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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. Recently, 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 cases of MEN4 that have been described to date is PHPT followed by pituitary adenomas. Recently, somatic and germline mutations in *CDKN1B* were also identified in patients with sporadic PHPT, 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.

Keywords

Multiple Endocrine Neoplasia; MEN4; MEN1; Neuroendocrine tumors; CDKN1B; p27

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)

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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 #171400) 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 (Chandrasekharappa, et al. 1997; Larsson, et al. 1988), 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 (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 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 that do not have mutations in *MEN1* or *RET* (El-Maouche, et al. 2016).

A novel MEN syndrome was recently 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^{Kip1} (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 and germline mutations in *CDKN1B* were also identified in patients with sporadic PHPT, 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 talk about the role of p27 in other tumors.

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

In 2000, Franklin *et al.* (Franklin, et al. 2000) tested the theory that cyclin-dependent kinase inhibitor (CDKI) genes may function as tumor suppressors genes on mouse models. 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 (Philipp-Staheli, et al. 2001).

In 2002, an MEN-like syndrome in rats that did not involve mutations in the *MEN* or *RET* gene was described but the responsible genetic defect(s) remained unknown (Fritz, et al. 2002). The spontaneous development of multiple endocrine neoplasias (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 genome-wide linkage analysis that excluded *RET*, which is present on the same chromosomal region (Piotrowska, et al. 2004). In 2006, Pellegata *et al.* 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*^{-/-} 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 *et al.* 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 CDK inhibitors (CDKI) family that regulate cell cycle progression and arrest through their inhibitory function on several cyclin/CDKs, 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 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 inhibits and binds to CDK monomers while Cip/Kip proteins bind to both cyclin and CDK, and can be inhibitory or activating. The Cip/Kip family proteins activate cyclin D and CDK4 or CDK6 complexes. CDK4 are inhibitors and include p15 (encoded by *CDKN2B*), p16/p14 (encoded by *CDKN2A*), p18 (encoded by *CDKN2C*) and p19 (encoded by *CDKN2D*). The kinase inhibitor proteins (CIP) includes p21 (encoded by *CDKN1A*), p27 and p57 (encoded by *CDKN1C*) (Sherr and 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 (Figure 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 (Andreu, et al. 2005; Donovan, et al. 2001; Pagano, et al. 1995). 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. 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 and Reed 1996), and/or increased degradation (Chiappetta, et al. 2007; Pagano et al. 1995), 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 (Figure 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* (Figure 1). It is known that menin regulates the expression of p27 by forming a transcriptional activation complex with methyltransferase (MLL2) and RNA polymerase II (POL III). 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 (Figure 1). In one study, Borsari et al. showed that *MEN1* biallelic inactivation could be directly related to down-regulation of p27 expression through the inhibition of *CDKN1B* gene transcription (Borsari, 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 studies for confirmation. These studies demonstrated that the MEN4-associated deletions led to a truncated p27 protein that is very unstable and rapidly degraded, in part, by the proteasome (Sherr and Roberts 1999). Missense mutations, on the other hand, led to a reduced binding to interacting partners or decreased nuclear localization (Table 1) (Sherr and Roberts 1999). Overall, *CDKN1B* mutations causing MEN4 affect p27's cellular localization, stability or binding with Cdk2 or Grb2 (Agarwal, et al. 2009b).

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 (Malanga, 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 and Stratakis 2009). It was there that MEN4 was accepted as the latest member of the MEN syndromes affecting humans (Lee and 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% (Agarwal et al. 2009b; Georgitsi, et al. 2007; Molatore, et al. 2010).

The first case of MEN4 in humans was reported in 2006 by Pellegata *et al* (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 old, 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 (Pellegata et al. 2006). Family screening showed that the proband's older sister presented with a renal angiomyolipoma 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 *et al.* studied 37 patients (36 Dutch, 1 German) with a MEN1-like phenotype who did not have *MEN1* gene mutations or deletions. They identified a 19-bp duplication in exon 1 (c.59_77dup, 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 *et al.* (Agarwal et al. 2009b) 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*. The c.-7G>C and c.595T>C variants showed decreased expressivity 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 (Agarwal et al. 2009b; Moeller, et al. 2003). 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 adrenocortical 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.P69L) in a 79-year-old Caucasian woman of Italian ancestry with multiple typical and metastatic bronchial NET tumors, subcutaneous epigastric lipoma, nonfunctioning pituitary microadenoma, parathyroid adenoma, and papillary thyroid carcinoma (pT1bN1M0) (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, Malang *et al.* 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 *menin* mutation-negative MEN1 (Malanga et al. 2012).

Belar *et al.* 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 *et al.* 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 *et al.* characterized this germline mutation in *CDKN1B* c.374_375delCT (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 *et al.* 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 non-functioning pNET (Occhi et al. 2013). Subsequently, Sambugaro *et al.* 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 *et al.* identified two point mutations (~2% of the cases studied) in *CDKN1B*; p.I119T (c.356T>C) and p.K96Q (c.286A>C) in two patients from an *AIP*-negative FIPA family (Tichomirowa, et al. 2012). These variants

altered p27 function or structure *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 *et al.* studied 147 patients with typical parathyroid adenomas causing PHPT and found three germline *CDKN1B* variants (c.-80C>T, c.-29_-26delAGAG, c.397C>A) with reduction of *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 *MEN1*) and a MEN2-like phenotype (with *RET* polymorphisms p.G691S and p.A982C) (El-Maouche *et al.* 2016). Since only 19 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 (Figure 2, 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 *MEN1* mutation-positive and 30 *MEN1* mutation-negative cases, all with the 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 *menin* mutation-negative MEN1 cases should undergo a careful assessment for possible MEN4; still, confirmation of an MEN4 diagnosis should only be made with genetic testing for *CDKN1B* mutations.

1. 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 and 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.

2. 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 non-functional, somatotropinoma,

prolactinoma or corticotropinoma. The age of diagnosis for these lesions also varies widely, from 30–79 years (see 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 (Crona, et al. 2015; Occhi et al. 2013; Sambugaro et al. 2015). Mutations in *CDKN1B* in sporadic gigantism or acromegaly and among pediatric patients with pituitary adenomas appear to be very rare (Scherthaner-Reiter, et al. 2016; Stratakis, et al. 2010). Genetic alterations of *CDKN1B* in somatotropinoma, corticotropinoma, non-functioning pituitary adenomas and sporadic pNET are also very infrequent (Dahia, et al. 1998; Ikeda, et al. 1997; Lee and Pellegata 2013; Lindberg, et al. 2007; Stratakis et al. 2010; Takeuchi, et al. 1998). 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 (Verges, et al. 2002; Stratakis et al. 2010;), and very rarely in MEN2B (Kasturi, et al. 2017). Interestingly, one study found the common *CDKN1B* rs2066827 polymorphism to play a role in corticotropinoma susceptibility and tumorigenesis through a yet unidentified mechanism or, maybe, epigenetic factors (Sekiya, et al. 2014). The management of pituitary tumors in MEN4 is like other sporadic or familial cases. Routine surveillance for the development of pituitary tumors in patients with MEN4 should be performed on a case-by-case basis and following existing guidelines for other MENs.

3. 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).

4. 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 non-functional nodular hyperplasia (Gatta-Cherifi, et al. 2012), primary bilateral adrenocortical hyperplasia (PBMAH) (Stratakis and 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 non-functional 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.

5. 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, 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. 2009b). 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 (Hossain, et al. 2009; Kirsch et al. 2009; Morris, et al. 2005). 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 intratumor heterogeneity (Crona et al. 2015; Francis, et al. 2013), 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 (Chu, et al. 2008; Lloyd, et al. 1999). Oncogenic activation of phosphatidylinositol 3-kinase (PI3K) (Liang and 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 above, 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 MEN2, 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 Figure 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. *CDKN1B* somatic mutations are frequent in NETs and other non-endocrine neoplasms pointing to potential use of this knowledge in molecularly targeted therapies for these lesions.

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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

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/genetics-laboratories

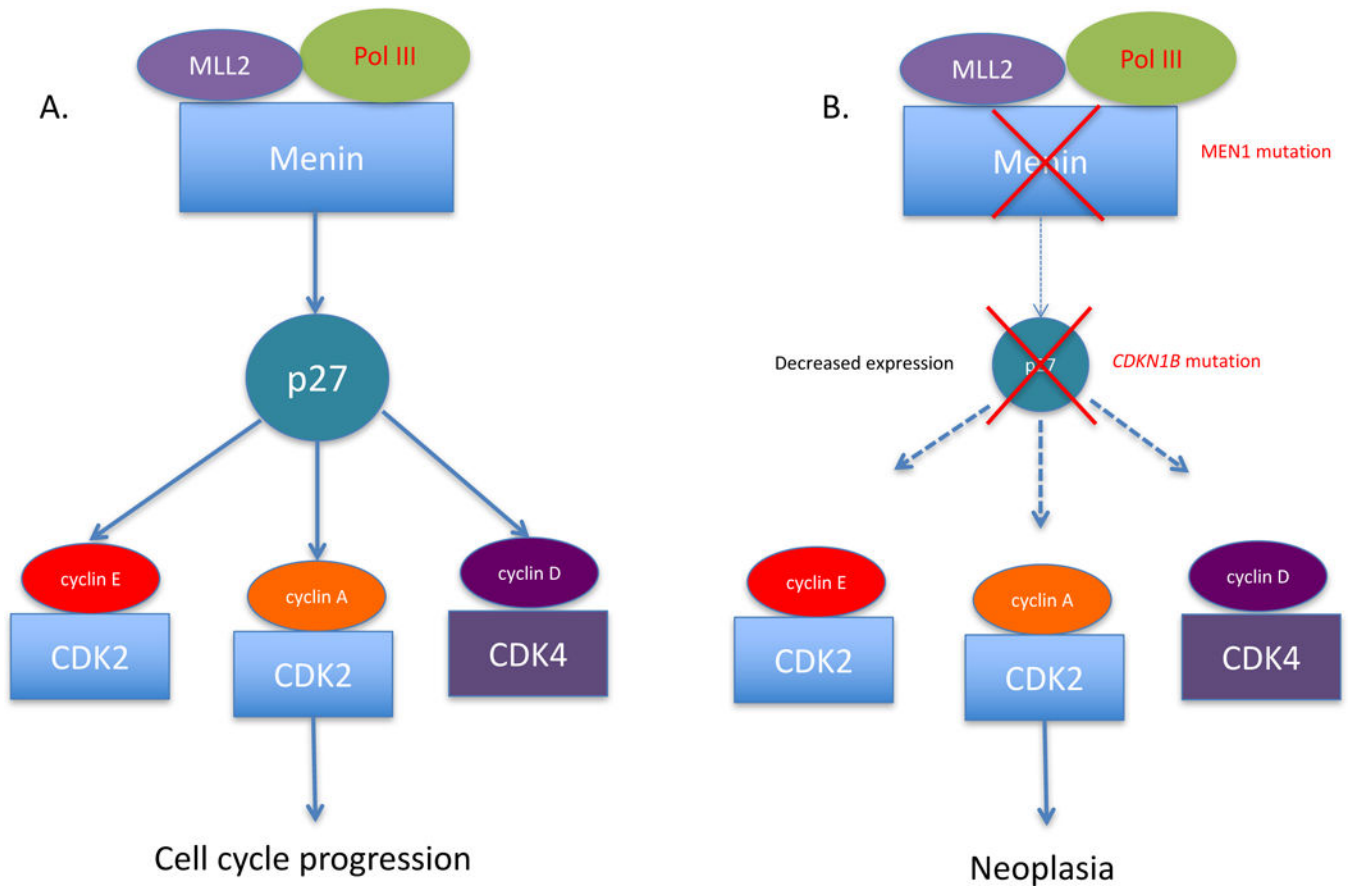


Figure 1.

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 methyltransferase (MLL2) and RNA polymerase II (Pol III). 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 neoplasia.

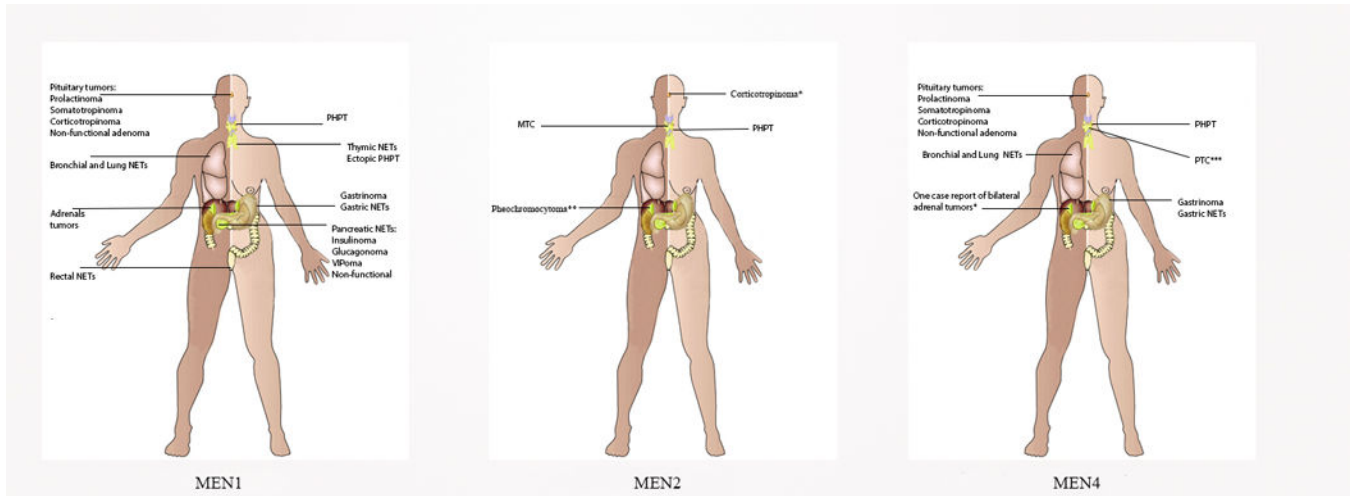


Figure 2.

The variable clinical phenotypes of MENs.

*Recent case of Cushing Disease reported in MEN2B

**Pheochromocytomas have been described in mouse models of MEN4

*** On case of metastatic papillary thyroid carcinoma

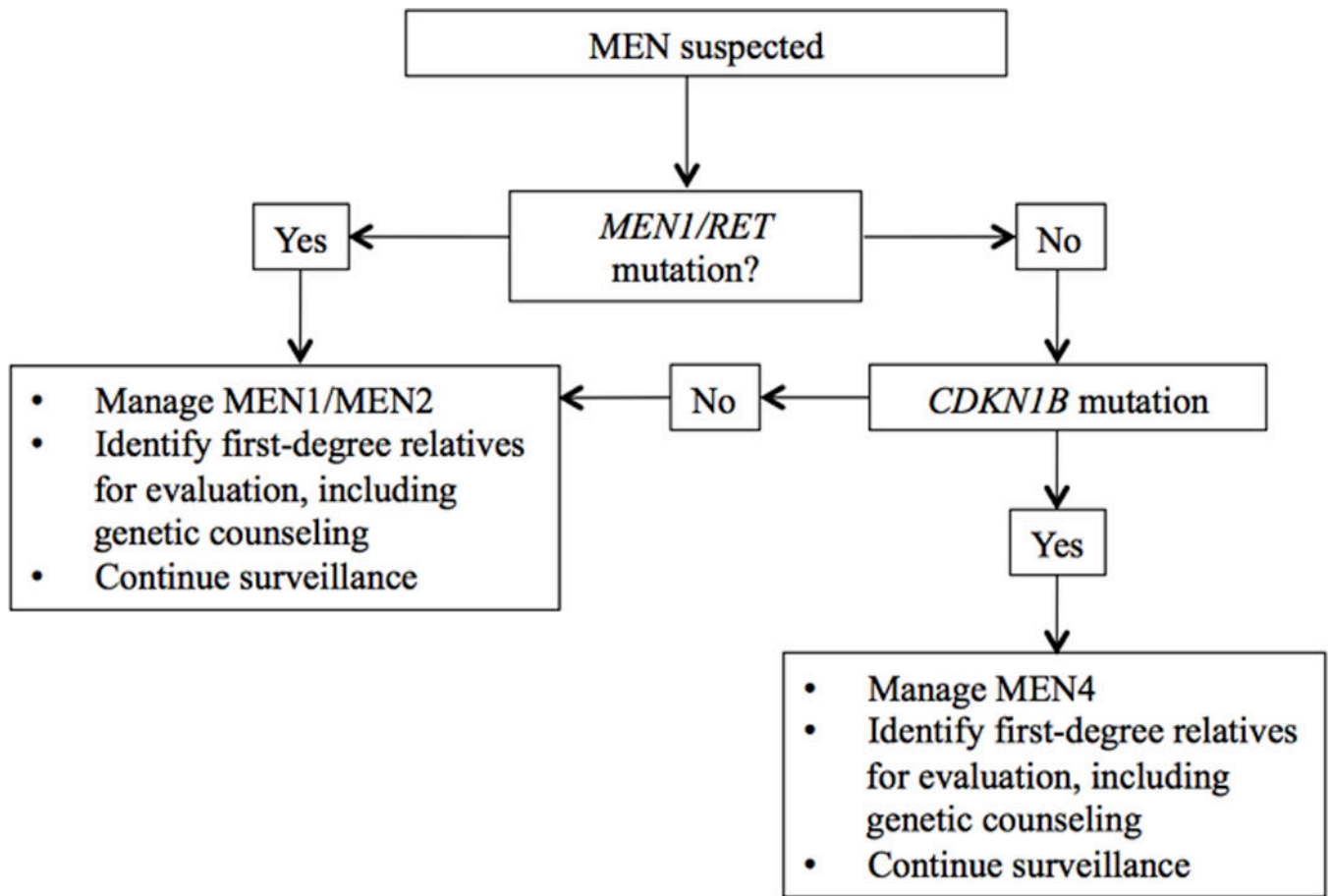


Figure 3.

An approach to screening in MEN4. Index cases, or individuals with MEN1-like features and negative MEN1 testing should be offered genetic counseling and testing for MEN4 (CDKN1B). 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.

Table 1

Established cases of MEN4. Abbreviation: ACTH, Adrenocorticotropic hormone; FIPA, familial isolated pituitary adenoma; GH, growth hormone; NET, neuroendocrine tumor; PHPT, primary hyperparathyroidism; NA, not available

<i>CDKN1B</i> Mutation	Age	Sex	Race	Clinical Manifestations	Family History	Ref.
p.W76X (c.692G>A)	48	F	Caucasian	Acromegaly PHPT	3 family members with the same p27 variant. Father had acromegaly (not tested for the p27 variant). Sister had renal angiomyolipoma (positive for same p27 variant); her son has testicular cancer	(Pellegata et al. 2006)
p.K25fs (c.59_77dup)	47	F	Caucasian	Small-cell neuroendocrine carcinoma at age 45 ACTH-dependent Cushing syndrome (Cushing disease) PHPT Multiple sclerosis at age 46	Negative	(Georgitsi et al. 2007)
ATG-7G>C in the 50-UTR	61	F	NA	Bilateral nonfunctional adrenal masses Uterine fibroids PHPT	Two asymptomatic daughters (ages 47 and 48), positive for same p27 variant	(Agarwal, et al. 2009a)
p.P95S (c.283C>T)	50	F	NA	Zollinger-Elison Syndrome with masses in duodenum and tail of pancreas PHPT	NA	(Agarwal et al. 2009a)
Stop>Q (c.595T>C)	50	F	NA	PHPT	Monozygotic twin sisters positive for same variant with PHPT (1 parathyroid tumor) at age 66. Aunt and cousin have PHPT, not tested for p27	(Agarwal et al. 2009a)
p.P69L (c.678C>T)	79	F	Caucasian	Papillary thyroid carcinoma with neck lymph node metastases Bilateral multiple lung metastatic from bronchial carcinoma PHPT Non-functioning pituitary microadenoma Subcutaneous epigastric lipoma Type 2 diabetes mellitus	Negative	(Molatore et al. 2010)
p.G9R (c.25G>A)	68	M	Caucasian	PHPT	NA	(Costa-Guda, et al. 2011)
p.P133T (c.397C>A)	58	F	Caucasian	PHPT (1 parathyroid tumor)	NA	(Costa-Guda, et al. 2011)
heterozygous GAGA deletion in the 5'-UTR (ATG-32-29del) (c.32_29del(GAGA))	69	F	Hispanic	Gastric NET PHPT	NA	((Malanga et al. 2012)
p.A55T (c.163G>A)	42	F	Hispanic	Zollinger-Elison Syndrome with gastrinoma and hepatic metastases	Negative	(Belar, et al. 2012)

<i>CDKN1B</i> Mutation	Age	Sex	Race	Clinical Manifestations	Family History	Ref.
				PHPT		
p.S125X (c.374_375delCT)	53	F	Caucasian	Gastrointestinal NET PHPT	The patient's 35-year-old son, who had no MEN4-associated clinical features, was the first reported male mutation carrier in <i>p27</i>	(Tonelli, et al. 2014)
p.E126D (c.378G>C)	15	F	Caucasian	Early-onset PHPT Recurrent renal calculi and hypercalcemia	Mother (aged 46) and maternal grandfather (aged 74 years) carried the same missense mutation but both were normocalcemic with normal PTH levels	(Elston, et al. 2015)
4-bp deletion within the 5' -UTR (c.-456_-453delCCCTT)	62	F	Caucasian	Acromegaly (somatotropinoma) Well-differentiated nonfunctional pancreatic NET	Negative	(Occhi et al. 2013)
heterozygous mutation in the 5' - UTR region (c.-29_-26delAGAG)	6	F	Caucasian	Gigantism (somatotropinoma)	Negative	(Sambugaro, et al. 2015)
p.I119T (c.356T>C)	NA	NA	NA	<i>AIP</i> mutation-negative FIPA, presenting with a GH-secreting adenoma	The p.I119T change was found in one member of a two person homogeneous FIPA family with somatotropinomas	(Tichomirowa, et al. 2012).
p.K96Q (c.286A>C)	NA	NA	NA	<i>AIP</i> mutation-negative FIPA, presenting with hyperprolactinemia, due to a suspected prolactinoma that was treated chronically with cabergoline when referred Breast cancer at the age of 41	The unaffected sister of this patient was also a carrier of this variant.	(Tichomirowa, et al. 2012).
c.-80C>T	NA	NA	NA	PHPT	Negative	(Borsari et al. 2017).
c.-29_-26delAGAG	NA	NA	NA	PHPT	Negative	(Borsari et al. 2017).
c.397C>A	NA	NA	NA	PHPT	Negative	(Borsari et al. 2017).

Phenotypic spectrum across the MEN syndromes. Abbreviation: CD, Cushing disease; MTC, Medullary Thyroid Carcinoma; NET, Neuroendocrine tumors; PHPT, primary hyperparathyroidism; PGL, paraganglioma; PTC, Papillary Thyroid Carcinoma.

Table 2

MEN Type	Pituitary Tumors	Acromegaly	CD	PHPT	MTC	PTC	Pheochromocytoma	PGL	Adrenal tumors	Pancreatic NET	Lung/Bronchial NET	Gastric NET	Thymic NET	Gastrinoma	Skin
MEN1	+	+	+	+					+	+	+	+	+	+	+
MEN2A				+	+		+	+							+
MEN2B	+		+		+		+								+
MEN4	+	+	+	+		*	***		+	+	+	+		+	

* A single case report of Cushing disease in a patient with MEN2B

** A single case report of metastatic PTC in a patient with MEN4

*** Not yet found in humans