-bp deletion that modifies the regulatory uORF in the 5’UTR of the CDKN1B leads to an in vivo and in vitro reduction of p27 expression (Occhi et al. 2013). The common CDKN1B rs2066827 polymorphism (described later) may influence the clinical outcome (i.e.: tumor formation) of patients with MEN1 (Longuini et al. 2014), a finding that should be ascertained in further studies.

MEN4 in humans

In 2008, MENX was renamed to MEN4 during the 11th International Workshop on MENs in Delphi, Greece (Alevizaki & Stratakis 2009). It was there that MEN4 was accepted as the latest member of the MEN syndromes affecting humans (Lee & Pellegata 2013). To date, only 19 cases having CDKN1B germline mutations have been reported in the medical literature (Table 1). The incidence of CDKN1B mutations in patients with a MEN1-related phenotype is difficult to estimate, but it is likely to be in the range of 1.5–3.7% (Georgitsi et al. 2007, Agarwal et al. 2009, Molatore et al. 2010). Immunohistochemical staining of affected tissues in MEN4 did not detect expression of the p27 protein, suggesting that other mechanisms, likely posttranslational, such as phosphorylation and ubiquitination, may regulate p27 stability in these tumors.

The first case of MEN4 in humans was reported in 2006 by Pellegata and coworkers (Pellegata et al. 2006) in a 3-generation family with a negative mutation in MEN1. The family history consisted of acromegaly in the father, severe hypertension (possibly due to endocrine hypertension) in the brother who died at 39 years and MEN1-like features in the proband, who was a 48-year-old Caucasian female with a 3-cm
somatotropinoma causing acromegaly. The pathology revealed an invasive pituitary adenoma that stained for growth hormone with a high mitotic activity and cell atypia. Later, the same patient developed PHPT, likely due to parathyroid hyperplasia. Sequencing of the CDKN1B gene showed a germline heterozygous nonsense mutation at codon 76 (c.692G>A, p.W76X), causing premature truncation of the p27 protein (Pellegrata et al. 2006). Family screening showed that the proband’s sister presented with a renal angiomylipoma at age 55 years, with no p27 staining, and was also a carrier of the mutation (Molatore et al. 2010). Her son had testicular cancer.

Subsequently, Georgitsi and coworkers studied 37 patients (36 Dutch, 1 German) with a MEN1-like phenotype who did not have MEN1 gene mutations or (Georgitsi et al. 2007). They identified a 19-bp duplication in exon 1 (c.59_77dup19, p.K25fs; heterozygous frameshift mutation at codon 25) of CDKN1B in a 47-year-old Dutch woman with a small-cell neuroendocrine cervical carcinoma that was first diagnosed at the age of 45 years and in which LOH of wild-type CDKN1B was observed. The patient also had a corticotropinoma causing Cushing disease at 46 years of age, and PHPT that was diagnosed a year later (Georgitsi et al. 2007). This second report further expanded the clinical spectrum of MEN4.

In 2009, Agarwal and coworkers identified three potentially pathogenic changes in CDKN1B (c.-7G>C; c.283C>T, p.P95S; c.595T>C, p.X199QextX*60 or stop > Q) after screening a total of 196 consecutive index cases of clear or suspected MEN1 and no identifiable germline mutations in MEN1 (Agarwal et al. 2009). The c.-7G>C and c.595T>C variants showed decreased expression of p27 when compared to wild-type p27, while the c.283C>T variant did not affect the protein expression, but rather its ability to bind Grb2 (Moeller et al. 2003, Agarwal et al. 2009). The patient with the c.-7G>C variant (mutation at the –7 position in the Kozak sequence ATG-7G>C) had a parathyroid tumor, bilateral adenocortical masses (first and only report so far of adrenal tumors in MEN4) and uterine fibroids, while the patient with the c.283C>T variant had PHPT and masses in both the duodenum and pancreas. Later in 2010, Molatore et al. extended their preliminary observation and reported a novel germline missense variant in CDKN1B (c.678C>T, p.E126D) in a 62-year-old female with a renal angiomylipoma, hepatic angiomyolipomas and a germline frameshift mutation (c.692G>A, p.W76X) in CDKN1B causing acromegaly and a well-differentiated nonfunctioning pNET (Molatore et al. 2010). Subsequently, the same group studied 90 patients with presumably sporadic PHPT as the sole presentation of MEN4, and reported two novel mutations in CDKN1B. The first patient was a 68-year-old man with PHPT that had a heterozygous germline single-nucleotide change at base 25 in CDKN1B exon 1 (c.25G>A, p.G9R). The second patient was a 53-year-old woman with mild PHPT and a heterozygous single-nucleotide substitution (c.397C>A, p.P133T) in CDKN1B (Costa-Guda et al. 2011). A year later, Malanga and coworkers reported on a 69-year-old female with a gastric NET and PHPT who was positive for a heterozygous GAGA deletion in the 5’-UTR of CDKN1B. This patient was found after screening 15 Spanish index cases with MEN1-negative patients showing a MEN-like phenotype (Malanga et al. 2012).

Belar and coworkers studied 79 different cases of sporadic and familial cases with MEN1 phenotype and identified a novel missense mutation (c.163G>A, p.A55T) in CDKN1B in a Spanish woman with a corticotropinoma, Zollinger-Ellison syndrome (ZES) with hepatic metastasis that was diagnosed at 42 years of age and PHPT at 51 years (Belar et al. 2012). In a different report, Tonelli and coworkers described a 53-year-old Italian woman that had presented with PHPT and gastrointestinal NET due to a germline frameshift mutation in CDKN1B (c.371delCT) (Tonelli et al. 2014). The patient’s 35-year-old son, who had no MEN4-associated clinical features, was the first reported male mutation carrier in CDKN1B. In 2015, Pardi and coworkers characterized this germline mutation in CDKN1B (c.374_375Del, p.S125X) confirming the pathogenic role of this mutation in MEN4 (Pardi et al. 2015). Early-onset PHPT was identified in a 15-year-old girl with a germline CDKN1B variant (p.E126D) that was predicted to be damaging. There was no family history to suggest a syndromic association (Elston et al. 2015). This report is believed to describe the youngest published case of MEN4 to date.

More recently, a number of MEN4 cases with pituitary involvement have been described. Occhi and coworkers identified a 4-bp deletion (c.-456_-453delCCTT) within the 5′-UTR of CDKN1B in a 62-year-old female with acromegaly and a well-differentiated nonfunctioning pNET (Occhi et al. 2013). Subsequently, Sambugaro and coworkers identified a patient with gigantism, first diagnosed at 6 years of age, and later confirmed to be due to a novel heterozygous mutation in the CDKN1B 5′-UTR region (c.-29_-26delAGAG) that led to reduction in CDKN1B mRNA levels (Sambugaro et al. 2015). In a total of 124 affected subjects with a pituitary adenoma,
Tichomirowa and coworkers identified two point mutations (~2% of the cases studied) in \textit{CDKN1B}; p.1119T (c.356T > C) and p.K96Q (c.286A > C) in two patients from an \textit{AIP}-negative FIPA family (Tichomirowa et al. 2012). These variants altered p27 function or structure \textit{in vitro}, but it should be noted that the p.K96Q variant did not segregate with pituitary adenomas in one kindred.

A recent study further expanded on the clinical and genetic spectrum of a MEN1-like syndrome. Borsari and coworkers studied 147 patients with typical parathyroid adenomas causing PHPT and found three germline \textit{CDKN1B} variants (c.-80C > T, c.-29_-26delAGAG, c.397C > A) with reduction of \textit{CDKN1B} gene transcription rate, and loss of p27 expression in the tumor carrying the c.-29_-26delAGAG variant (Borsari et al. 2017). Co-existence of MEN4 with other MEN syndromes have not been reported to date, although possible, as described in a case report of a patient with clinical findings of MEN1 (harboring a germline mutation in \textit{MEN1}) and a MEN2-like phenotype (with \textit{RET} polymorphisms p.G691S and p.A982C) (El-Maouche et al. 2016). Since only 19 established cases of MEN4 have been reported in the medical literature, the clinical penetrance and precise tumor spectrum of MEN4 are still to be defined.

**Clinical manifestations of MEN4**

Although considerable overlap in the clinical manifestations of the MEN syndromes exists (Fig. 2 and Table 2), the relatively small number of cases reported so far does not allow conclusion to be drawn on the possible clinical differences between MEN4 and the other MEN syndromes. A recent study of 293 \textit{MEN1} mutation-positive and 30 \textit{MEN1} mutation-negative cases, all with the \textit{MEN1} phenotype showed that the mutation-negative cohort developed disease manifestations later in life with improved life expectancy (de Laat et al. 2016). Although the mutation-negative cohort might have had MEN4, it is difficult to draw conclusion on life expectancy or penetrance of disease from this cohort. It appears that the \textit{MEN1}-negative patients showing a MEN-like phenotype should undergo a careful assessment for possible MEN4; still, confirmation of an MEN4 diagnosis should only be made with genetic testing for \textit{CDKN1B} mutations.
Primary hyperparathyroidism (PHPT)

PHPT due to parathyroid neoplasia affects approximately 80% (15/19 cases, Table 1) of the reported cases of MEN4 to date. PHPT occurs at a later age in MEN4 than in MEN1 (mean age ~56 years vs ~25 years, respectively) with a female predominance (Lee & Pellegata 2013). Interestingly, none of the reported cases of MEN4 to date had PHPT recurrence after surgical resection, which might indicate that PHPT in MEN4 might represent an overall milder disease spectrum than MEN1. The indications for parathyroid surgery in MEN4 are the same as for MEN1, although there are no specific guidelines to date on management of PHPT in MEN4. The surgical approach in MEN4-related PHPT should be individualized; some patients may be treated as in MEN1 and undergo three-and-a-half gland resection with close follow-up for disease recurrence.

Pituitary adenomas

Pituitary involvement in MEN4 is the second most common manifestation of the disease, affecting approximately 37% of the reported cases to date (7/19 cases, Table 1). The types of pituitary adenomas in MEN4 vary, including nonfunctional, somatotropinoma, prolactinoma or corticotropinoma. The age of diagnosis for these lesions also varies widely, from 30 to 79 years (Table 2). In general, pituitary tumors in MEN4 are present with reduced aggressiveness, but may exert a variable degree of morbidity depending on the hormonal functional status, size and presence of invasion or mitotic index. Conversely, pituitary adenomas are characterized by a larger size and a more aggressive presentation in MEN1.

Gigantism or acromegaly is reported in MEN4 (Occhi et al. 2013, Crona et al. 2015, Sambugaro et al. 2015). Mutations in CDKN1B in sporadic gigantism or acromegaly and among pediatric patients with pituitary adenomas appear to be very rare (Stratakis et al. 2010, Schernthaner-Reiter et al. 2016). Genetic alterations of CDKN1B in somatotropinoma, corticotropinoma, nonfunctioning pituitary adenomas and sporadic pNET are also very infrequent (Ikeda et al. 1997, Dahia et al. 1998, Takeuchi et al. 1998, Lindberg et al. 2007, Stratakis et al. 2010, Lee & Pellegata 2013). Cushing disease has been reported in MEN4 due to a heterozygous 19-bp duplication (c.59_77dup19) in CDKN1B, leading to a truncated protein (Georgitsi et al. 2007). It should be noted that corticotropinomas are also observed in MEN1.
Neuroendocrine duodeno-pancreatic tumors

Only a few cases of NETs in the context of MEN4 have been reported to date (7/19 cases, Table 1). These include duodeno- or gastric-pNETs, that could be nonfunctioning or hormonally active and may secrete several substances, including gastrin, insulin, ACTH or vasoactive intestinal polypeptide (VIP). NETs in MEN may be associated with various clinical syndromes. Gastrin-secreting tumors (gastrinomas) lead to peptic and gastric ulcerations due to excess release of gastrin levels and are the leading cause of NET in MEN1. The clinical syndrome associated with this is ZES, which has been reported in two cases of MEN4 (Table 1). In MEN4, there are no reported cases of insulinoma, VIPoma, glucagonoma, ectopic-ACTH-secreting NET or malignant transformation of pNETs. In MEN1, NETs are usually multiple with an uncertain behavior. It appears that there is a decreased penetrance of pNETs in MEN4 when compared to MEN1. The diagnosis and management of pNETs in MEN4 is similar to that in MEN1 (Thakker et al. 2012).

Adrenal neoplasia

Adrenal neoplasia is a frequent finding in MEN1 but figures for MEN4 are not available. MEN1 also predisposes to primary aldosteronism (Beckers et al. 1992), bilateral adrenal nonfunctional nodular hyperplasia (Gatta-Cherifi et al. 2012), primary bilateral adrenocortical hyperplasia (PBMAH) (Stratakis & Boikos 2007) and adrenocortical cancer (Gatta-Cherifi et al. 2012), which has not been reported in MEN4. While adrenal tumors are found in mouse MENX, only one case of nonfunctional bilateral adrenal nodules was reported in MEN4 (Table 2). Routine surveillance for the development of ACTH-independent Cushing syndrome, primary aldosteronism or pheochromocytoma (only reported in rats with MENX (Molatore et al. 2010)) in patients with MEN4 should be performed on a case-by-case basis.

Other

Testicular cancer and neuroendocrine cervical carcinoma have been reported in MEN1 but not in MEN4. Likewise, skin manifestations that are commonly reported in MEN1, such as lipomas, angiofibromas and collagenomas have not been reported in MEN4. Finally, CDKN1B has been implicated in primary ovarian insufficiency: one study found two nonsynonymous variants of CDKN1B; p.V109G, a polymorphism, and p.I119T, a mutation with a potential deleterious effect requiring functional studies for confirmation (Ojeda et al. 2011).

CDKI and human neoplasia

Endocrine tumors have been associated with over-expression of cyclins and/or loss of function of CDKI. Importantly, CDKN1B and CDKN2C are transcriptional gene targets of menin (Milne et al. 2005). In a study of 196 patients with MEN1 (but no MEN1 gene mutations), the relative frequency of the various CDKI mutations were 1, 0.5, 0.5 and 1.5% for p15 (CDKN2B), p18 (CDKN2C), p21 (CDKN1A) and p27, respectively (Agarwal et al. 2009). This report was the first to document a missense variant (p.V31L) in CDKN2C in an endocrinopathy. Other authors have confirmed the very rare or non-existent association between CDKN2C and parathyroid neoplasia (Tahara et al. 1997) and a frequent promoter methylation of CDKN2C in pituitary adenomas (Kirsch et al. 2009). Moreover, CDKN2C has been found significantly under-expressed in various pituitary tumors, including corticotropinomas, prolactinomas and somatotropinomas (Morris et al. 2005, Hossain et al. 2009, Kirsch et al. 2009). In most patients with atypical manifestations of MEN1, the disease seems to occur due to genetic causes other than CDKN1B, implicating other genetic alternations that are yet to be identified (Ozawa et al. 2007).

Somatic changes in CDKN1B without mutations in the remaining wild-type allele, as well as the reduced expression of p27 protein without CDKN1B mutations in various human tumors support the concept that CDKN1B is a likely tumor suppressor gene that confers tumorigenicity in haploinsufficiency. Nonsense mutations were discovered in adult T-cell leukemia/lymphoma (p.W76X) (Morosetti et al. 1995) and breast cancer (p.Q104X) (Spirin et al. 1996), while a missense change (p.I119T) was found in a myeloproliferative
disorder (Pappa et al. 2005). Somatic alterations in CDKN1B represent the second most common mutated gene in hairy-cell leukemia (Dietrich et al. 2015). Other studies have identified recurrent somatic frameshift mutations and deletions in CDKN1B of small intestinal NET with inter- and intra-tumor heterogeneity (Francis et al. 2013, Crona et al. 2015), supporting its role as a haploinsufficient tumor suppressor gene. Several potentially functional single-nucleotide polymorphisms (SNPs; −838C>A, −79C>T and 326T>G) were associated with a variety of human cancers including prostate, breast and thyroid cancer (Landa et al. 2010). One study found that the CDKN1B rs2066827 polymorphism may be associated with decreased susceptibility to ovarian cancer (Lu et al. 2015).

It is known that downregulation of p27, through a mechanism that enhances proteasome-mediated degradation, is associated with tumor progression, aggressiveness, poor clinical outcome and decreased survival in these malignancies (Lloyd et al. 1999, Chu et al. 2008). Oncogenic activation of phosphatidylinositol 3-kinase (PI3K) (Liang & Slingerland 2003), proto-oncogene tyrosine-protein kinase Src or MAPKs inactivate p27 or accelerate its proteolysis in human cancers (Busse et al. 2000, Donovan et al. 2001). These findings highlight the potential role of tyrosine kinase inhibitors for the management of aggressive NETs associated with MEN4 or somatic p27 mutations.

Genetic testing and counseling for MEN4
The identification of the genes responsible for MEN1, MEN2 and MEN4 has enabled the genetic diagnosis and, thus, early detection of patients with suspected endocrine tumor syndromes. Genetic testing in clinical practice for affected patients and their families with MENs has now become routine. In addition, sequencing for MEN1 and RET is now included in clinical exome and genome sequencing of other cases and reported as secondary findings (Kalia et al. 2017).

Genetic screening is useful in identifying carriers or at-risk family members who can then be monitored for the clinical manifestations of the respective syndrome(s). Negative genetic testing offers reassurance to those who do not carry the mutation and prevents unnecessary screening. Preimplantation and/or prenatal screening may also be offered. Patient and family counseling should incorporate patient’s values and attitudes toward their disease, underscoring the risks and benefits of genetic screening and counseling, psychosocial interventions and service delivery. An experienced genetic counselor and team should provide a comprehensive assessment, including education and discussions on preventing and screening options.

As we mentioned previously, since the identification of mutations in CDKN1B as causative for MEN4, only 19 cases have been reported in the medical literature (Table 1). Thus, guidelines and recommendations for MEN4 are lacking and difficult to formulate given the paucity of cases described in the literature. Moreover, the limited analysis of relatives with CDKN1B that do not manifest with any signs or symptoms suggestive of MEN4 is consistent with an incomplete penetrance of the disease.

In clinical practice, if a clinician encounters patients with asymptomatic or symptomatic PHPT that are young (typically <30 years old), with multigland disease, parathyroid carcinoma or atypical adenoma, or those with a family history or evidence of syndromic disease and negative for MEN1 or RET, genetic testing for CDKN1B should be pursued. However, no guidelines exist. As we already know from MEN2, the genetic status of suspected pre-symptomatic patients provides survival benefits based on preemptive management of the potential morbidities (Brandi et al. 2001). On the other hand, MEN1-related tumors have no effective prevention except for prophylactic thymectomy for thymic NET (Brandi et al. 2001, Thakker et al. 2012).

An approach to screening in MEN4 is outlined in Fig. 3. Index cases or individuals with MEN1-like features and negative MEN1 testing should be offered genetic counseling and testing for MEN4 (CDKN1B) in accredited laboratories. Screening should also be offered to a first-degree relative with or without MEN1 features. The identification of a germline CDKN1B mutation should prompt periodic clinical, biochemical and radiological screening for MEN4. Mutations leading to the MEN4 phenotype are transmitted in an autosomal dominant fashion, and each sibling has a 50% risk of having the mutation. If neither parent carries the mutation, the risk to siblings is low, but the possibility of germline mosaicism or de novo mutations exists. It is advisable to refer the patient and/or family members to a tertiary center with expertise in these rare conditions. It is also important for the proband or at-risk family members to receive genetic counseling and testing for risk stratification of affected and unaffected individuals.
Summary
Over the last two decades, significant progress has been made in the understanding of the molecular and genetic mechanisms of tumor pathogenesis in MENs. In this review, we presented the genetic and clinical features of MEN4, being the newest member to join the MEN family of conditions. MEN4 is a rare syndrome with clinical features that overlap with the other MENs. The discovery of CDKN1B mutations that cause MEN4 enabled personalized approaches to diagnosis, risk stratification and appropriate treatment for individuals with MEN and other sporadic tumors affecting various organs and systems. Index cases or individuals with MEN1-like features and negative MEN1 testing should be offered genetic counseling and testing for MEN4. Screening should also be offered to a first-degree relative with or without MEN1 features. The identification of a germline CDKN1B mutation should prompt periodic clinical, biochemical and radiological screening for MEN4.

Selected accredited laboratories for MEN molecular analysis

United States
- Esoterix Molecular Endocrinology, Calabasas Hills, California
  www.esoterix.com
- Molecular Genetics Laboratory, Emory, Atlanta, Georgia
  www.geneticslab.emory.edu
- Molecular Genetics Laboratory, Mayo Clinic, Rochester, Minnesota
  www.mayoclinic.org
- PreventionGenetics, Marshfield, Wisconsin
  www.preventiongenetics.com
- Quest Diagnostics, Nichols Institute, San Juan Capistrano, California
  www.education.questdiagnostics.com
- GeneDx, Gaithersburg, Maryland
  www.genedx.com
- InVitae Corp., San Francisco, California
  www.invitae.com

Europe
- Reference Laboratory Genetics, Spain
  www.reference-laboratory.es
- Service Hormonologie Métabolisme Nutrition Oncologie, Institut de Biochimie et Biologie Moléculaire, CHRU de Lille – Centre de Biologie Pathologie Génétique, France
  www.chru-lille.fr
- Pränatal-Medizin München, Germany
  www.de.praenatal-medizin.de
- Molecular Genetics Laboratory, Oxford Medical Genetics Laboratories, The Churchill Hospital, England
  www.ouh.nhs.uk/services/referrals/genetics/geneticslaboratories
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General information

- www.orpha.net/consor/cgt-bin/index.php
- www.eddnal.com

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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