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Beyond MEN1, When to Think About MEN4? Retrospective Study on 5600 Patients in the French Population and Literature Review

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Abstract

Context: Germline *CDKN1B* variants predispose patients to multiple endocrine neoplasia type 4 (MEN4), a rare MEN1-like syndrome, with <100 reported cases since its discovery in 2006. Although *CDKN1B* mutations are frequently suggested to explain cases of genetically negative MEN1, the prevalence and phenotype of MEN4 patients is poorly known, and genetic counseling is unclear.

Objective: To evaluate the prevalence of MEN4 in MEN1-suspected patients and characterize the phenotype of MEN4 patients.

Design: Retrospective observational nationwide study. Narrative review of literature and variant class reassessment.

Patients: We included all adult patients with class 3/4/5 *CDKN1B* variants identified by the laboratories from the French Oncogenetic Network on Neuroendocrine Tumors network between 2015 and 2022 through germline genetic testing for MEN1 suspicion. After class reassessment, we compared the phenotype of symptomatic patients with class 4/5 *CDKN1B* variants (ie, with genetically confirmed MEN4 diagnosis) in our series and in literature with 66 matched MEN1 patients from the UMD-MEN1 database.

Results: From 5600 MEN1-suspected patients analyzed, 4 with class 4/5 *CDKN1B* variant were found (0.07%). They presented with multiple duodenal NET, primary hyperparathyroidism (PHPT) and adrenal nodule, isolated PHPT, PHPT, and pancreatic neuroendocrine tumor. We listed 29 patients with *CDKN1B* class 4/5 variants from the literature. Compared with matched MEN1 patients, MEN4 patients presented lower NET incidence and older age at PHPT diagnosis.

Conclusion: The prevalence of MEN4 is low. PHPT and pituitary adenoma represent the main associated lesions, NETs are rare. Our results suggest a milder and later phenotype than in MEN1. Our observations will help to improve genetic counseling and management of MEN4 families.

Key Words: *CDKN1B*, MEN4, MEN1, multiple endocrine neoplasia, pituitary, parathyroid, neuroendocrine tumor

Abbreviations: 5'UTR, 5' untranslated region; ACMG, American College of Medical Genetics and Genomics; *CDKN1B*, cyclin dependent kinase inhibitor 1B; dpNET, duodeno-pancreatic neuroendocrine tumor; GTE, French Group of Endocrine Tumors; MEN1, multiple endocrine neoplasia type 1; MEN4, multiple endocrine neoplasia type 4; MRI, magnetic resonance imaging; NET, neuroendocrine tumor; NGS, next-generation sequencing; PA, pituitary adenoma; PHPT, primary hyperparathyroidism; pNET, pancreatic neuroendocrine tumor; TENGEN, French Oncogenetic Network of Neuroendocrine Tumors.

Genetic predisposition is involved in a minority of patients presenting with pituitary adenoma (PA), primary hyperparathyroidism (PHPT), and/or neuroendocrine tumors (NET). The most frequently mutated gene is *MEN1*, with an incidence of ~1 to 3:100 000 patients, resulting in multiple endocrine neoplasia type 1 (MEN1, OMIM #131100) (1). Patients with MEN1 mostly present with 1 or multiple diseases, including PHPT (>90%), duodenopancreatic NET (~55–70%), PA (~40%), and other less frequent features (2, 3). The disease is transmitted in an autosomal-dominant manner. Approximately 10% of patients presenting with the MEN1 phenotype lack a *MEN1* mutation, which defines MEN1 phenocopies (4). Some of these can present with mosaic *MEN1* mutations or a variant located in the 5' untranslated region (5'UTR) or deep introns of the gene (5–8). They can also present variants in other endocrine tumor-predisposing genes, such as cell division cycle 73 (*CDC73*) or glial cells missing transcription factor 2 (*GCM2*) in cases of isolated PHPT, or aryl hydrocarbon receptor interacting protein (*AIP*) in cases of familial isolated PA (7, 9). Finally, the involvement of other genes may explain some of these phenotypes. In 2006, a cyclin-dependent kinase inhibitor 1B (*CDKN1B*) variant was identified in a family of patients with MEN1 phenocopies. The resulting disease was named multiple endocrine neoplasia type 4 (MEN4, OMIM #610755) (10). *CDKN1B* is located on chromosome 12 (12p13) and comprises 2 exons encoding the 198 amino

acid p27^{kip1} protein. p27^{kip1} is a cyclin-dependent kinase inhibitor that is normally activated by menin (encoded by *MEN1*) to negatively regulate the cell cycle and limit the G1-S phase transition of CDK2 and CDK4 (11). Deleterious mutations in *MEN1* or *CDKN1B* lead to decreased p27^{kip1} expression, triggering uncontrolled cell-cycle progression (12, 13).

The number of reported cases of MEN4 is small (<100), mainly as case reports, with few series (14). Moreover, there are currently no recommendations to guide the management of these patients.

In this study, we report patients with *CDKN1B* variants identified in the French Oncogenetic Network on Neuroendocrine Tumors (TENGEN) laboratory network and managed in French tertiary centers as part of the GTE network (French Group of Endocrine Tumors). We aimed to investigate the germline *CDKN1B* sequence variations identified in these patients, together with their associated detailed phenotypes, to understand the severity and age of onset of MEN4, and to establish genotype/phenotype correlations. We also analyzed *CDKN1B* variants reported in the literature and reclassified according to the American College of Medical Genetics and Genomics (ACMG) criteria (15). We pooled patients with *CDKN1B* likely pathogenic and pathogenic variants (class 4 and class 5) (ie, patients with genetically confirmed MEN4) from our series and literature to compare them with MEN1 patients from the national French UMD-MEN1 database, using a matching procedure.

Methods

Patient Selection

This retrospective, multicenter study was approved by the scientific board of the Groupe d'étude des Tumeurs Endocrines (GTE). This study was approved and registered with the French Commission Nationale de l'Informatique et des Libertés Data Protection Agency, no. 2214454v0 (July 19, 2019) and conducted in accordance with the guidelines of the Declaration of Helsinki. Patients received information and a nonopposition note for the study. All had previously provided signed informed consent allowing germline DNA analysis during 1-on-1 genetic counseling. They were index cases analyzed in the context of the following indications: clinical diagnosis of MEN1 (at least 2 MEN1-related major lesions in 1 patient), familial PA or isolated macroPA before 30 years of age, familial history of isolated PHPT or isolated PHPT before 50 years of age, and isolated thoracic and/or duodenopancreatic NET before age 50 years (French recommendations for genetic testing ANPGM-TENGEN-SFE, available on <https://www.reseau-gte.org/tengen/>).

The inclusion criteria were (1) patients with a *CDKN1B* variant initially classified as class 3, 4, or 5 identified by targeted panel next-generation sequencing (NGS) and (2) those diagnosed and managed in France.

A noninclusion criterion was the presence of a pathogenic or likely pathogenic variant identified in a gene known to predispose to endocrine tumors (such as *MEN1*, *VHL*, *PRKARIA*).

The clinical, biological, and imaging data were pseudo-anonymized and collected between January 1, 2020, and January 31, 2023, after contacting the referent physicians.

CDKN1B Sequencing and Variant Classification

Variants were recorded according to the Human Genome Variation Society nomenclature (NM_004064.5; assembly GRCh37/hg19 version of the reference human genome). Sequence variants in *CDKN1B* were reinterpreted and reclassified into 1 of the 5 classes of pathogenicity: class 1, benign; class 2, likely benign; class 3, uncertain significance; class 4, likely pathogenic; class 5, pathogenic, according to the ACMG-Association of Molecular Pathology guidelines, considering open-access population database frequencies (dbSNP, gnomAD, ESP, ExAc); in silico prediction software packages; available familial, clinical, and biological data; patient variant databases (Clinvar, HGMDPro); and scientific publications.

Review of Published *CDKN1B* Cases and Variants Classification Reassessment

We performed a narrative review of publications indexed in the PubMed database from January 1, 2006, to June 30, 2023, using the terms “multiple endocrine neoplasia,” “MEN4,” and “*CDKN1B*” to list symptomatic patients harboring class 3-4-5 *CDKN1B* variants. We collected data on the genotypes and phenotypes of these patients. Because ACMG variant classification is susceptible to evolving, we actualized the classification of these variants according to the ACMG guidelines. The molecular biologists of the TENGEN network reassessed the 52 patients thus listed with class 3-4-5 *CDKN1B* variants and endocrine tumors.

Matching With the French UMD-MEN1 Patient Database

Given the similarities between the phenotypic spectra of MEN4 and MEN1, we compared the outcome of genetically confirmed MEN4 and MEN1 patients. The phenotypic presentation of patients with *CDKN1B* class 4-5 variants (ie, patients with genetically confirmed MEN4) from our series together with those reported in the literature was matched with that of patients with MEN1 included in the national UMD-MEN1 database as of March 2023. Briefly, this database collects and centralizes the clinical data of all patients undergoing genetic diagnosis in 1 of the 6 TENGEN centers performing *MEN1* genetic screening (Lille, Lyon, Marseille, Toulouse, Angers, Paris), in connection with the clinical national MEN1-GTE database (University of Burgundy, Dijon). The French UMD MEN1 database project was approved by the French supervisory authority Commission Nationale de l'Informatique et des Libertés (registration no. 908361) and the national ethics committee (Comité Consultatif pour le Traitement de l'Information en matière de Recherche dans le domaine de la Santé; no. 07.421) and registered under no. 91513.

We performed a propensity score matching procedure with the MatchIt package in R Studio according to (16) and realized a 2:1 nearest neighbor MEN1:CDKN1B propensity score matching without replacement with a propensity score estimated using logistic regression of the treatment on the following covariates: sex (men-women), patient type (proband-relative), class variant (4,5), and age at last follow-up (years). Regarding the last covariate, age at last follow up for patients already reported in the literature was defined, if not mentioned as is in the publication, at the age at diagnosis of the last endocrine tumor.

Statistical Analysis

Qualitative variables are presented as numbers (percentages), and quantitative variables are expressed as means (95% CI) or medians (range) according to normal distribution or not. Descriptive statistics were performed using GraphPad Prism, version 9.4.1 (GraphPad Software). Group comparisons were performed using χ^2 or *t*-tests for parametrical values and Fisher test for nonparametrical values. The probability of the occurrence of different lesions (expressed in % [SD]) according to age was estimated using the Kaplan–Meier method and analyzed with log-rank tests. Statistical significance was set at $P < .05$.

Results

Variants

Between January 2015 and June 2022, 5600 NGS analyses were performed in 6 molecular biology laboratories of the French TENGEN Network analyzing *CDKN1B* in index cases for the previously mentioned indications (in detail, 20% for PA exploration [1120/5600], 64% for PHPT 3584/5600, and 16% for a MEN1/4 clinical diagnosis 896/5600). These 5600 analyses revealed 50 patients with a *CDKN1B* variant initially classed as class 3/4/5 variant. After reclassification according to the ACMG guidelines, we retained 4 class 4/5 *CDKN1B* variants (4/5600, 0.07%, Table 1): c.313G > T, p.(Glu105*), c.320del, p.(Gln107Argfs*12), c.201_202del, p.(His67Glnfs*57), and c.285del, p.(Gly97Valfs*22). All

Table 1. French *CDKN1B* class 4-5 variants and phenotypic outcomes

Nucleotide variation	Localization	Variation type	ACMG class proposition	Protein variation	Frequency in gnomAD (all: %)	Sex	Pituitary lesion	PHPT	NET	Adrenal lesion
c.201_202 del	Exon 1	Frameshift	5	p.(His67Gln fs*57)	NA	M	—	+	—	—
c.285del	Exon 1	Frameshift	5	p.(Gly97Valfs*22)	NA	F	—	+	+	—
c.313G > T	Exon 1	Nonsense	5	p.(Glu105*)	NA	F	—	—	+	—
c.320del	Exon 1	Frameshift	5	p.(Gln107Argfs*12)	0.000401	M	—	+	—	+

GnomAD version v2.1.1.

Abbreviations: ACMG, American College of Medical Genetics and Genomics; NET, neuroendocrine tumor; PHPT, primary hyperparathyroidism; SNV, single nucleotide variant.

class 4/5 variants identified here were nonsense or frameshift variants leading to truncated proteins or to absence of protein by the nonsense mediated decay mechanism. The other variants were reclassified as class 3 or 2 (Supplementary Table 1) (17). One patient presented with 2 class 3 *CDKN1B* variants. Five variants have not been previously reported (c.-465G > C, c.-317G > A, c.-231C > T, c.313G > T, c.285del, and c.402G > C).

In comparison, in these 5600 NGS analyses, 381 patients were identified with a class 4/5 *MEN1* variant (6.8%). No patient presented with a combination of *CDKN1B* and *MEN1* class 4-5 variant. The ratio class 4-5 *CDKN1B* variant/class 4-5 *MEN1* variant is 0.07/6.9 \approx 0.01.

Phenotypic Analysis

Detailed clinical data were available for 22 patients harboring 23 different variants, who were included in the phenotypic analysis (Fig. 1). All were index cases and no detailed information regarding the screening of relatives was available.

Class 4-5 Variants

The 4 patients harboring a class 4 or 5 variant presented the following outcomes (Table 1):

- A woman was diagnosed at age 68 years with 3 non-functional, asymptomatic, localized duodenal G1 NETs. Molecular exploration was therefore performed and revealed a c.313G > T, p.(Glu105*) *CDKN1B* class 5 variant.
- A 63-year-old man was diagnosed a mild PHPT (calcium level 104 mg/L [2.6 mmol/L] [reference values: 88-104 mg/L, 2.2-2.6 mmol/L], phosphorus level 23 mg/L [0.7 mmol/L] [reference values: 25-45 mg/L, 0.8-1.45 mmol/L], PTH 110 pg./mL, 24-hour urinary calcium levels 193 mg/4.8 mmol) because of a unique 6-mm parathyroid adenoma. He presented with a history of urinary lithiasis at 21 years of age and was also diagnosed with densitometric osteoporosis (T-score -2.8). He was also incidentally diagnosed with a unilateral, 18-mm benign adrenal nodule at age 62 years, with mild glucocorticoid secretion (physiological urinary free cortisol, 8 AM cortisol after 1-mg dexamethasone suppression test 3.8 μ g/dL [105 nmol/L] [N < 1.8 μ g/dL (50 nmol/L)]). In this context, molecular screening was performed and identified a c.320del, p.(Gln107Argfs*12) *CDKN1B* class 5 variant.
- A 71-year-old man was diagnosed PHPT (known since 30 years of age according to the patient, calcium levels

141 mg/L [3.5 mmol/L], phosphorus levels 27 mg/L [0.9 mmol/L], PTH 94 pg/mL) resulting from a unique 15-mm parathyroid adenoma. He also presented with a suspicion of acromegaly, with mild IGF1 elevation (<120% the upper limit of normal), but without abnormality on magnetic resonance imaging (MRI). Because of the supposed young age at diagnosis of PHPT and suspicion of acromegaly, molecular screening was performed and identified a c.201_202del, p.(His67Gln fs*57) *CDKN1B* class 5 variant.

- Finally, a woman was diagnosed at age 42 years a 22-mm nonfunctional, asymptomatic, localized G1 pancreatic neuroendocrine tumor (pNET) and benefited from surgery. She also later benefited from thyroid surgery for a multinodular goiter responsible for hyperthyroidism at age 50 years, during which a centimetric parathyroid lesion corresponding to a parathyroid adenoma was fortuitously discovered and resected. The patient's calcium and phosphorus levels were normal before surgery. Because of the cooccurrence of parathyroid adenoma and pNET before age 50 years, an NGS exploration was performed and revealed a c.285del, p. p.(Gly97Valfs*22) *CDKN1B* class 5 variant.

CDKN1B Variants of Unknown Significance

Detailed clinical data were available for 18 patients presenting a class 3 *CDKN1B* variant, of unknown significance (Table 2). The sex ratio was balanced (9 men and 9 women) and the median age at first manifestation was 38 years (range, 15-71 years). The most frequent tumor was PA (11 patients, 73%), followed by PHPT (9 patients, 50%) and duodenopancreatic NET (1 patient, 10%).

Eleven of 15 screened patients (73%) presented with PA. The median age at diagnosis was 33 years (range, 16-71) (Table 3). Seven patients presented an endocrine secretion (2 prolactinomas, 3 somatotrophinomas, and 2 corticotropinomas). The median lesion size was 10.8 mm (range, 3-30 mm), 6 patients (55%) presented with a macroadenoma, and cavernous sinus invasion was observed in 3 patients (Supplementary Table S2) (17).

PHPT was observed in 9 of the 18 screened patients (50%), with a median age at diagnosis of 44 years (range, 33-71) (Table 4). Blood calcium levels were mildly elevated (median, 105 mg/L or 2.62 mmol/L), 5 of the 9 patients (55%) presented with normocalcemic PHPT. The median 24-hour urinary calcium level was 260 mg (6.5 mmol), PTH levels were higher than the normal upper limit of the assays in all patients. Sixty-seven percent and 22% of patients had 1 and 2

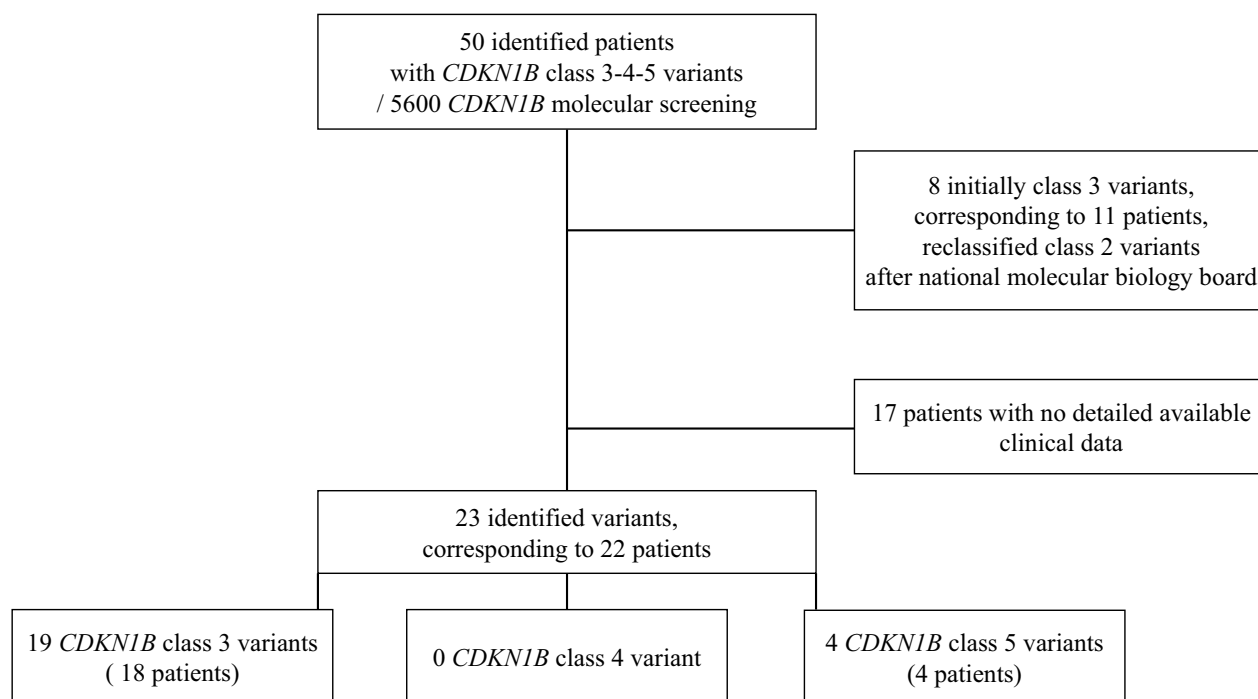


Figure 1. Flow chart of the study.

parathyroid lesions, respectively. Six patients benefited from parathyroid surgery; adenoma was observed in 4 patients and parathyroid hyperplasia in 2 patients (Supplementary Table S3) (17).

One patient was diagnosed with a unique, localized, non-functional, incidentally discovered at 50 years of age, with a size of 17 mm, and grade 2 pNET (Ki-67:9%). One patient, also diagnosed with PA and PHPT, presented at age 66 years an incidentally discovered benign, unilateral 9-mm adrenal lesion, without endocrine secretion. Three patients presented with multiple endocrine tumors: both with PA and PHPT.

Narrative Review of the Literature

The literature review identified 26 studies, which are included in this review and comprise 77 patients harboring class 3-4-5 *CDKN1B* variants with or without clinical manifestations (14, 18-39). Most patients were recently reviewed by Halperin et al (40) and Singeisen et al (41), who also included new patients, and 2 other cases have been recently published (42). Of the 77 patients reported in the literature, from which we subtracted the 2 included in the present study, 23 (30%) presented no symptoms related to endocrine tumors and were excluded from the analysis because they were identified in familial molecular screening given the presence of a *CDKN1B* variant in the family, screening for a germline genetic predisposition to develop nonendocrine cancer, or during the evaluation of a patient with overgrowth and neurodevelopmental delay. We reassessed the classification of 28 variants of the 52 symptomatic patients in literature (Table 3 and supplemental Supplementary Table S1) (17). We therefore reviewed parameters of the 29 patients presenting with a class 4-5 variant, confirming the molecular diagnosis of MEN4 (n = 5 and 24, respectively) (Table 3, Supplementary Fig. S1, and Table 4) (17). Two patients were reported in a

pediatric study (35): they developed isolated microcorticotropinomas diagnosed at age 12 and 15 years, respectively.

Among these 29 MEN4 patients, 17 were women (58%). Fifteen were index cases and 14 were related. In the 15 reported MEN4 proband patients, the first manifestation was PHPT in 8 patients (53%, median age at diagnosis = 40.5 years, interquartile range, 12.2), PA in 5 patients (33%, median age at diagnosis = 30, interquartile range, 30), and NET in 2 patients (13%, median age at diagnosis = 39 years).

Of these, PA was diagnosed in 13 patients (44%, 9 women and 4 men) at a median age of 40 years (range, 12-67) (26, 18, 14, 42, 35, 39, 27, 20, 10). Most adenomas were secreting, including 4 corticotropinomas (2 pediatric cases), 2 prolactinomas (including 1 suspicion without MRI abnormality), 2 somatotrophinomas, and 5 nonfunctional PA.

In 10 of the 13 patients with available data regarding imaging procedure, 8 were diagnosed with microadenoma and 2 with macroadenoma.

PHPT was diagnosed in 25 patients (86%, 16 women and 9 men) at a median age of 47 years (range, 29-71) (10, 14, 18-20, 27, 34, 39, 40, 42). The median serum ionized calcium levels, available for 13 patients, was 1.36 mmol/L (range, 1.3-1.5 mmol/L). Total serum calcium levels were available for 4 patients, with a median value of 3.1 mmol/L (range, 2.8-3.4 mmol/L). The 2 patients for which bone density scan was reported presented with osteopenia or osteoporosis.

Morphological exploration results, available for 11 patients, identified 1, 2, 3, and 4 lesions in 5 (45%), 2 (18%), 2 (18%), and 2 (18%) patients, respectively. Pathological examinations identified parathyroid adenoma in 6 patients, including 1 atypical parathyroid adenoma, association of adenoma and hyperplasia in 2 patients.

NET was identified or suspected in 8 patients (27%, 6 women and 2 men) at a median age of 56 years (range, 31-70), with duodenopancreatic NET diagnosed in 4 patients, among

Table 2. French *CDKN1B* class 3 variants and phenotypic outcomes

Nucleotide variation	Localization	Variation type	TENGEN ACMG Proposition	Number of patients harboring this variant	Protein variation	Frequency in gnomAD (all: %)	Sex	Pituitary lesion	PHPT	NET	Adrenal lesion
c.-465G > C	5' UTR	SNV	3	1	p.(?)	NA	F	+	+	NA	NA
c.-317G > A	5' UTR	SNV	3	1	p.(?)	NA	M	—	+	NA	—
c.-231C > T	5' UTR	SNV	3	1	p.(?)	NA	M	+	—	NA	NA
c.-29_-26del	5' UTR	Deletion	3	1	p.(?)	NA	M	+	—	—	—
c.25G > A/ c.236T > G	Exon 1/Exon 1	Missense/ Missense	3	1	p.(Gly9Arg)/ p.(Val79Gly)	0.0004/0.002	M	NA	+	—	—
c.31C > T	Exon 1	Missense	3	1	p.(Pro11Ser)	0.004	M	—	+	—	—
c.154 A > G	Exon 1	Missense	3	1	p.(Met52Val)	NA	M	+	—	—	—
c.155T > C	Exon 1	Missense	3	1	p.(Met52Thr)	0.0141	F	+	+	—	—
c.195G > T	Exon 1	Missense	3	1	p.(Gln65His)	0.00212	F	NA	+	NA	NA
c.206C > T	Exon 1	Missense	3	1	p.(Pro69Leu)	0.000398	F	+	+	—	+
c.232G > A	Exon 1	Missense	3	1	p.(Glu78Lys)	NA	M	NA	+	—	NA
c.272C > T	Exon 1	Missense	3	1	p.(Pro91Leu)	0.000399	F	—	+	—	—
c.341 C > G	Exon 1	Missense	3	1	p.(Pro114Arg)	0.000404	F	—	—	+	—
c.386A > G	Exon 1	Missense	3	1	p.(His129Arg)	0.00319	M	+	—	NA	NA
c.402G > C	Exon 1	Missense	3	1	p.(Lys134Asn)	NA	F	+	—	NA	NA
c.475 + 18A > T	Intron 1	Exon skipping	3	1	p.(?)	4.3 × 10 ⁻⁸	F	+	—	NA	NA
c.476A > G	Exon 2	Missense	3	1	p.(Asp159Gly)	0.000796	F	+	—	NA	NA
c.586C > T	Exon 2	Missense	3	1	p.(Arg196Cys)	0.000796	M	+	—	—	—

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Abbreviations: ACMG, American College of Medical Genetics and Genomics; NA, not available; NET, neuroendocrine tumor; PHPT, primary hyperparathyroidism; SNV, single nucleotide variant; TENGEN, Oncogenetic Network on Neuroendocrine Tumors; UTR, untranslated region.

which 1 presented with Zollinger-Ellison syndrome and 2 with multifocal lesions (14, 18, 20, 26, 27, 39, 40). The other localizations of NETs included the lungs, thymus, and small intestine. One patient presented with cervical neuroendocrine carcinoma. Three patients presented with tumor metastases.

Adrenal lesions were described in 3 patients (7%): 1 recently reported a case of unilateral, cortisol-secreting, benign lesion, and a second patient with a bilateral adrenal mass, with unknown secretory status (39, 42).

Other nonendocrine neoplasia were reported in 2 patients: 1 case of metastatic Gleason 6 prostate adenocarcinoma at age 60 years in a patient with cooccurring *NF1* variant and 1 case of differentiated thyroid cancer diagnosed at age 64 years (20, 34).

Comparison of the Clinical Presentation of MEN4 Patients With MEN1 Patients From the French UMD-MEN1 Database

After pooling the data of MEN4 patients with class 4-5 *CDKN1B* variants reported in our series ($n = 4$) and in the literature ($n = 29$), we performed a matching procedure between these 33 MEN4 patients and 66 MEN1 patients from the previously reported UMD-MEN1 database, adjusted for sex, class variant, patient type (proband vs relative), and age at last follow-up (Table 4, Supplementary Fig. S2) (17). There was no difference between the proportion of patients diagnosed with PA and age at diagnosis (*CDKN1B* vs *MEN1*: 39.3 vs 44, $P = 0.65$; mean age [95% CI] at diagnosis: 44.8 [32.6-57] vs 37.7 [32.5-42.9], $P = .26$), also for adrenal lesion

(9.0 vs 24, $P = .07$; 52.0 [30.5-73.5] vs 44.6 [38.2-51], $P = .28$). PA cumulated incidence in *CDKN1B* patients was estimated to 6% at age 20 years (95% CI, 1.5-22.1), 18.6% at age 40 years (8.8-36.8), and 30.3% at age 60 years (16.8-50.7), without difference with MEN1-matched patients (Fig. 2A).

There was no difference regarding the occurrence of PHPT (84.8 vs 89, $P = .51$), although mean age at diagnosis was significantly older in *CDKN1B* patients (50.8 [45.7-55.9] vs 43 [39.4-46.5], $P = .01$). PHPT cumulated incidence in *CDKN1B* patients was estimated to 0% at age 20 years, 22.6% at age 40 years (95% CI, 11.4-41.6), 63% at age 60 years (46.2-79.6), and significantly different with MEN1-matched patients ($P = .028$, Fig. 2B). More detailed comparison regarding phenotypic parameters at diagnosis was not performed because of the important lack of data in patients previously reported.

MEN4 patients presented significantly less NET than *MEN1* patients (30.3 vs 62, $P = .003$), diagnosed at an older age (55.5 [46.1-64.9] vs 42.6 [37.8-47.3], $P = .01$). NET-cumulated incidence in *CDKN1B* patients was estimated to 0% at age 20 years and age 40 years, and 17.7% at age 60 years (95%, 7.0-40.8), which is significantly different with MEN1-matched patients ($P = .004$) (Fig. 2C).

Discussion

We used multicenter French molecular biology laboratories and tertiary clinical referral center networks to provide the largest series of proband patients harboring *CDKN1B*

Table 3. CDKN1B class 4-5 variants and phenotypic outcomes reported in the literature (after classification reassessment by TENGEN molecular biologists)

Ref	Nucleotide variation	Localization	Variation type	TENGEN ACMG class proposition	Protein Variation	Frequency in gnomAD (all: %)	Sex	Pituitary lesion	PHPT	NET	Adrenal lesion
26	c.-454_-451delTTCC	5'UTR	Deletion	4	p.(?)	NA	F	+	—	+	—
18	c.59_77dup19	Exon 1	Frameshift	5	p.(Ser27Glyfs*104)	NA	F	+	+	+	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	F	+	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	F	—	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	F	—	+	+	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	M	+	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	F	—	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	M	+	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	F	—	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	F	—	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	M	—	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	F	—	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	F	—	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	F	—	+	—	—
14	c.121_122delTT	Exon 1	Frameshift	5	p.(Leu41Asnfs*83)	NA	M	—	+	—	—
42	c.169C > T	Exon 1	Nonsense	5	p.(Gln57*)	NA	M	—	+	—	—
39	c.179G > A	Exon 1	Nonsense	5	p.(Trp60*)	NA	F	+	+	+	+
20	c.206C > T	Exon 1	Missense	4	p.(Pro69Leu)	NA	NA	+	+	+	—
10	c.227G > A	Exon 1	Nonsense	5	p.(Trp76*)	NA	F	+	+	—	—
42	c.280_281delinsG	Exon 1	Frameshift	5	p.(Pro94Alafs*25)	NA	M	—	+	—	+
34	c.285dupC	Exon 1	Frameshift	5	p.(Lys96Glnfs*29)	NA	M	—	+	—	—
40	c.320delA	Exon 1	Frameshift	5	p.(Gln107Argfs*12)	0.00000410	M	—	+	+	—
40	c.320delA	Exon 1	Frameshift	5	p.(Gln107Argfs*12)	0.00000411	M	—	+	—	—
35	c.320delA	Exon 1	Frameshift	5	p.(Gln107Argfs*12)	0.00000412	M	+	—	—	—
27	c.374_375delCT	Exon 1	Deletion	5	p.(Ser125*)	NA	F	+	+	+	—
39	c.374_375delCT	Exon 1	Deletion	5	p.(Ser125*)	NA	M	+	—	+	—
35	c.376G > C	Exon 1	Missense	4	p.(Glu126Gln)	NA	F	+	—	—	—
19	c.595T > C	Exon 2	Nonsense	4	p.(*199Glnext60)	NA	F	—	+	—	—
19	c.595T > C	Exon 2	Nonsense	4	p.(*199Glnext60)	NA	F	—	+	—	—

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Abbreviations: ACMG, American College of Medical Genetics and Genomics; NA, not available; NET, neuroendocrine tumor; PHPT, primary hyperparathyroidism; SNV, single nucleotide variant; TENGEN, Oncogenetic Network on Neuroendocrine Tumors; UTR, untranslated region.

Table 4. Phenotypic outcomes of pooled MEN4 patients from our series and reported in the literature, matched with MEN1 patients

	<i>CDKN1B</i> (n = 33)	<i>MEN1</i> (n = 66)	<i>P</i>
Sex			.57
Male	14 (42)	32 (48)	
Female	19 (58)	34 (52)	
Patient type			.88
Proband	19 (57)	39 (59)	
Relative	14 (23)	27 (41)	
Class variant			.13
4	5 (15)	4 (6)	
5	28 (85)	62 (94)	
Mean age (95% CI) at last follow up	53.8 (48.6–59.1)	53.8 (50.3–57.5)	.99
PA	13 (39.3)	29 (44)	.65
Mean age (95% CI) at PA diagnosis	44.8 (32.6–57.0)	37.7 (32.5–42.9)	.26
PHPT	28 (84.8)	59 (89)	.51
Mean age (95% CI) at PHPT diagnosis	50.8 (45.7–55.9)	43.0 (39.4–46.5)	.01
NET	10 (30.3)	41 (62)	.003
Mean age (95% CI) at NET diagnosis	55.5 (46.1–64.9)	42.6 (37.8–47.3)	.01
Adrenal lesion	3 (9)	16 (24)	.07
Mean age (95% CI) at adrenal lesion diagnosis	52.0 (30.5–73.5)	44.6 (38.2–51)	.28

Abbreviations: MEN, multiple endocrine neoplasia; NET, neuroendocrine tumor; PA, pituitary adenoma; PHPT, primary hyperparathyroidism.

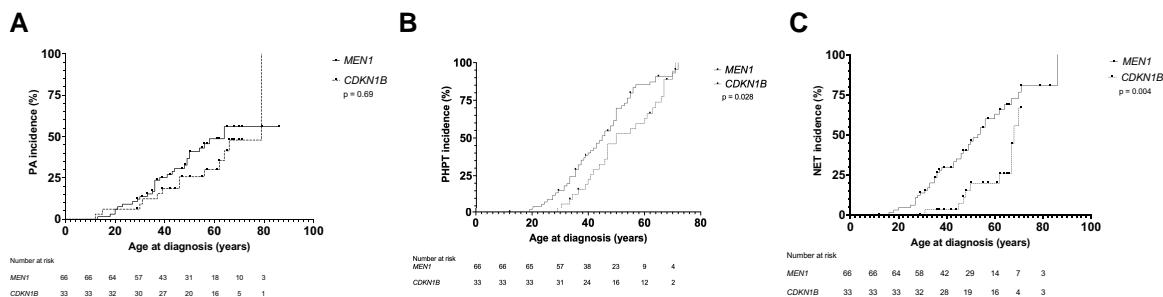


Figure 2. Cumulated incidence of endocrine tumors in pooled MEN4 patients from our series and reported in the literature, and matched MEN1 patients. (A) Pituitary adenoma (PA); (B) primary hyperparathyroidism (PHPT); and (C) neuroendocrine tumors (NET). Solid lines represent the cumulative risk.

variants that has been reported to date; however, most present with class 3 variants of unknown significance and with the most detailed phenotype characterization possible.

The strength of our study is its “real-world” setting; that is, the variants came from systematic and exhaustive reports of molecular analyses performed nationwide. This resulted in a predominance of class 3 variants of unknown significance rather than the pathogenic class 4-5 variant reported in the literature, which could represent some part of publication bias. We included only adult patients in the phenotype analysis; nevertheless, no minor index case has been identified with a class 4-5 variant by the TENGEN laboratories during the same period (data not shown). We did not observe hotspot variants or recurrent genotype-phenotype relationships (data not shown). Halperin et al recently reported a higher risk of PHPT in patients with indel variants compared with the risk in patients with missense mutations (40). Because of the small number of patients, we could not compare these data with those of our patients. In our series, the prevalence

of *CDKN1B* class 4 or 5 variants is low (<0.1% of all the NGS in our series, approximately 100 times rarer than *MEN1*); therefore, the question of *CDKN1B* testing and its indications remains open. Nevertheless, in the NGS era, the cost of concomitant *CDKN1B* sequencing with other genes in panel involved in *MEN1*-related endocrine tumors is negligible, which helps to maintain it. On the other hand, more data are needed to adapt the genetic counseling and the management of these MEN4 patients, which is why we performed a literature review.

Interestingly, the reassessment of previously reported variants “declared” 23 patients initially considered as harboring a class 5-4-3 to class 3 or 2, mostly missense variants. This variant reassessment procedure is helpful and necessary, given the regular actualization of knowledge and has already been done in other diseases (43-45). Confirmed class 4-5 variants in our series and in the literature are mostly deletion/duplication leading to truncated nonfunctional proteins or to the absence of protein by nonsense mediated decay mechanism rather than

missense variants, a point that should be considered when one interprets future *CDKN1B* variants.

Because we described here all genetically confirmed MEN4 patients reported in literature, it is redundant to discuss these findings with the available literature on patients with *CDKN1B* variants. We chose to match the genetically confirmed MEN4 patients from our series and literature with MEN1 patients after adjusting with potential confounding covariates such as age at last follow up, taking advantage of the large French UMD-MEN1 database. Regarding this last point, mean age at last follow up was approximately 54 years old for MEN4 and 56 years old for MEN1, which limits conclusions because some endocrine tumors can be diagnosed in the second half of life. Nevertheless, this matching brought us interesting results that could suggest recommendations to guide the management of *CDKN1B*-mutated (MEN4) patients, by comparing them with the MEN1 management guidelines (46).

Regarding PA, apart from the pediatric study focusing on corticotropinoma, we did not observe significant difference between MEN4 and matched MEN1 patients regarding PA occurrence and age at diagnosis, PA subtype, or lesion size (47, 48). We were nevertheless intrigued to observe that 4 MEN4 genetically confirmed patients developed a corticotropinoma, including 2 pediatric cases, although this subtype of PA is exceptionally caused by hereditary mutations. Instead, driver *USP8* somatic pathogenic variants are found in up to 62% of corticotropinomas, and recurrent *BRAF* and *USP48* mutations were described, varying from 0% to 23% and from 10% to 16% of *USP8* wild-type corticotropinomas, respectively (49-53). Therefore, because somatic *USP8*, *USP48*, and *BRAF* mutations were not ruled out in these 4 MEN4 patients, we think there is still uncertainty regarding the role of *CDKN1B* in corticotropinoma tumorigenesis, even if identified variants were classed as pathogens or likely pathogens. MEN1 guidelines recommend annual prolactin and IGF-1 dosage and pituitary MRI every 3 years starting at age 5 years; pituitary screening in MEN4 asymptomatic patient could be similar, with the possibility of also screening for hypercortisolism.

The proportion of MEN4 patient with PHPT is similar to these of matched MEN1 patients; however, it seems to occur significantly later, which questions the age above which *CDKN1B* variant research should no longer be carried out: it is typically placed at age 40 to 50 years in other endocrine tumor susceptibility genes and at age 30 years in isolated PHPT, but these thresholds could potentially miss a number of patients (54, 55). As expected, serum calcium levels followed a bimodal distribution, with mild values when relative subjects were screened, whereas hypercalcemia was often above 2.9 mmol/L in probands presenting with PHPT. Morphopathological results in MEN4 patients differs from that in MEN1 patients, with most PHPT patients presenting with 1 or 2 lesions, mostly adenoma, whereas parathyroid disease in MEN1 is typically caused by multiglandular hyperplasia (2, 56). In MEN4 patients, both in our series and in the literature, imaging modalities were heterogeneous, notably the use of ¹⁸F choline positron emission tomography-computed tomography, which is of interest in MEN1 (57). Finally, because of the retrospective nature of our study, data on follow-up and potential PHPT recurrence were lacking; therefore, we could not guide surgical management. To summarize, an annual calcium and PTH test seems to be adapted in MEN4 patients as in MEN1. The age when to

begin surveillance is again questionable, suggested at 8 years in MEN1 but never observed before age 29 years in MEN4 patients. There is currently no argument to suggest different surgical modalities in MEN4 patients with PHPT other than in sporadic forms, especially no rationale to propose subtotal parathyroidectomy as recommended in MEN1 patients (58). Moreover, there is a clear lack of exploration of bone impact, and future studies should investigate this point, because 3 of the 4 patients that benefited from a bone density scan presented significant abnormality, and we could hypothesize that a long duration of PHPT in MEN4 patients may lead to important alterations of bone mineral density, as observed in MEN1 patients (59, 60).

When looking at NET screening, we observed that NETs are significantly less frequent and occur later than in MEN1. The prevalence of NETs may be underestimated in our study because of the absence of systematic abdominal MRI or functional imaging such as somatostatin receptor positron emission tomography-computed tomography, whose diagnostic performance seems better than that of computed tomography, especially for MEN1 (61). The small number of patients with duodeno-pancreatic neuroendocrine tumors (dpNET) limits conclusions, but high-grade and metastatic disease seems to be uncommon, at least at diagnosis, as observed in MEN1 patients (62). Screening for dpNET could perhaps be more relaxed than in MEN1, in which annual MRI is proposed before age 10 years, even if MRI is a nonirradiating procedure and makes it possible to concomitantly study adrenal glands. Because of the absence of prolonged follow up and unknown risk of progression, we are not able to propose management recommendations, and we actually do not know if conservative management in localized, below 2 cm in size, but dpNET is of interest in MEN4 patients by homology with recent MEN1 dpNET guidelines (63).

We did not observe small intestine NET in our series, which could have been considered given the presence of somatic *CDKN1B* mutations at these NET locations (64).

Because of the exceptional occurrence of lung or thymic NET and adrenal lesion in *CDKN1B* patient, we are not able to suggest any proposal as to the methods and timing of monitoring and management.

The prevalence of adrenal lesions was lower in our study compared with matched MEN1 patients, which is also associated with an increased risk of cancer not observed in MEN4 patients, although it is difficult to draw conclusions given the small number of patients and the lack of follow-up data (65).

Some studies have reported an increased risk of developing nonendocrine cancers, such as prostate or colorectal cancer, in patients harboring germline *CDKN1B* variants (29, 66). Protein p27^{kip1} levels are reduced in several cancers; however, we did not observe recurrent nonendocrine cancer in our series, although the small number of patients and the absence of prolonged follow-up are limitations in our study (67).

Despite being the largest *CDKN1B* variant series reported to date, our study has several limitations, notably its retrospective nature and the associated lack of follow-up of genetic conditions that may evolve with the occurrence of additional endocrine tumors at an older age. Because of the multicenter origin of the patients, medical practices were not homogeneous; therefore, the reasons for requesting molecular analysis can differ between centers, and some patients did not benefit from the search for NET or adrenal lesions. We were also

unable to collect the clinical data associated with some of the identified variants. *CDKN1B* loss-of-heterozygosity screening, which is helpful for classification of variants of unknown significance, was not systematically performed after surgery, especially pituitary and parathyroid surgery. Furthermore, the narrative review of the literature is necessarily associated with publication bias, heterogeneity in clinical management of patients, and missing data, which is why we excluded asymptomatic patients with *CDKN1B* class 3/4/5 variants in the literature, being aware that this approach could overestimate the observed cumulative incidence. Nevertheless, it allowed us to strengthen our comparison with MEN1.

Conclusion

We report the largest series of patients harboring *CDKN1B* variants to date and performed an actualized review of cases previously reported in the literature. Patients with *CDKN1B* class 4-5 variants (ie, patients with genetically confirmed MEN4 in literature and in our series) were finally pooled and matched with MEN1 patients. The prevalence of MEN4 among MEN1-suspected patients is low (<0.1%), representing a small proportion of MEN1 phenocopies, and the clinical presentations differ slightly between these 2 diseases with decreased of NET incidence and an older age at PHPT diagnosis in MEN4. Other genes may be involved in MEN1 phenocopies, and whole-exome or whole-genome sequencing will likely allow for their discovery. Finally, even if we can suggest some proposal to help management and follow up, additional studies including new patients with longer follow-up periods are needed to define guidelines for these rare patients.

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Disclosures

None.

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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