UMD-MEN1 Database: An Overview of the 370 *MEN1* Variants Present in 1676 Patients From the French Population

Pauline Romanet,[1] Amira Mohamed,[2] Sophie Giraud,[3] Marie-Françoise Odou,[4] Marie-Odile North,[5] Morgane Pertuit,[2] Eric Pasmant,[5] Lucie Coppin,[6] Céline Guien,[7] Alain Calender,[3] Françoise Borson-Chazot,[8] Christophe Béroud,[9] Pierre Goudet,[10,11] and Anne Barlier[1]

[1]Aix Marseille Univ, APHM, INSERM, MMG, Laboratory of Molecular Biology, Hospital La Conception, 13385 Marseille, France; [2]Laboratory of Molecular Biology, Hospital La Conception, APHM, 13385 Marseille, France; [3]Genetics Department, Hospices Civils de Lyon, University Hospital (HCL), East Pathology Center, Lyon, B-A3, 59 Bld Pinel, 69677 Bron Cedex, France; [4]Service de Biochimie et Biologie Moléculaire "Hormonologie, Métabolisme-Nutrition, Oncologie", Centre de Biologie Pathologie, Centre Hospitalier Universitaire Lille, Lille F-59037 Cedex, France; [5]Service de Génétique et Biochimie Moléculaires, Hôpital Cochin, Assistance Publique-Hôpitaux de Paris, 27 Rue du Faubourg Saint Jacques, 75014 Paris, France; [6]Univ. Lille, INSERM, CHU Lille, UMR-S 1172, - JPARC - Jean-Pierre Aubert Research Center, F-59000 Lille, France; [7]Aix Marseille Univ, APHM, INSERM, MMG, U 1251 Bioinformatic Team, 13385 Marseille, France; [8]Hospices Civils de Lyon, Fédération d'Endocrinologie, Université Claude Bernard Lyon 1, HESPER EA 7425, F-69008 Lyon, France; [9]Aix Marseille Univ, APHM, INSERM, MMG, Department of Genetics, Hospital La Timone Enfants, 13385 Marseille, France; [10]Department of Endocrine Surgery, University Hospital of Dijon, and INSERM, U866, 21079, Dijon, France; and [11]Epidemiology and Clinical Research in Digestive Oncology Team, and INSERM, CIC1432, Clinical Epidemiology Unit, University Hospital of Dijon, Clinical Investigation Center, Clinical Epidemiology/Clinical Trials Unit, 21079 Dijon, France

ORCiD numbers: 0000-0003-1775-9569 (P. Romanet); 0000-0002-3740-6173 (A. Barlier).

Context: Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disease caused by mutations in the *MEN1* gene characterized by a broad spectrum of clinical manifestations, of which the most frequent are primary hyperparathyroidism, pituitary adenomas, and neuroendocrine tumors.

Objective: The aim of this work was to facilitate interpretation of variants and improve the genetic counseling and medical care of families of patients with MEN1.

Design, Setting, and Patients: The TENGEN network (Oncogenetics Network of Neuroendocrine Tumors) has interpreted and collected all allelic variants and clinical characteristics of the *MEN1*-positive patients identified through genetic testing performed in the French population from 1997 to 2015. Patients and their variants were registered in the locus-specific UMD-MEN1 database (www.umd.be/MEN1/).

Main Outcomes: Variant classification, age-related penetrance, and odds ratios.

Results: A total of 370 distinct variants reported in 1676 patients, including 181 unpublished variants, have been registered. This database analysis revealed a low frequency (6.6%) of benign or likely benign missense variants in *MEN1*. Eight families (1.9%) had members with familial isolated

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Abbreviations: ADRE, adrenal tumor; BV, benign variant; DP-NET, duodenopancreatic neuroendocrine tumor; FIHP, familial isolated hyperparathyroidism; HPTH, hyperparathyroidism; INCa, Institut National de lutte contre le Cancer; indel, insertion and deletion; LBV, likely benign variant; LPV, likely pathogenic variant; MEN1, multiple endocrine neoplasia type 1; microRGT, microrearrangement; MLPA, multiplex ligation-dependent probe amplification; NV, new variant; PIT, pituitary adenoma; PV, pathogenic variant; RGT, rearrangement; TENGEN, Oncogenetics Network of Neuroendocrine Tumors; UMD, Universal Mutation Database; UMD-MEN1, Universal Mutation Database for the *MEN1* gene; VUS, variant of uncertain significance.

hyperparathyroidism and harbored the same mutations as that found in families with authentic MEN1. An association existed between large rearrangements and an earlier onset of the disease, whereas no difference was observed between truncating and nontruncating variants.

Conclusion: The UMD-MEN1 database provides an exhaustive overview of the *MEN1* variants present in the French population. For each variant, a classification is publicly available. Clinical data collections allow the determination of genotype-phenotype correlation and age-related penetrance of lesions in the cohort. (***J Clin Endocrinol Metab* 104: 753–764, 2019**)

Multiple endocrine neoplasia type 1 (MEN1) is an inherited disease that predisposes carriers to primary hyperparathyroidism (HPTH), duodenopancreatic neuroendocrine tumors (DP-NETs), pituitary adenomas (PITs), adrenal tumors (ADREs), and thymic or bronchial neuroendocrine tumors (1). MEN1 is caused by a heterozygous mutation in *MEN1*, a tumor suppressor gene located in chromosome 11q13 (2, 3). *MEN1* encodes menin, a 610 amino acid protein expressed in numerous tissues (4, 5). Menin is a nuclear protein with several molecular functions, such as chromatin, protein, and DNA binding. This protein is also involved in many biological processes, such as negative regulation of the cell cycle, DNA repair, cytoskeletal components, regulation of transcription (menin inhibits the transcriptional activation by JunD), and regulation of telomerase activity (4–7).

MEN1 disease may display various clinical associations; the criteria for diagnosis were first established in Gubbio, Italy, and then regularly updated (8–10). MEN1 disease is usually described as an autosomal-dominant tumoral syndrome that is very progressive with a high penetrance during the lifespan (9, 11, 12). The clinical expression of the disease is variable, depending on the type of developed tumors. HPTH is present in 90% to 95% of cases (8, 10, 13–15). DP-NETs occur in 30% to 70% of patients with MEN1, and the third major manifestation, PIT, is reported in 30% to 40% of patients with MEN1 (10). The penetrance for all clinical features increases to 95% at age 40 years (10, 16) An increased risk for breast cancer has also been described in women with *MEN1* mutations (17).

In 2012, a group of experts, including physicians, surgeons, and geneticists from international centers, provided guidelines for the evaluation and treatment of and genetic testing for the *MEN1* gene (10). They redefined the bases for MEN1 diagnosis according to three categories of criteria: (i) clinical criterion: a patient with two or more MEN1-associated endocrine tumors (*i.e.,* HPTH, DP-NET, or PIT); (ii) familial criterion: a patient with one MEN1-associated tumor and a first-degree relative with MEN1; and (iii) genetic criterion: an individual who has a *MEN1* mutation but does not have clinical or biochemical manifestations of MEN1 (*i.e.,* a mutant gene carrier) (10).

The group proposed three different situations in which the *MEN1* mutational analysis should be undertaken (10): (i) in index cases with clinical MEN1; (ii) in index cases with suspected or atypical MEN1, which includes patients with HPTH before the age of 30 years, patients with multigland or multiple diseases in the same gland at any age (*e.g.*, multigland parathyroid disease), or patients harboring two or more MEN1-associated tumors, including one other than HPTH, PIT, and DP-NET (*e.g.*, HPTH plus ADRE); and (iii) in first-degree relatives of known *MEN1* mutation carriers, whether asymptomatic or not.

Since the beginning of genetic testing, and owing to early and suitable therapies, the discovery of a causal variant in the *MEN1* gene has reduced the morbidity of patients with MEN1 (8, 10, 18, 19). The *MEN1* gene presents a broad spectrum of variants, including large deletions, and truncating, missense, or splicing point mutations (20). No mutational hot spot has been defined, but some recurrent mutations have been described (20–23). The genotype-phenotype relationship remains under debate. In some kindred, only HPTH appears to develop in *MEN1* variants carriers; this situation of *MEN1*-mutation–related disease is referred as familial isolated hyperparathyroidism (FIHP), a rare, heritable disorder, characterized by hypercalcemia, inappropriately high PTH levels, and isolated parathyroid tumors with no evidence of hyperfunction of any other endocrine tissues (24, 25). FIHP seems to be more associated with *MEN1* missense variants (20). The age-dependent penetrance and the variability of intra- and interfamilial expression of the disease increase the difficulty of interpretation of allelic variants in the *MEN1* gene, particularly regarding sporadic patients with incomplete diagnosis criteria (22). In this context, the discovery of nontruncating *MEN1* variants may be a challenge for interpretation.

Here, we present the French Universal Mutation Database (UMD) for the *MEN1* gene (UMD-MEN1) developed with the UMD-Software. This project, funded by the Institut National de lutte contre le Cancer (INCa) and the French Ministry of Health, was initiated by a French national consortium and received the effective participation of the four French laboratories performing a comprehensive *MEN1* molecular analysis.

The UMD-MEN1 database is a locus-specific database designed to provide centralized and updated sequencing data for *MEN1* and interactive tools for the interpretation of sequencing variants in an attempt to classify the new variants (NVs) in one of the five classes of pathogenicity in accordance with international recommendations (26).

Materials and Methods

Organization of the *MEN1* gene analysis in France

To facilitate access to health care, the French Ministry of Health, via INCa, organized the genetic screening of French patients by funding regional platforms of molecular biology. Four laboratories belonging to the French Oncogenetics Network of Neuroendocrine Tumors (TENGEN) performed, until 2015, the totality of the *MEN1* gene testing, according to the international recommendations (10, 27). In France, *MEN1* genetic testing is performed in patients with clinical MEN1 or suspected MEN1, including patients in whom isolated HPTH has developed before the age of 50 years, isolated PIT before the age of 30 years, isolated DP-NET, regardless of age, isolated bronchial or thymic carcinoid tumor, regardless of age.

Laboratory practices for *MEN1* gene molecular analysis

The genetic analyses were performed after written informed consent was given by the patients during a one-on-one genetic counseling session. The sequencing of the full coding sequences and exon-intron junctions (−10 to +10 nucleotides from the splicing sites) of the MEN1 gene were performed in all index cases. Genetic analyses were achieved using Sanger sequencing or targeted next-generation sequencing from blood leukocyte DNA. In case of negative sequencing screening, large rearrangements (RGTs; *i.e.*, a deletions or duplications of at least one *MEN1* exon) were screened by quantitative multiplex PCR of short fluorescent fragments or multiplex ligation-dependent probe amplification (MLPA; MRC-Holland, Amsterdam, Netherlands).

The screening of relatives was based on the targeted research for the familial variants. Genotypes were double-checked on two independent biological samples.

UMD-MEN1 database: data collection and implementation for MEN1 variants

Project and online publishing was approved by the French supervisory authority, Commission Nationale pour l'Informatique et les 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.

An anonymized number was created for each patient and a second number was generated for each family. Genetic and phenotypic data were collected for patients with a *MEN1* variant. Patients (index cases or relatives) with negative *MEN1* genetic testing or patients who only had *MEN1* polymorphisms were not included in the database.

Clinical description

The laboratories in charge of the analysis collected clinical data from all patients harboring a *MEN1* variant. Clinical data included the date of birth, the MEN1-related clinical manifestation, the age at which the patient experienced manifestations of MEN1 disease, and the presence of a family history of MEN1 spectrum disorders. Cosegregation data were collected when available.

Molecular data

All variants were annotated using the same reference transcript (NM_130799) in the human genome GRCh37. They were named according to the Human Genome Variation Society nomenclature before implementation in the process (28). For all variants, the molecular data included the variant and the notion of variant co-occurrence. *In silico* predictions, including conservation level, SIFT, Polyphen 2, UMD-Predictor, and splicing consequence estimates, were collected.

Variant classification

Each variant was classified using a process consistent with the guidelines of the American College of Medical Genetics and Genomics in one of the following five classes (26): class 1: benign variant (BV); class 2: likely benign variant (LBV); class 3: variant of uncertain significance (VUS); class 4: likely pathogenic variant (LPV); and class 5: pathogenic variant. Because of the many examples in the literature of pathogenic midintronic or synonymous variants, these types of variants were not excluded from the analysis and were classified using the same method used for missense variants and microrearrangements (microRGTs) (29).

Systematic literature and database reviews were performed. Four additional pieces of information were also collected according to the case-by-case relevance: (i) screening of parents and relatives: to determine the *de novo* nature of the variant or to assess the variant cosegregation in multiple affected members; (ii) clinical phenotype and age of onset; (iii) additional molecular testing, including the sequencing of genes involved in phenocopies (*i.e.*, *AIP*, *HRPT2*, *CaSR*, and *CDKN1B*), screening of large RGTs by MLPA (the *MEN1* MLPA kits also explored *AIP* or *CDKN1B*) (18); and (iv) functional analysis: splicing analysis by direct RNA sequencing, search for a loss of heterozygosity in tumors, and *in vitro* testing of the mutation (stability and splicing).

Each NV with the all the related data was submitted to a consensus interpretation by the TENGEN expert group during a biannual meeting to classify it and the NV then was included in the UMD-MEN1 database.

Database description

Search tools

Database query is possible by the exon number, by the type of mutation, or directly by the amino acid or nucleotide position.

Interpretation tools

For each position and variant, all cases were listed and linked to the literature references. *In silico* predictions, conservation level, SIFT, and UMD-Predictor estimates were directly

integrated into the UMD database structure. They were automatically activated when the "summary" of the corresponding variation was opened. A table and graphical view of splicing analyses from Human Splicing Finder is available for each intronic variant (30).

Statistical analysis

Statistical analyses were performed using Prism, version 6.0 (GraphPad Software, La Jolla, CA). Patients' characteristics were compared using the two-tailed Fisher exact test for qualitative variables. The age-related penetrance of the MEN1-related lesions and the first MEN1 manifestation were estimated using the Kaplan-Meier method and analyzed with the log-rank test (aka, Mantel-Cox test). Statistical significance was set at $P < 0.05$.

Results

Characterization of the *MEN1*-positive patients reported in the UMD-MEN1 database

From 1997 to 2015, 5754 index cases in France and 2065 relatives of index cases positive for *MEN1*mutation were screened for *MEN1* gene mutations (INCa database). Over that period, the laboratories declared to the health authorities that *MEN1* genetic testing was positive in 721 index cases (12.5%). The cooperative effort made by the TENGEN group has led to the recovery of the molecular data of 680 index cases testing positive for *MEN1* that were referred in the UMD-MEN1 database, underlining the completeness of *MEN1* data collection in the French population. As expected in an autosomal-dominant disorder, the genetic testing was positive in half of *MEN1*-screened relatives (n = 996 MEN1-positive relatives for 2065 screened relatives; 48.2%).

Clinical data were available for 95.7% of *MEN1*-positive patients referred in the database (1604 of 1676; Table 1, Supplemental Table 1). Females represented 55.3% of the entries; they were overrepresented in the index cases category [OR, 1.396 (IC95% 1.196 to 1.629); $P < 0.001$, two-tailed Fisher exact test compared with the female/male proportion in the French population (31)], but not among patients' relatives [OR, 1.059 (IC95% 0.935 to 1.2)]. Follow-up data were available for 867 patients (51.7%) for a total duration of follow-up of 10,352 years (mean, 13.3 years; range, 0 to 18 years). The phenotype was undetermined for 72 patients. A total of 340 patients were asymptomatic; 1264 patients developed lesions: 102 with four major MEN1 lesions, 300 with three major MEN1 lesions, 413 with two major MEN1 lesions, 446 with one major MEN1 lesion, and 3 with atypical MEN1 (Supplemental Table 1). One-third of the MEN1-positive relatives were asymptomatic. The mean age at last follow-up of asymptomatic relatives was 28 years (range, 0 to 88 years). The age-related penetrance of the MEN1 manifestations was consistent with the previous published data (Fig. 1) (8–16). As expected, HPTH was the most common lesion in all patients (79.9% of index cases and 57.6% of MEN1-positive relatives). FIHP represented 1.9% of families (including eight index cases and 10 relatives).

Characterization of the variants in the *MEN1* gene reported in the UMD-MEN1 database

Altogether, 370 different variants from the 1676 entries were included in the UMD-MEN1 database (Fig. 2). To our knowledge, more than half of the *MEN1* variants in the database have not been reported before in the literature (n = 181 of 370; Supplemental Table 2).

Point variations

Nucleotide substitutions (*i.e.*, nonsense, missense, splice junction, midintronic, and synonymous variants) represented 64.9% of all *MEN1* variants (n = 240 of 370; Fig. 2). Missense variants were the most frequent type (33% of mutations; n = 122 of 370). Overall, 122 missense variants were registered and represented 26.2% of the index cases.

Microrearrangements

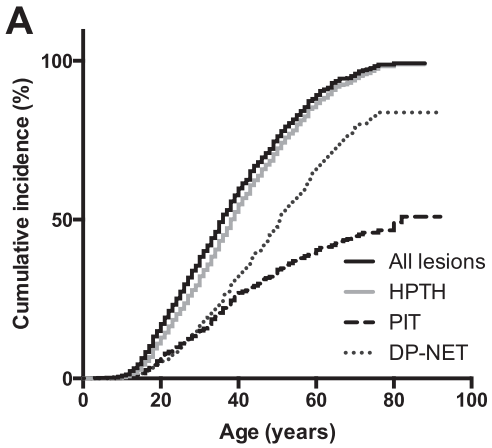
Microrearrangements [*i.e.*, deletions, insertions, duplications, insertions and deletions (indels) of one or few bases] represented one-third of the mutational events in the *MEN1* gene (n = 122 of 370 variants) and were present in 46.2% of the index cases. Most (88.5%) microRGTs led to a frameshift in the coding sequence inducing a premature codon stop and were, consequently, truncating mutations.

Large RGTs

A large RGT is a rare event. Eight distinct, large *MEN1* deletions were identified, ranging from one exon

Table 1. Clinical Characteristics of the 1676 *MEN1*-Positive Patients in the UMD MEN1 Database

Patient Type	No.	% of Total	Age at Molecular Diagnosis, Mean (Range), y	Female		Male		Patients With Follow-Up Data		Duration of Follow-Up, Mean (Range), y	Asymptomatic Patients	
				No.	%	No.	%	No.	%		No.	%
All	1676		37.5 (0–88)	927		738		867	51.7	13.4 (0–18)	340	
Index cases	680	40.6	44.5 (7–82)	400	58.8	269	39.6	310	45.6	13.3 (0–18)		
Relatives	996	59.4	32.0 (0–88)	527	52.9	467	46.9	557	55.9	13.2 (0–18)	340	34.1



B

Age (years)	All lesions Penetrance (%)	All lesions uninjured patients (n)	HPTH Penetrance (%)	PIT Penetrance (%)	DP-NET Penetrance (%)	ADRE Penetrance (%)
5	0.1	1327	0.1	0,0	0,0	0.1
10	0.9	1294	0.4	0.1	0.5	0.1
20	17.1	1028	12.8	6.9	5.4	0.5
30	38.3	736	32.1	15.1	17.2	3.4
40	59.8	455	54.8	27.0	32.8	10.1
50	76.2	253	72.5	34.3	49.6	20.1
60	89.3	105	86.7	40.5	66.7	29.4
70	96.0	32	94.5	44.8	78.9	38.6
80	99.2	3	98.9	49.1	83.7	42.0

Figure 1. Age-related penetrance for MEN1 and MEN1-related lesions in *MEN1*-positive patients and listed in the UMD-MEN1 database (n = 1403). Index cases and relatives harboring *MEN1* VUSs or BV or LBV were not considered in this figure. (A) Graphical representation of age-related penetrance for the three major MEN1-related lesions (HPTH, PIT, DP-NET) and age-related penetrance for the first major manifestation. (B) Penetrance (reported as percentage) by age for MEN1 disease and the four major MEN1-related lesions. Uninjured patients, patients without diagnosis of an MEN1-related lesion.

to the entire gene deletion and represented 2.2% of the different variants in the UMD-MEN1 database. No large duplication was detected. A large RGT was found in 15 of the 680 *MEN1*-positive index cases (2.2%), and in 85 relatives of these 15 index cases (overall frequency of RGT in all relatives analyzed with or without a family member with the RGT, 85 of 996 relatives; 8.5%)

Pathogenicity of the MEN1 variants

Seven *MEN1* BVs were reported in the UMD-MEN1 database: two in the introns and five in the coding sequence (Table 2) (32). All previously were reported as polymorphisms.

Of the variants referenced in the UMD MEN1 database, 73% were pathogenic variants (PVs) or LPVs (Fig. 3). Despite the classification process, 16.2% were VUSs; LBVs or BVs represented 10.8% of the MEN1 variants, and they were principally midintronic, synonymous, or missense variants.

LPVs or PVs represented 60.9% of the MEN1 missense variants. One-third of the missense MEN1 variants were VUSs, mainly owing to the lack of clinical and segregation data. LBVs and BVs were uncommon in the *MEN1* gene, including the missense variants.

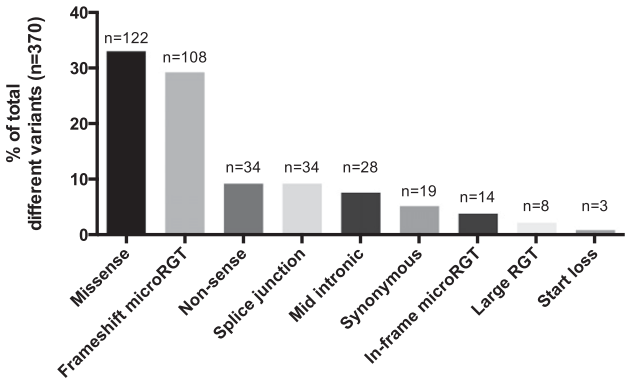


Figure 2. Repartitions in percentage of the different variants of *MEN1* by type of molecular event (n = 370 different variants). Definitions of terms are as follows: splice junction: intronic nucleotide variations in extreme positions (*i.e.*, −10 to +10 nucleotides from the exons); midintronic: intronic nucleotide variations in the intron center.

Co-occurring variants

Excluding BVs, five co-occurring variants were reported in the UMD-MEN1 database (Table 3). The occurrence of a VUS with LPV or PV was used to reclassify the VUS as an LBV according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines (26).

Analysis of the *MEN1* mutations spectrum in the French population

Molecular data collection confirmed a large mutational spectrum from *MEN1*-positive patients (Table 4). Frameshift microRGTs (*i.e.*, microdeletions, microduplications, microinsertions, indels with frameshift consequence) represented the most frequent type of *MEN1* variants identified in the index cases (43.8%); the second most frequent type was missense variants (26.2%).

Excluding BVs, most variants were identified in only one index case (74%; n = 269 of 363) or two index cases (13%; n = 47 of 363), underlining the occurrence of *MEN1* private mutations in MEN1 patients. Only 47 variants were identified in three or more index cases (13%); among them, only six variants were recurrent *MEN1* PVs with a frequency >1.5% in the index cases referenced in the UMD-MEN1 database (Table 5).

Table 2. Molecular Description of the Seven BVs Referred to in the UMD-MEN1 Database and Minor Allele Frequency Reported in the gnomAD Browser

Genomic Change (GRCH37)	Intron/Exon	Nucleotide Change NM_130799	Amino Acid Change	Molecular Event	gnomAD Minor Allele Frequency, %
Chr11:g.64577620G>C	Intron 1	c.-23-16C>G		Midintronic[a]	16.65
Chr11:g.64577147G>A	2	c.435C>T	p.(Ser145Ser)	Synonymous	2.86
Chr11:g.64575505C>T	3	c.512G>A	p.(Arg171Gln)	Missense	1.22
Chr11:g.64572602G>A	9	c.1254C>T	p.(Asp418Asp)	Synonymous	38.55
Chr11:g.64572557A>G	9	c.1299C>T	p.(His433His)	Synonymous	0.76
Chr11:g.64572403C>G	Intron9	c.1350+103G>C		Midintronic	31.82
Chr11:g.64572018T>C	10	c.1621G>A	p.(Ala541Thr)	Missense	6.6

[a]Intronic nucleotide variations in the intron center (beyond the −10 or +10 positions).

Variants associated with FIHP

The UMD-MEN1 database revealed eight families with FIHP (Table 6). Variants involved in FIHP were frameshift microRGTs or nonsense variants in two families and variants affecting splicing in three families. Missense variants were reported in only one FIHP family. Splicing variants were then overrepresented in French FIHP families compared with the MEN1 families [OR, 5.49 (95% CI, 1.283 to 23.49); Fisher exact test $P = 0.04$)].

Location of the MEN1 variation events across menin

Even if the variants were localized throughout the gene, 65.1% of the causal or likely causal *MEN1* variants identified in the French population were located in exons 2 and 10, including four of six of the most frequent variants of *MEN1* (Fig. 4; Table 5). Exon 10 harbors the two most frequently mutated nucleotides: position 1546 is the seat of frameshift microRGTs (31 deletions and 8 duplications) in 39 index cases (5.8%), and position 1378 showed a nonsense change in 18 index cases and a frameshift microRGT in 5 index cases. The LBVs involving the coding sequence also more frequently occurred in exon 10 (n = 13 of 29).

Genotype and phenotype correlations

We compared the age of onset of MEN1-related lesions in three populations of patients: those with large RGTs, those with truncating variants, and those with nontruncating variants. Variants affecting splice junction, synonymous or midintronic variants, or variants causing start-codon loss were not included in the analysis, because of the lack of systematic cDNA sequencing, to determine if they caused a premature stop codon. Index cases and relatives harboring *MEN1* variants of unknown significance or benign, or likely benign, variants were also not considered in this analysis. The three populations were similar in terms of age at last follow-up and age at molecular diagnosis (Supplemental Fig. 1) for index cases and relatives.

All patients with MEN1 with large *MEN1* RGTs had earlier onset of lesions than did patients with MEN1 with nontruncating or truncating variants (Fig. 5). HPTH and PITs manifested earlier in patients harboring large *MEN1* RGTs than in patients with nontruncating or truncating variants (Fig. 5), but the age at onset of DP-NETs was similar. For the patients harboring large *MEN1* RGTs, the median ages, reported as years (range), at occurrence of the first lesion, HPTH, PIT, DP-NET, and ADRE were, respectively, 28 (10 to 74), 30 (10 to 74), 22 (14 to 60), 34 (12 to 68), and 43 (21 to 55). The first lesion of *MEN1* developed earlier in patients with truncating variants than in patients with nontruncating variants (log-rank test $P = 0.012$), but the age at onset of each major MEN1-related manifestation was similar. For the patients

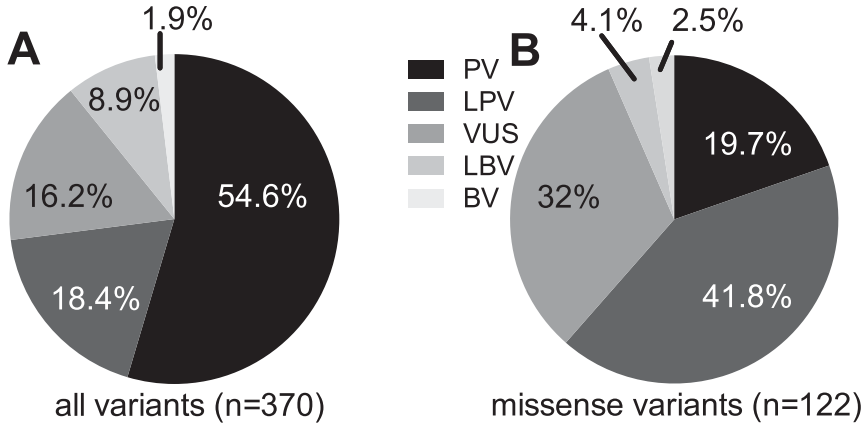


Figure 3. Repartition of the total *MEN1* variants referred in the UMD MEN1 database by class of pathogenicity. (A) Repartition of the 370 different *MEN1* variants by class of pathogenicity. (B) Repartition of the 122 *MEN1* missense variants by class of pathogenicity.

Table 3. Co-Occurrence of Variants Within the Same Patient in the UMD-MEN1 Database and Clinical Characteristics of the Patients

	First Variant: PV, LPV, or VUS			Second Variant: LBV or VUS			Patients' Description		
	Nucleotide Change	Amino Acid Change	Variant Classification	Nucleotide Change	Amino Acid Change	Variant Classification	Phenotype of Patient	Age of Onset (y)	Sex
1	c.842G>A	p.(Gly281Glu)	LPV	c.655-6C>A	p.(?)	VUS	PIT	17	Female
2	c.654+1G>A	p.(?)	PV	c.61C>A	p.(Arg21Ser)	LBV	DP-NET, HPTH	30	Female
3	c.629C>T	p.(Thr210Ile)	VUS	c.609C>T	p.(Asn203Asn)	LBV	DP-NET, ADRE	82	Female
4	c.1382_1390dup	p.(Glu461_Glu463dup)	PV	c.1541C>T	p.(Pro514Leu)	LBV	HPTH	34	Female
5	c.628_631del	p.(Thr210Serfs*13)	PV	c.1-6C>T	p.(?)	LBV	HPTH, DP-NET, PIT, ADRE	39	Male

harboring truncating variants, the median ages, reported as years (range) of occurrence of the first lesion, HPTH, PIT, DP-NET, and ADRE were, respectively, 34 (4 to 80), 36 (4 to 80), 33 (12 to 82), 39 (9 to 76), and 43.5 (14 to 80). For the patients with nontruncating variants, the median ages were, respectively, 36 (3 to 76), 38 (12 to 76), 33 (11 to 72), 44 (12 to 76), and 47 (3 to 75).

Discussion

Position of the UMD-MEN1 database in public databases and database update

UMD-MEN1 is a public, open-access database accessible through the framework of the Human Genome Variation Society (www.hgvs.org or www.umd.be/MEN1/). With 370 variant entries, the UMD-MEN1 database is the first database collecting the *MEN1* variants and proposing molecular interpretation at no charge for all registered variants by an expert group. With 181 unreported *MEN1* variants, the UMD-MEN1 database complements the knowledge of the *MEN1* mutation spectrum. As with all UMD databases, the UMD-MEN1 database allows interpretation of sequence variants with online interactive *in silico* tools, such as SIFT, UMD-predictor, or Human Splicing Finder. For registered patients, age at MEN1 onset is the only accessible clinical data.

Database update

Implementations of NVs are performed when they are communicated to the curator. Twice a year, the TENGEN group reviews the classification of NVs. The curator updates the literature, molecular data, clinical data, and results of functional analysis. The UMD-MEN1 database is then upgraded.

The UMD-MEN1 database provides an overview of *MEN1* variants present in the French population

With data collected from 680 *MEN1*-positive index cases from 1997 to 2015, the UMD-MEN1 database provides a comprehensive overview of *MEN1* variants present in the French population. The relative low frequency of *MEN1* variants in the tested index cases is explained by the broad indication for *MEN1* genetic screening. Classification of patients according to their status as index cases or relatives enables accurate statistical analysis of variant frequency according to the types of molecular events.

In accordance with previous studies, we found *MEN1* variants are distributed all along the gene. Overrepresentation of variants in exons 2 or 10 has been classically attributed to the proportionally larger size of these two exons. Nevertheless, variants in exons 2 or 10 represent 50% of the *MEN1* microRGTs and punctual variants, highlighting the requirement to entirely cover the *MEN1* coding sequence in high-throughput sequencing strategies. Six recurrent variants were reported in the UMD-MEN1 database with a frequency >1.5% (Table 5). The relatively high frequency of five of these six

Table 4. Type and Number of Variation Events of the *MEN1* Gene From Patients Referenced in the UMD-MEN1 Database

	All Patients		Index Cases		Relatives	
	No.	%	No.	%	No.	%
Total	1676		680		996	
Frameshift microRGT	593	35.4	298	43.8	375	37.7
Missense	375	22.4	178	26.2	197	19.8
Nonsense	283	16.9	100	14.7	183	18.4
Splice junction[a]	172	10.3	67	9.9	105	10.5
Large RGT	100	6	15	2.2	85	8.5
Midintronic[b]	71	4.2	64	9.4	7	0.7
Synonymous	46	2.7	29	4.3	17	1.7
In-frame microRGT	42	2.5	16	2.4	26	2.6
Start loss	4	0.2	3	0.4	1	0.1

[a]Intronic nucleotide variations in extreme positions (−10 to +10 nucleotides from the exons).

[b]Intronic nucleotide variations in the intron center (beyond −10 or +10 nucleotides from the exons).

Table 5. UMD-MEN1 Variants With a Frequency >1.5% in Index Cases

Exon	Nucleotide Change	Amino Acid Change	Type of Event	Index Cases in UMD-MEN1 Database		Frequency in Patients With MEN1 Reported in Lemos and Thakker,[20] %
				No.	Frequency,[a] %	
10	c.1546dup	p.(Arg516Profs*15)	Frameshift duplication	31	6	2.7
2	c.249_252delGTCT	p.(Ile85Serfs*33)	Frameshift deletion	18	3.5	4.5
10	c.1378C>T	p.(Arg460*)	Nonsense	18	3.5	2.6
9	c.1252G>A	p.(Asp418Asn)	Missense	14	2.7	NR
3	c.628_631delACAG	p.(Thr210Serfs*13)	Frameshift deletion	12	2.3	2.5
2	c.292C>T	p.(Arg98*)	Nonsense	10	1.9	1.5

Abbreviation: NR, not reported.

[a]Frequencies of variants are expressed regarding the 517 index cases harboring a PV or LPV in the UMD-MEN1 database.

variants has been previously highlighted (20). We also identified one frequent variant in exon 9 in the French population, c.1252G>A, p.(Asp418Asn), suggesting a founder effect in France.

Large RGTs were reported with a frequency of 2.2% in index cases, underlying the need to perform copy-number variation screening by MLPA or next-generation sequencing in cases of patients with MEN1 without variants identified in the *MEN1* coding sequence. According to the literature, no mutation could be found in 10% to 30% of patients suspected of having MEN1, despite an extensive analysis of the *MEN1* locus (20, 33, 34).

The UMD-MEN1 database confirmed that the most frequent variant types in index cases with *MEN1* genetic testing were frameshift microRGTs (43.8%) and missense variants (26.2%) (20). Interpretation of missense variants may be difficult, in particular in sporadic presentation with incomplete phenotype. The implemented process for interpretation allowed classification of 68% of the missense variants as (likely) benign or (likely) pathogenic. Missense variants reported as PVs or LPVs represented 61.5% of the missense variants, against 6.6% for the BV or LBVs. The low number of BVs or LBVs is in agreement with the low number of known polymorphisms identified in the *MEN1* gene (*i.e.*, four in the coding region) (35). Missense BVs or LBVs are frequently identified in exon 10. For these variants, *in silico* three-dimensional modeling was not contributive, because of a probable moving loop in the protein structure (PyMOL), supporting the evidence of a benign effect (36). The remaining 32% of variants were classified as VUS, awaiting supplemental data from cosegregation studies or functional studies.

Genotype-phenotype correlations

The clinical data in the UMD-MEN1 database allowed us to establish age-related penetrance of the first MEN1 manifestations and age-related penetrance of HPTH, PIT, DP-NET, and ADRE. Because of the large number of families and the various types of mutant events, the UMD-MEN1 database cohort was relevant and accurate for the description of the *MEN1*-positive patients' outcome.

The UMD-MEN1 database revealed a phenotype difference between patients harboring large RGTs and those with truncating or nontruncating variants. All considered patients with MEN1 with large RGTs

Table 6. *MEN1* Variants in Families With FIHP in the UMD-MEN1 Database

Family	Familial Variant	Type of Variant	Class	Index Cases		*MEN1*-Positive Relatives	
				Age at HPTH Diagnosis, (y)	Age at Last Follow-Up (y)	Age at HPTH Diagnosis, (y)	Age at Last Follow-Up (y)
F1	c.79_88del, p.(Leu27Argfs*89)	Frameshift mRGT	PV	28	42	36	44
F2	c.202_206dup, p.(Asp70Profs*51)	Frameshift mRGT	PV	43	43	21	21
F3	c.1069G>C, p.(Asp357His)	Missense	PV	47	63	15;20	21;36
F4	c.957C>A, p.(Tyr319*)	Nonsense	PV	40	40	76	76
F5	c.1213C>T, p.(Gln405*)	Nonsense	PV	35	55	32	32
F6	c.783+1G>A, p.(?)	Splice junction[a]	PV	31	31	14;61	14;61
F7	c.1050-1G>C, p.(?)	Splice junction	PV	43	43	42	42
F8	c.1351-1G>T, p.(?)	Splice junction	PV	NA	89	25	63

Abbreviations: mRGT, microrearrangement; NA, not available.

[a]Intronic nucleotide variations in extreme position (−10 to +10 nucleotides from the exons).

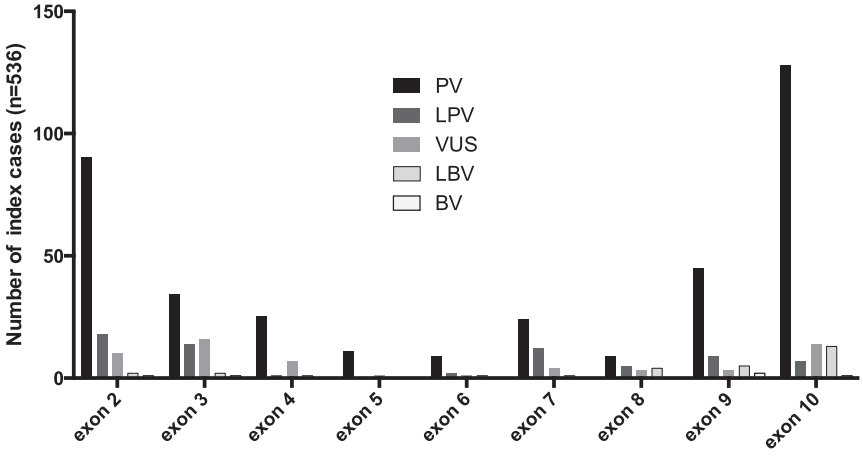


Figure 4. Repartition by exons and classification of pathogenicity of the variants identified in the 536 index cases with *MEN1* variants in the coding sequence.

experienced earlier first MEN1 manifestation. HPTH and PIT developed at an earlier age than in patients with truncating or nontruncating variants. This difference of PIT outcomes is not due to the association of *AIP* gene deletion, located in 11q13.2 near the 11q13.1 *MEN1* locus and involved in hereditary familial pituitary adenomas (37). In more than half of patients (those most recently tested), MLPA did not found an *AIP* deletion associated with *MEN1* deletion. Overall, these data support earlier molecular screening in families with large RGTs.

FIHP was a rare event in families in the UMD-MEN1 database (18 *MEN1*-positive patients in 8 of 416 families; 1.9%). FIHP was not preferentially associated with missense variants in our series, in contrast to the findings of others (20, 24). Each of the variants identified in FIHP was also reported in at least one *MEN1*-positive index case in the database harboring the other major MEN1-related lesions, except for the variants of the families F6 and F7 (Table 5). Both F6 and F7 variants affected splicing and were reported in the literature on patients with MEN1 (38, 39). In the first case, phenotypic description of the patient was not available; in the second case, the patient was reported as harboring HPTH, PIT, and DP-NET. In the F6 and F7 families, four of five patients were younger than 50 years and were still subject to development of secondary MEN1-related lesions. These data did not allow for adapting the clinical treatment of the *MEN1*-positive patients in the FIHP families to be different than the treatment in MEN1 families.

The UMD-MEN1 database can improve the genetic counseling and medical care of families of patients with MEN1

The identification of the *MEN1* gene in 1997 modified the landscape of MEN1 disease in MEN1 families by providing a predictive test for the risk of MEN1 lesions developing in relatives (2). Today, because of the discovery of VUS, *MEN1* genetic testing remains challenging in patients and families in which genetic analysis was uninformative. For example, in the *MEN1* ClinVar dataset, 37.2% of the *MEN1* variants are VUS (n = 256 of 688); this proportion increases to 80.6% in the category of missense variants (n = 187 of 232). In the UMD-MEN1 database, the collaborative work among laboratories, geneticists, and physicians led only 60 of all 370 variants (16.2%) and only 39 of 122 as missense variants (32%), to be qualified as VUS (Fig. 3). Theoretically, VUS is a transitional classification state. VUSs should not be used in medical decisions for the patient or relatives. Concerted efforts between geneticists and physicians have to be undertaken to resolve the VUS classification, incorporating a detailed phenotypic description. Testing additional family members for segregation analysis or functional analysis could result in the classification of these variants, but this is not always practicable. Extensive segregation studies are often difficult to perform, owing to the lack of compliance of patients and their relatives, the time required for the physicians and laboratories, and the expense. The interpretation of segregation studies may be difficult, because of the age-dependent penetrance and expressivity of the disease. The accumulation of data from several families harboring the same variants should compensate for lack of intrafamilial data. That is why sharing data on genes, variants, and phenotypes, through databases, is key to offering optimal care to patients and their families.

In conclusion, the UMD-MEN1 database is a locus-specific database designed to provide centralized and updated sequencing data for *MEN1* and interactive tools for the interpretation of sequence variants. Thanks to a publicly funded national collaborative network (the TENGEN group), the UMD-MEN1 database provides an exhaustive overview of the *MEN1* variants present in the French population. Knowledge sharing, standardization efforts, and implementation have contributed to the extensive expertise of the TENGEN group in the classification of *MEN1* variants. To date, the database contains data on 370 different variants from 1676 patients, including 181 NVs. The UMD MEN1 database is also open to other laboratories worldwide. For each variant, a classification, resulting from a consensus interpretation based on the clinical data collection from

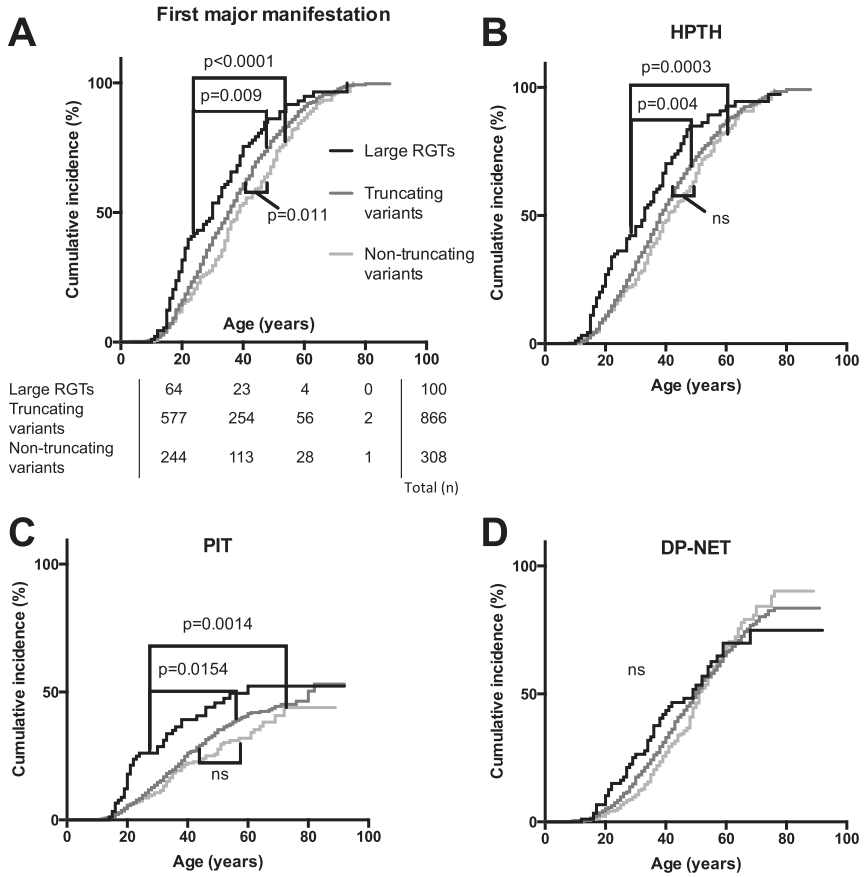


Figure 5. Characterization of the outcome of 1274 *MEN1*-positive patients by type of variant. Index cases and relatives harboring *MEN1* VUSs, BVs, or LBVs were not considered in this figure. In addition, patients harboring LPVs and PVs affecting splice junction, synonymous or midintronic variants, or variants causing start-codon loss were not included (n = 129). (A) Age-related incidence of the first major manifestation by type of identified variants in patients. The number of uninjured patients harboring each type of variant, reported by age (years); and the number of total patients harboring each type of variant are reported in the table below the graph. (B) Age-related incidence of HPTH by type of identified variants in patients. (C) Age-related incidence of PIT by type of identified variant in patients. (D) Age-related incidence of DP-NET by type of identified variant in patients. Definitions of terms are as follows: nontruncating variants: missense variants and in-frame microRGTs; uninjured patients: patients without diagnosis of MEN1-related lesion. ns, nonsignificant.

patients and a standardized analysis, is publicly available. The UMD-MEN1 database represents a public knowledge base, allowing access to original data and interactive tools to help geneticists and molecular biologists interpret their sequencing results in patients with MEN1.

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All data generated or analyzed during this study are deposited in the UMD-MEN1 database.

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Correspondence and Reprint Requests: Anne Barlier, PhD, MD, Aix-Marseille Université, INSERM, MMG, UMR 1251 Faculté de Médecine La Timone, boulevard Jean Moulin, 13385 Marseille cedex 05, France. E-mail: anne.barlier@univ-amu.fr.

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References

1. Wermer P. Genetic aspects of adenomatosis of endocrine glands. *Am J Med*. 1954;**16**(3):363–371.
2. Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, Crabtree JS, Wang Y, Roe BA, Weisemann J, Boguski MS, Agarwal SK, Kester MB, Kim YS, Heppner C, Dong Q, Spiegel AM, Burns AL, Marx SJ. Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science*. 1997;**276**(5311):404–407.
3. Marx S, Spiegel AM, Skarulis MC, Doppman JL, Collins FS, Liotta LA. Multiple endocrine neoplasia type 1: clinical and genetic topics. *Ann Intern Med*. 1998;**129**(6):484–494.
4. Balogh K, Rácz K, Patócs A, Hunyady L. Menin and its interacting proteins: elucidation of menin function. *Trends Endocrinol Metab*. 2006;**17**(9):357–364.
5. Yang Y, Hua X. In search of tumor suppressing functions of menin. *Mol Cell Endocrinol*. 2007;**265-266**:34–41.
6. Dreijerink KMA, Mulder KW, Winkler GS, Höppener JWM, Lips CJM, Timmers HTM. Menin links estrogen receptor activation to histone H3K4 trimethylation. *Cancer Res*. 2006;**66**(9):4929–4935.
7. Kim H, Lee J-E, Cho E-J, Liu JO, Youn H-D. Menin, a tumor suppressor, represses JunD-mediated transcriptional activity by association with an mSin3A-histone deacetylase complex. *Cancer Res*. 2003;**63**(19):6135–6139.
8. Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, Gheri RG, Libroia A, Lips CJ, Lombardi G, Mannelli M, Pacini F, Ponder BA, Raue F, Skogseid B, Tamburrano G, Thakker RV, Thompson NW, Tomassetti P, Tonelli F, Wells SA Jr, Marx SJ. Guidelines for diagnosis and

therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab*. 2001; **86**(12):5658–5671.
9. Marini F, Falchetti A, Del Monte F, Carbonell Sala S, Gozzini A, Luzi E, Brandi ML. Multiple endocrine neoplasia type 1. *Orphanet J Rare Dis*. 2006;**1**(1):38.
10. Thakker RV, Newey PJ, Walls GV, Bilezikian J, Dralle H, Ebeling PR, Melmed S, Sakurai A, Tonelli F, Brandi ML; Endocrine Society. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metab*. 2012;**97**(9):2990–3011.
11. White ML, Doherty GM. Multiple endocrine neoplasia. *Surg Oncol Clin N Am*. 2008;**17**(2):439–459.
12. Goudet P, Bonithon-Kopp C, Murat A, Ruszniewski P, Niccoli P, Ménégaux F, Chabrier G, Borson-Chazot F, Tabarin A, Bouchard P, Cadiot G, Beckers A, Guilhem I, Chabre O, Caron P, Du Boullay H, Verges B, Cardot-Bauters C. Gender-related differences in MEN1 lesion occurrence and diagnosis: a cohort study of 734 cases from the Groupe d'etude des Tumeurs Endocrines. *Eur J Endocrinol*. 2011;**165**(1):97–105.
13. Benson L, Ljunghall S, Akerström G, Oberg K. Hyperparathyroidism presenting as the first lesion in multiple endocrine neoplasia type 1. *Am J Med*. 1987;**82**(4):731–737.
14. Calender A, Giraud S, Cougard P, Chanson P, Lenoir G, Murat A, Hamon P, Proye C. Multiple endocrine neoplasia type 1 in France: clinical and genetic studies. *J Intern Med*. 1995;**238**(3):263–268.
15. Trump D, Farren B, Wooding C, Pang JT, Besser GM, Buchanan KD, Edwards CR, Heath DA, Jackson CE, Jansen S, Lips K, Monson JP, O'Halloran D, Sampson J, Shalet SM, Wheeler MH, Zink A, Thakker RV. Clinical studies of multiple endocrine neoplasia type 1 (MEN1). *QJM*. 1996;**89**(9):653–670.
16. Bassett JHD, Forbes SA, Pannett AAJ, Lloyd SE, Christie PT, Wooding C, Harding B, Besser GM, Edwards CR, Monson JP, Sampson J, Wass JAH, Wheeler MH, Thakker RV. Characterization of mutations in patients with multiple endocrine neoplasia type 1. *Am J Hum Genet*. 1998;**62**(2):232–244.
17. Dreijerink KMA, Goudet P, Burgess JR, Valk GD; International Breast Cancer in MEN1 Study Group. Breast-cancer predisposition in multiple endocrine neoplasia type 1. *N Engl J Med*. 2014;**371**(6): 583–584.
18. Turner JJO, Christie PT, Pearce SHS, Turnpenny PD, Thakker RV. Diagnostic challenges due to phenocopies: lessons from Multiple Endocrine Neoplasia type1 (MEN1). *Hum Mutat*. 2010;**31**(1): E1089–E1101.
19. Pieterman CRC, Schreinemakers JMJ, Koppeschaar HPF, Vriens MR, Rinkes IHMB, Zonnenberg BA, van der Luijt RB, Valk GD. Multiple endocrine neoplasia type 1 (MEN1): its manifestations and effect of genetic screening on clinical outcome. *Clin Endocrinol (Oxf)*. 2009;**70**(4):575–581.
20. Lemos MC, Thakker RV. Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. *Hum Mutat*. 2008;**29**(1): 22–32.
21. Thakker RV. Multiple endocrine neoplasia type 1 (MEN1) and type 4 (MEN4). *Mol Cell Endocrinol*. 2014;**386**(1-2):2–15.
22. Thevenon J, Bourredjem A, Faivre L, Cardot-Bauters C, Calender A, Le Bras M, Giraud S, Niccoli P, Odou MF, Borson-Chazot F, Barlier A, Lombard-Bohas C, Clauser E, Tabarin A, Pasmant E, Chabre O, Castermans E, Ruszniewski P, Bertherat J, Delemer B, Christin-Maitre S, Beckers A, Guilhem I, Rohmer V, Goichot B, Caron P, Baudin E, Chanson P, Groussin L, Du Boullay H, Weryha G, Lecomte P, Schillo F, Bihan H, Archambeaud F, Kerlan V, Bourcigaux N, Kuhn JM, Vergès B, Rodier M, Renard M, Sadoul JL, Binquet C, Goudet P. Unraveling the intrafamilial correlations and heritability of tumor types in MEN1: a Groupe d'étude des Tumeurs Endocrines study. *Eur J Endocrinol*. 2015;**173**(6): 819–826.
23. Thevenon J, Bourredjem A, Faivre L, Cardot-Bauters C, Calender A, Murat A, Giraud S, Niccoli P, Odou M-F, Borson-Chazot F, Barlier A, Lombard-Bohas C, Clauser E, Tabarin A, Parfait B, Chabre O, Castermans E, Beckers A, Ruszniewski P, Le Bras M, Delemer B, Bouchard P, Guilhem I, Rohmer V, Goichot B, Caron P, Baudin E, Chanson P, Groussin L, Du Boullay H, Weryha G, Lecomte P, Penfornis A, Bihan H, Archambeaud F, Kerlan V, Duron F, Kuhn J-M, Vergès B, Rodier M, Renard M, Sadoul J-L, Binquet C, Goudet P. Higher risk of death among MEN1 patients with mutations in the JunD interacting domain: a Groupe d'etude des Tumeurs Endocrines (GTE) cohort study. *Hum Mol Genet*. 2013;**22**(10):1940–1948.
24. Pannett A A J, Kennedy AM, Turner JJO, Forbes SA, Cavaco BM, Bassett JHD, Cianferotti L, Harding B, Shine B, Flinter F, Maidment CGH, Trembath R, Thakker RV. Multiple endocrine neoplasia type 1 (MEN1) germline mutations in familial isolated primary hyperparathyroidism. *Clin Endocrinol (Oxf)*. 2003;**58**(5): 639–646.
25. Wassif WS, Moniz CF, Friedman E, Wong S, Weber G, Nordenskjöld M, Peters TJ, Larsson C. Familial isolated hyperparathyroidism: a distinct genetic entity with an increased risk of parathyroid cancer. *J Clin Endocrinol Metab*. 1993;**77**(6): 1485–1489.
26. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;**17**(5):405–423.
27. Groupe d'étude des Tumeurs Endocrines (GTE). Arbres décisionnels d'explorations génétiques. Available at: www.reseau-gte.org. Accessed 24 September 2018.
28. den Dunnen JT, Dalgleish R, Maglott DR, Hart RK, Greenblatt MS, McGowan-Jordan J, Roux AF, Smith T, Antonarakis SE, Taschner PE. HGVS recommendations for the description of sequence variants: 2016 update. *Hum Mutat*. 2016;**37**(6):564–569.
29. Lenglet M, Robriquet F, Schwarz K, Camps C, Couturier A, Hoogewijs D, Buffet A, Knight SJL, Gad S, Couvé S, Chesnel F, Pacault M, Lindenbaum P, Job S, Dumont S, Besnard T, Cornec M, Dreau H, Pentony M, Kvikstad E, Deveaux S, Burnichon N, Ferlicot S, Vilaine M, Mazzella J-M, Airaud F, Garrec C, Heidet L, Irtan S, Mantadakis E, Bouchireb K, Debatin K-M, Redon R, Bezieau S, Bressac-de Paillerets B, Teh BT, Girodon F, Randi M-L, Putti MC, Bours V, Van Wijk R, Göthert JR, Kattamis A, Janin N, Bento C, Taylor JC, Arlot-Bonnemains Y, Richard S, Gimenez-Roqueplo A-P, Cario H, Gardie B. Identification of a new *VHL* exon and complex splicing alterations in familial erythrocytosis or von Hippel-Lindau disease. *Blood*. 2018;**132**(5):469–483.
30. Desmet F-O, Hamroun D, Lalande M, Collod-Béroud G, Claustres M, Béroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. *Nucleic Acids Res*. 2009;**37**(9):e67.
31. Institut National de la Statistique et des Études Économiques (INSEE). 2017 Demographic Report. https://www.insee.fr/en/statistiques/3533493. Accessed 19 October 2018.
32. gnomAD. Available at: gnomad.broadinstitute.org/. Accessed 24 September 2018.
33. Cebrián A, Ruiz-Llorente S, Cascón A, Pollán M, Díez JJ, Picó A, Tellería D, Benítez J, Robledo M. Mutational and gross deletion study of the MEN1 gene and correlation with clinical features in Spanish patients. *J Med Genet*. 2003;**40**(5):e72.
34. de Laat JM, van der Luijt RB, Pieterman CRC, Oostveen MP, Hermus AR, Dekkers OM, de Herder WW, van der Horst-Schrivers AN, Drent ML, Bisschop PH, Havekes B, Vriens MR, Valk GD. MEN1 redefined, a clinical comparison of mutation-positive and mutation-negative patients. *BMC Med*. 2016;**14**(1):182.
35. Thakker RV. Multiple endocrine neoplasia type 1 (MEN1). *Best Pract Res Clin Endocrinol Metab*. 2010;**24**(3):355–370.
36. Huang J, Gurung B, Wan B, Matkar S, Veniaminova NA, Wan K, Merchant JL, Hua X, Lei M. The same pocket in menin binds both

MLL and JUND but has opposite effects on transcription. *Nature*. 2012;**482**(7386):542–546.
37. Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TML, Salmela PI, Paschke R, Gündogdu S, De Menis E, Mäkinen MJ, Launonen V, Karhu A, Aaltonen LA. Pituitary adenoma predisposition caused by germline mutations in the AIP gene. *Science*. 2006;**312**(5777):1228–1230.
38. Pannett AAJ, Thakker RV. Somatic mutations in MEN type 1 tumors, consistent with the Knudson "two-hit" hypothesis. *J Clin Endocrinol Metab*. 2001;**86**(9):4371–4374.
39. Morelli A, Falchetti A, Martineti V, Becherini L, Mark M, Friedman E, Brandi ML. MEN1 gene mutation analysis in Italian patients with multiple endocrine neoplasia type 1. *Eur J Endocrinol*. 2000;**142**(2):131–137.