UMD-MEN1 database: an overview of the 370 MEN1 variants present in 1,676 patients from the French population

Pauline Romanet, Amira Mohamed, Sophie Giraud, Marie-Françoise Odou, Marie-Odile North, Morgane Pertuit, Eric Pasmant, Lucie Coppin, Celine Guien, Alain Calender, et al.

To cite this version:

Pauline Romanet, Amira Mohamed, Sophie Giraud, Marie-Françoise Odou, Marie-Odile North, et al.. UMD-MEN1 database: an overview of the 370 MEN1 variants present in 1,676 patients from the French population. Journal of Clinical Endocrinology and Metabolism, Endocrine Society, 2019, 104 (3), pp.753-764. 10.1210/jc.2018-01170. hal-01975538

HAL Id: hal-01975538
https://hal-amu.archives-ouvertes.fr/hal-01975538

Submitted on 9 Jan 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
UMD-MEN1 database: an overview of the 370 MEN1 variants present in 1,676 patients from the French population

Pauline Romanet, Amira Mohamed, Sophie Giraud, Marie-Françoise Odou, Marie-Odile North, Morgane Pertuit, Eric Pasmant, Lucie Coppin, Céline Guieu, Alain Calender, Françoise Borson-Chazot, Christophe Béroud, Pierre Goudet, Anne Barlier

The Journal of Clinical Endocrinology & Metabolism
Endocrine Society

Submitted: May 29, 2018
Accepted: October 15, 2018
First Online: October 18, 2018

Advance Articles are PDF versions of manuscripts that have been peer reviewed and accepted but not yet copyedited. The manuscripts are published online as soon as possible after acceptance and before the copyedited, typeset articles are published. They are posted "as is" (i.e., as submitted by the authors at the modification stage), and do not reflect editorial changes. No corrections/changes to the PDF manuscripts are accepted. Accordingly, there likely will be differences between the Advance Article manuscripts and the final, typeset articles. The manuscripts remain listed on the Advance Article page until the final, typeset articles are posted. At that point, the manuscripts are removed from the Advance Article page.

DISCLAIMER: These manuscripts are provided "as is" without warranty of any kind, either express or particular purpose, or non-infringement. Changes will be made to these manuscripts before publication. Review and/or use or reliance on these materials is at the discretion and risk of the reader/user. In no event shall the Endocrine Society be liable for damages of any kind arising references to, products or publications do not imply endorsement of that product or publication.
UMD-MEN1 database: 370 variants in 1676 patients

UMD-MEN1 database: an overview of the 370 MEN1 variants present in 1,676 patients from the French population

Pauline Romanet¹, Amira Mohamed², Sophie Giraud³, Marie-Françoise Odou⁴, Marie-Odile North⁵, Morgane Pertuit², Eric Pasmant⁵, Lucie Coppin⁶, Céline Guien⁷, Alain Calender³, Françoise Borson-Chazot⁸, Christophe Béroud⁹, Pierre Goudet¹⁰, Anne Barlier¹.

¹Aix Marseille Univ, INSERM, MMG, Laboratory of Molecular Biology Hospital La Conception, Marseille, France.

Email address: pauline.romanet@univ-amu.fr, anne.barlier@univ-amu.fr

²Laboratory of Molecular Biology, Hospital La Conception, APHM, Marseille

Email address: amira.mohamed@ap-hm.fr, morgane.pertuit@ap-hm.fr

³Genetics Department, Hospices Civils de LYON (HCL), University Hospital, East Pathology Center, LYON, B-A3, 59 Blvd Pinel, 69677, BRON Cedex, France.

Email address: alain.calender@chu-lyon.fr, sophie.giraud@chu-lyon.fr.

⁴Service de Biochimie et Biologie Moléculaire « Hormonologie, Métabolisme-Nutrition, Oncologie », Centre de Biologie Pathologie, CHU Lille, Bd du Pr J Leclercq, Lille F-59037 Cedex, France.

Email address: marie-francoise.odou@chru-lille.fr


Email address: marie-odile.north@aphp.fr; eric.pasmant@aphp.fr

⁶Univ. Lille, Inserm, CHU Lille, UMR-S 1172 - JPARC - Jean-Pierre Aubert Research Center, F-59000 Lille, France.

Email address: lucie.coppin@chru-lille.fr

⁷Aix Marseille Univ, INSERM, MMG, U 1251 Bioinformatic team, Marseille, France.

Email address: celine.guien@univ-amu.fr

⁸Hospices Civils de Lyon, Fédération d’Endocrinologie, Université Claude Bernard Lyon 1, HESPER EA 7425, F-69008 Lyon, France.

Email address: francoise.borson-chazot@chu-lyon.fr

⁹Aix Marseille Univ, INSERM, MMG, Department of genetics Hospital La Timone Enfants, Marseille, France.

Email address: christophe.beroud@univ-amu.fr

¹⁰Department of Endocrine Surgery, University Hospital of Dijon, and INSERM, U866, 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, Dijon, France.

Email address: pierre.goudet@chu-dijon.fr

ORCiD numbers:
0000-0003-1775-9569

ROMANET
PAULINE
0000-0002-3740-6173
Received 29 May 2018. Accepted 15 October 2018.

**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 is to facilitate interpretation of variants and improve the genetic counseling and medical care of MEN1-patients’ families.

**Design, Setting, and Patients:** The TENGEN network (French 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. They were registered in a locus-specific database called the UMD-MEN1 database (www.umd.be/MEN1/).

**Main Outcomes:** variant classification, age-related penetrance, odds ratio.

**Results:** Three hundred seventy distinct variants reported in 1,676 patients, including 181 unpublished variants, have currently been registered. This database analysis pointed out the low frequency of benign or likely benign missense variants in MEN1 (only 6.6%). Eight families (1.9%) presented a familial isolated hyperparathyroidism and harbored the same mutation found in authentic MEN1-families. An association exists between large rearrangements and an earlier onset of the disease, whereas no difference was observed between truncating and non-truncating variants.

**Conclusion:** 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.

The French TENGEN network has interpreted 370 MEN1 variants and registered 1676 MEN1-positive patients in the UMD-MEN1 database. Clinical data were analyzed to determine the patients’ outcome.

**INTRODUCTION:**

Multiple endocrine neoplasia type 1 (MEN1) is an inherited disease that predisposes carriers to primary hyperparathyroidism (HPTH), duodeno-pancreatic 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 the 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, and 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, and the third major manifestation, PIT, is reported in 30 to 40% of the MEN1 patients (10). The penetrance for all clinical
features increases to 95% at age 40 (10,16) An increased risk for breast cancer has also been described in \textit{MEN1}-mutated women (17).

In 2012, a group of experts, including physicians, surgeons, and geneticists from international centers, provided guidelines for the evaluation, treatment, and genetic testing for the \textit{MEN1} gene (10).

They redefined the basis for \textit{MEN1} diagnosis as: 1) Clinical criteria: a patient with 2 or more \textit{MEN1}-associated endocrine tumors (i.e., HPTH, DP-NET, or PIT); 2) Familial criteria: a patient with 1 \textit{MEN1}-associated tumor and a first-degree relative with \textit{MEN1};

3) Genetic criteria: an individual who has a \textit{MEN1} mutation but does not have clinical or biochemical manifestations of \textit{MEN1} (i.e., a mutant gene carrier) (10).

They proposed four different situations in which the \textit{MEN1} mutational analysis should be undertaken (10):

- in index cases with clinical \textit{MEN1} (see above);
- in index cases with suspected or atypical \textit{MEN1}, which includes:
  - patients with HPTH before the age of 30;
  - patients with multi-gland or multiple diseases in the same gland at any age (example: multi-gland parathyroid disease);
  - or patients harboring 2 or more \textit{MEN1}-associated tumors, including one other than HPTH, PIT, and DP-NET (example HPTH + ADRE).
- in first-degree relatives of known \textit{MEN1} mutation carriers, whether asymptomatic or not.

Since the beginning of genetic testing, and due to early and suitable therapies, the discovery of a causal variant in the \textit{MEN1} gene has reduced the morbidity of the \textit{MEN1}-related patients (8,10,18,19).

The \textit{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, \textit{MEN1} variants carrier appears to develop only HPTH, this situation of \textit{MEN1}-mutation related disease is referred as Familial Isolated Hyperparathyroidism (FIHP), a rare heritable disorder, characterized by hypercalcaemia, 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 \textit{MEN1} missense variants (20). The age-dependent penetrance and the variability of intra- and inter-familial expression of the disease increase the difficulty of interpretation of allelic variants in the \textit{MEN1} gene, particularly regarding sporadic patients with incomplete diagnosis criteria (22). In this context, the discovery of non-truncating \textit{MEN1} variants may be a high challenge for interpretation.

Here we present the French Universal Mutation Database for the \textit{MEN1} gene (UMD-MEN1) developed with the UMD-Software. This project funded by the \textit{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 \textit{MEN1} molecular analysis. The UMD-MEN1 database is a locus-specific database designed to provide centralized and updated sequencing data for \textit{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 the international recommendations (26).

**MATERIALS AND METHODS:**

Organization of the \textit{MEN1} gene analysis in France:
To facilitate access to health care, the French Ministry of Health via the INCa organized the genetic screening of French patients by funding regional platforms of molecular biology. Four laboratories belonging to the TENGEN network (French oncogenetics network of neuroendocrine tumors) performed, until 2015, the totality of the MEN1 gene testing, according to the international recommendations (10 and GTE (French Tumeur Endocrine Group), available on http://www.reseau-gte.org). In France, MEN1 genetic testing is performed in patients with clinical MEN1 or suspected MEN1, including patients developing isolated HPTH before the age of 50, isolated PIT before the age of 30, isolated neuroendocrine duodenopancreatic tumor, regardless of age, or in patients with isolated bronchial or thymic carcinoid tumor regardless of age.

**Laboratory practices for MEN1 gene molecular analysis:**

The genetic analyses are performed after written informed consent of 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 (NGS) 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 polymerase chain reaction (PCR) of short fluorescent fragments (QMPSF) or multiplex ligation-dependent probe amplification (MLPA®, MRC-Holland, Amsterdam, Holland).

The screening of relatives is 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**

Both project and on-line publishing were approved by the French supervisory authority CNIL (Commission Nationale pour l’Informatique et les Libertés, registration no.908361, 17 juillet 2009) and the national ethics committee CCTIRS (Comité Consultatif pour le Traitement de l’Information en matière de Recherche dans le domaine de la Santé, no. 07.421, 22 November 2007) and registered under n° 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 presented only with 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 of the manifestations, and the presence of a family history of MEN1 spectrum disorders. Co-segregation 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 HGVS nomenclature before implementation in the process (27). 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 five classes below (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)
- class 5: pathogenic variant (VP).

Due to the many examples in the literature on pathogenic mid-intronic or synonymous variants, this type of variants were not excluded from the analysis and were classified using the same method than that used for missense variants and microrearrangements (28).

A systematic literature and database review was performed. Four additional pieces of information were also collected according to the case-by-case relevance:

- screening of parents and relatives: to determine the de novo nature of the variant or to assess the variant co-segregation in multiple affected members;
- clinical phenotype and age of onset;
- additional molecular testing including the sequencing of genes involved in phenocopies (AIP, HRPT2, CaSR and CDKN1B), large RGTs screening by MLPA (please note that the MEN1 MLPA kits also explored AIP or CDKN1B) (29);
- functional analysis: splicing analysis by direct RNA sequencing, search for a loss of heterozygosity (LOH) in tumors, and in vitro testing of the mutation (stability and splicing).

Each newly identified variant (NV) with the whole related data was submitted to a consensus interpretation by the French TENGEN expert group during a biannual meeting to classify it them and was then implemented in the UMD-MEN1 database.

Database description

Search tools
Database query is possible by positioning in the coding sequence, 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 are automatically activated when opening the “summary” of the corresponding variation. A table and graphical view of splicing analyses from HSF (Human Splicing Finder) is available for each intronic variant (30).

Statistical analysis:
Statistical analyses were performed using Prism v6.0 (GraphPad Software, La Jolla, USA). Patients’ characteristics were compared using the two-tailed Fisher’s 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 (Mantel Cox). Statistical significance was set for a p-value inferior to 0.05.

RESULTS:

1 – Characterization of the MEN1-positive patients reported in the UMD-MEN1 database:

From 1997 to 2015, 5,754 index cases and 2,065 relatives of MEN1-mutation positive index cases were screened for the MEN1 gene mutations in France (data from the INCa database). Over that period, the laboratories declared to the health authorities that the 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 MEN1-positive tested index cases, 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 2,065 screened relatives, 48.2%).
Clinical data were available for 96.1% of MEN1-positive patients referred in the database (1,605/1,676; Table 1 and Supplemental Table 1). Females represented 55.3% of the entries; they were overrepresented in the index cases category (OR: 1.396 (1.196-1.629), p<0.001, two-tailed Fisher exact test compared to the female/male proportion in the French population – INSEE 2018 Demographic Profile), but not in the relatives (OR 1.059 (0.935-1.2)). 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 (min 0 to max 18 years)). The phenotype was undetermined for 72 patients. 340 patients were asymptomatic. 1264 patients have developed lesions, including: 102 patients with 4 major MEN1 lesions, 300 with 3 major MEN1 lesions, 413 with 2 major MEN1 lesions, 446 symptomatic with 1 major MEN1-lesions, and 3 patients with atypical MEN1 (Supplemental Table 1). One third of the MEN1-positive relatives were asymptomatic. The mean age of 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 (Figure 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 8 index cases and 10 relatives).

2 – Characterization of the variants in the MEN1 gene reported in the UMD-MEN1 database
Altogether, 370 different variants from the 1,676 entries were included in the UMD-MEN1 database (Figure 2). More than half of the MEN1 variants presented in the database were never reported before in the literature (n=181/370, Supplemental Table 2).

Point variations:
Nucleotide substitutions (non-sense, missense, splice junction, mid-intronic, and synonymous variants) represented 64.9% (n=240/370; Figure 2) of the whole MEN1 variants. Missense variants were the most frequent type, and represented 33% of mutations (122/370). Overall, 122 missense variants were registered, and represented 26.2% of the index cases.

Microrearrangements (microRGTs):
MicroRGTs (deletions, insertions, duplications, indels of one or few bases) represented one third of the mutational event in the MEN1 gene (33%, 122/370 variants) and was present in 46.2% of the index cases. Over 88% (88.5%) of the microRGTs led to a frameshift in the coding sequence inducing a premature codon stop and were consequently truncating mutations.

Large RGTs:
Large RGT is a rare event. Eight distinct large MEN1 deletions were identified, ranging from one exon to the entire gene deletion, and represented 2.2% of the different variants in the UMD-MEN1 database. No large duplication was detected. Large RGT was found in 15 of the 680 MEN1-positive index cases (2.2%), and in 85 relatives belonging to these 15 index cases (overall frequency of RGT in all relatives analyzed with or without a family member with the RGT, 85/996 relatives, 8.5%)

Pathogenicity of the MEN1 variants:
Seven MEN1 BVs were reported in the UMD-MEN1 database: 2 in the introns and 5 in the coding sequence (Table 2). All have been previously reported as polymorphisms.

Seventy-three percent of the variants referenced in the UMD MEN1 database were PVs or LPVs (Figure 3). Despite the classification process, 16.2% of the variants remained of uncertain significance (VUSs); LBVs, or BVs represented 10.8% of the MEN1 variants; they were principally mid-intronic, synonymous, or missense variants.

LPVs or PVs represented 60.9% of the MEN1 missense variants. One third of the missense MEN1 variants were VUS, mainly due to the lack of clinical and segregation data.
We found that LBVs and BVs were uncommon in the MEN1 gene, including the missense variants.

**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 LBV according to the ACMG-AMP guidelines (26).

3 – 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 (microdeletions, microduplications, microinsertions, indels with frameshift consequence) represented the most frequent type of MEN1 variants identified in the index cases (43.8%); the second one was missense variants (26.2%).

Excluding BVs, the majority of variants were identified in only one (74%, 269/363) or two index cases (13%, 47/363) underlining the occurrence of MEN1 private mutations in MEN1 patients. Only 47 variants were identified in 3 or more index cases (13%); among them, only 6 variants were recurrent MEN1 PVs with a frequency higher than 1.5% in the index cases referenced in the UMD MEN1 database (Table 5).

Variants associated with FIHP:
The UMD-MEN1 database revealed 8 families with FIHP (Table 6). Variants involved in FIHP were frameshift microRGT or non-sense variants in 2 families, and variants affecting splicing in 3 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 (IC: 1.283-23.49), p=0.04, Fisher’s exact test).

Location of the MEN1 variation events across the 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 4 of 6 of the most frequent variants of MEN1 (Figure 4 and 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 non-sense change in 18 index cases and a frameshift microRGT in five index cases. The LBVs involving the coding sequence were also more frequently setting in exon 10 (n=13/29).

4 – Genotype/phenotype correlations:
We compared the age of onset of MEN1-related lesions in 3 populations of patients: those with large RGT, those with truncating variants and those with non-truncating variants. Variants affecting splice junction, synonymous or mid-intronic variants, or variants causing start codon loss were not included in the analysis, because of the lack of systematic cDNA sequencing, in order to determine if they caused a premature stop codon or not. 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 term of age of last follow-up and age of molecular diagnosis (Supplemental Fig. 1) for index cases and relatives.

All MEN1-patients with large MEN1 RGT had earlier onset of lesions than MEN1 patients with non-truncating or truncating variants (Figure 5). Patients harboring large MEN1 RGTs presented earlier with HPTH and PIT than patients with non-truncating or truncating variants (Figure 5), but the age of onset of DP-NETs was similar. For the patients harboring large MEN1 RGTs, the median ages of occurrence of the first lesion, HPTH, PIT, DP-NET, and ADRE were respectively 28 years (range 10 to 74 years), 30 (10-74), 22 (14-60), 34 (12-68), and 43 (21-55). The patients with truncating variants developed also the first lesion of...
MEN1 earlier than the patients with non-truncating variants (log rank test (Mantel Cox) p=0.012), but the age of onset of each major MEN1-related manifestation was similar. For the patients harboring truncating variants, the median ages of occurrence of the first lesion, HPTH, PIT, DP-NET, and ADRE were respectively 34 years (range 4 to 80 years), 36 (4-80), 33 (12-82), 39 (9-76), and 43.5 (14-80). For the patients with non-truncating variants, the median ages were respectively 36 (range 3 to 76 years), 38 (12-76), 33 (11-72), 44 (12-76), and 47 (3-75).

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 (HGVS, https://www.hgvs.org) or at http://www.umd.be/MEN1/. With 370 variant entries, the UMD-MEN1 database represents 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 all UMD databases, the UMD-MEN1 database allows interpretation of sequence variants with online interactive in-silico tools, such as SIFT, UMD-predictor, or HSF. For registered patients, age of 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 the 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, the 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 higher than 1.5% (Table 5). The relatively high frequency of 5 of these 6 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% of index cases underlying the need to perform copy number variation screen by MLPA or NGS in case of MEN1-related patients without variants identified in MEN1 coding sequence. According to the literature, no mutation could be found in 10 to 30% of MEN1-suspected patients, despite of an extensive analysis of the MEN1 locus (20,31,32).

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 classifying 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\textit{MEN1} gene (i.e., 4 in the coding region) (33). Missense BVs or LBVs are frequently identified in exon 10. For these variants, \textit{in silico} 3D modeling was not contributive due to a probable moving loop in the protein structure (PyMOL), supporting the evidence of a benign effect (34). The remaining 32% of variants were classified as VUS, waiting for supplemental data as co-segregation studies.

\textbf{Genotype-phenotype correlations:}

The clinical data referred in the UMD-MEN1 database allowed the establishment of age-related penetrance of the first MEN1 manifestations and age-related penetrance of HPTH, PIT, DP-NET, and ADRE. Due to 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\textit{MEN1}-positive patients' outcome.

The UMD-MEN1 database revealed a phenotype difference between patients harboring large RGTs and those with truncating or non-truncating variants. All considered MEN1 patients with large RGTs experienced earlier first MEN1 manifestation. They developed the HPTH and PIT at an earlier age than that of the patients with truncating or non-truncating variants. This difference of PIT outcomes is not due to the association of\textit{AIP} gene deletion, located in 11q13.2 near the 11q13.1\textit{MEN1} locus and involved in hereditary familial pituitary adenomas (35). In over half of patients (the most “recent” ones), the used method (MLPA) did not found an\textit{AIP} deletion associated with\textit{MEN1} deletion. Overall these data support earlier molecular screening in the families with large RGTs.

FIHP was found to be a rare event in families from the UMD-MEN1 database (18\textit{MEN1}-positive patients in 8/416 families, 1.9%). FIHP was not found to be preferentially associated with missense variants in our series in contrast to others (20,24). Each of the variants identified in FIHP was also reported in at least one\textit{MEN1}-positive index cases in the database harboring the other major\textit{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 in\textit{MEN1} patients (36,37). In the first case, phenotypic description of the patient was not available, and in the second case the patient was reported as harboring HPTH, PIT, and DP-NET. In the F6 and F7 families, 4/5 patients were below 50 years and were still subject to develop secondary\textit{MEN1}-related lesions. These data did not allow adapting the clinical management of the\textit{MEN1}-positive patients in the FIHP families differently than in\textit{MEN1} families.

\textbf{The UMD-MEN1 database can improved the genetic counseling and medical care of\textit{MEN1}-patients' families.}

The identification of the\textit{MEN1} gene in 1997 has modified the landscape of\textit{MEN1} disease in\textit{MEN1} families by providing a predictive test for the risk of developing\textit{MEN1} lesions in relatives (2). Today,\textit{MEN1} genetic testing remained challenging in patients and families in which genetic analysis were uninformative, due to the discovery of VUS. For example, in the\textit{MEN1} ClinVar dataset, 37.2% of the\textit{MEN1} variants are called as VUS (256/688); rising to 80.6% in the category of missense variants (187/232). In the UMD-MEN1 database, the collaborative work between laboratories, geneticists and physicians led to qualify as VUS only 16.2% of all variants (60/370) and 32% of missense variants (39/122, Figure 3). Theoretically, VUS is a transitional classification state. VUSs should not be used in medical decisions for the patient or his relatives. Concerted efforts between geneticists and physicians have to be undertaken to resolve the VUS classification, as providing detailed phenotypic
description. Testing additional family members, for segregation analysis, or functional analysis could result in the classification of these variants, but were not always practicable. Extensive segregation studies are often difficult to perform due to the lack of compliance of patients and their relatives, the time required for the physicians and laboratories, and the expensive costs. The interpretation of segregation studies may be difficult due to the age-dependent penetrance and expressivity of the disease. The accumulation of data over several families harboring the same variants should compensate for lack of intra-familial data. That’s why sharing data on genes, variants and phenotypes, through databases, is key to offer optimal care to the patients and their families.

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 publically founded national collaborative network called 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 attributed to the extensive expertise of the TENGEN group in the classification of MEN1 variants. To date, the database contains data concerning 370 different variants from 1,676 patients, including 181 NVs. The UMD MEN1 database is also open to other laboratories in the world. For each variant, a classification, resulting from a consensus interpretation based on the clinical data collection from 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 MEN1-related patients.

ACKNOWLEDGMENT:

This study was conducted on behalf of the TENGEN group. We thank all the patients and their medical doctors and professors, who have placed their trust in us for more than 20 years. We especially thank the members of GTE for sharing the clinical data from the MEN1 database. Thank you to Grégoire Mondielli, PhD for performing the 3-D Modeling.

FUNDING SOURCE: All phases of this study were supported by grants from the Institut National de lutte contre le Cancer (INCa) and the French Ministry of Health.

AUTHORS’ CONTRIBUTIONS:

All the authors contributed to this article. PR performed the search and wrote the draft. PR, MFO, LC, MON, MP, AE, SG, and AB performed the genetics analysis. PR, MP, PG, and AM collected patients’ data. CB created the database. CB, PR, CG, and AM curated the database. AB conceptualized and designed the project and manuscript and coordinated the study. MFO, LC, MP, MON, AE, SG, AB, FBC, CG, CB, and PG critically reviewed and revised the manuscript to achieve the final format.

CORRESPONDENCE: Prof Anne Barlier, PhD, MD, Aix Marseille Univ, INSERM, MMG, UMR 1251 Prof Nicolas LEVY, Faculté de Médecine Nord, Boulevard Pierre Dramard, CS80011, 13344 Marseille cedex 05, France, Tel: +33 491 69 87 89, Fax: +33 491 69 89 20, anne.barlier@univ-amu.fr

DISCLOSURE SUMMARY

1. I, Prof Anne Barlier, on behalf of myself and my co-authors, hereby transfer and assign all right, title, and interest, including copyright and any moral rights, in and to the manuscript named in this submission “UMD-MEN1 database: an overview of the 370 MEN1 variants present in 1,676 patients from the French population” to the Endocrine Society (ES). If ES
ultimately declines to publish the Work in an ES journal, all rights in and to the Work will revert to the author(s).

2. I, and all co-authors, warrant that the Work intended for publication is original and has not been published other than as an abstract or preprint in any language or format and has not been submitted elsewhere for print or electronic publication consideration. We further warrant that the Work does not contain any material that is defamatory or the publication of which would violate any copyright or other personal, intellectual, property, contract, or proprietary right of any person or entity.

3. I warrant that each person listed as an author participated in the Work in a substantive way and is prepared to take public responsibility for it. All authors consent to the investigation of any improprieties that may be alleged regarding the Work. Each author further releases and holds harmless the Endocrine Society from any claim or liability that may arise therefrom.

4. I warrant that I am authorized to accept the terms of this agreement on behalf of myself and all co-authors. I accept the terms of the agreement on behalf of myself and all co-authors.

DISCLOSURE STATEMENTS:
The authors declare that they have nothing to disclose.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE:
All patients or their parents provided signed consent for genetic testing. The collection or sharing of molecular and clinical data was approved by the French supervisory authority CNIL (Commission Nationale pour l’Informatique et les Libertés, registration no.908361, 17 July 2009) and the national ethics committee CCTIRS (Comité Consultatif pour le Traitement de l’Information en matière de Recherche dans le domaine de la Santé, no. 07.421, 22 November 2007) and registered under n° 91513.

COMPETING INTERESTS:
The authors declare that they have no competing interests.

AVAILABILITY OF DATA AND MATERIALS:
All data generated or analyzed during this study are deposed in the UMD-MEN1 database.

REFERENCES:
18. Turner JJO, Christie PT, Pearce SHS, Turnpenny PD, Thakker R V. Diagnostic challenges due to phenocopies: Lessons from Multiple Endocrine Neoplasia type1 (MEN1). Hum Mutat. 2010;31(1).


Figure 1: Age-related penetrance for MEN1 and MEN1-related lesions in MEN1-positive patients referred in the UMD-MEN1 database (n=1403). Index cases and relatives harboring MEN1 variants of unknown significance or benign or likely benign variants were not considered in this figure. Uninjured patients: patients without diagnosis of MEN1-related lesion. 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 (in percentage) by age for the MEN1 disease and the four major MEN1-related lesions. ADRE: adrenal tumor, PIT: pituitary adenoma, DP-NET: duodeno-pancreatic neuroendocrine tumor, HPTH: primary hyperparathyroidism.

Figure 2: Repartitions in percentage of the different variants of MEN1 by type of molecular event (n=370 different variants). Frameshift microRGTs: microdeletion, microduplication, microinsertion, indels with frameshift of the coding sequence domain; splice junction: intronic nucleotide variations in extreme position (-10 to +10 nucleotides from the exons); large RGT: (large rearrangement): deletion or duplication of one or more exons; mid-intronic: intronic nucleotide variations in the intron center; in-frame microRGTs: microdeletion, microduplication, microinsertion, indels not leading to a frameshift of the coding sequence domain.

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. PV: pathogenic variant; LPV: likely pathogenic variant; VUS: variant of uncertain significance; LBV: likely benign variant; BV: benign variant.

Figure 4: Repartition by exons and classification of pathogenicity of the variants identified in the 536 index cases presented with MEN1 variants in the coding sequence. PV: pathogenic variant; LPV: likely pathogenic variant; VUS: variant of uncertain significance; LBV: likely benign variant; BV: benign variant.

Figure 5: Characterization of the outcome of 1274 MEN1-positive patients by type of variant. Index cases and relatives harboring MEN1 variants of unknown significance or benign or likely benign variants were not considered in this figure. In addition, patients harboring likely pathogenic and pathogenic variants affecting splice junction, synonymous or mid-intronic variants, or variants causing start codon loss were not included (n=129). Uninjured patients: patients without diagnosis of MEN1-related lesion. A: Age-related
incidence of the first major manifestation by type of identified variants in patients; the number of uninjured patients harboring each types of variant by age, and the number of total patients harboring each types of variant are given in the table below the graph 5-A. B: Age-related incidence of HPTH by type of identified variants in patients; C: Age-related incidence of PIT by type of identified variants in patients; D: Age-related incidence of DP-NET by type of identified variants in patients. Large RGT: deletion or duplication of one or more exons. Truncating variants: nonsense variants or frameshift microRGTs (microduplication, microdeletion, microinsertion indels with frameshift of the coding sequence domain). Non-truncating variants: missense variants, and in-frame microRGTs. DP-NET: duodeno-pancreatic neuroendocrine tumor; HPTH: primary hyperparathyroidism, PIT: pituitary adenoma, RGT: rearrangement, ns = non-significative.

Table 1: Clinical characteristics of the 1,676 MEN1-positive patients referred in the UMD MEN1 database.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean age of molecular diagnosis (years)</th>
<th>Female</th>
<th>Male</th>
<th>Number of patients with follow-up data</th>
<th>Mean duration of follow-up (years)</th>
<th>Asymptomatic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>37.5 (0-88)</td>
<td>927</td>
<td>738</td>
<td>867</td>
<td>51.7%</td>
<td>13.4 (0-18)</td>
</tr>
<tr>
<td>Index cases</td>
<td>40.6%</td>
<td>44.5 (7-82)</td>
<td>400</td>
<td>269</td>
<td>39.6%</td>
<td>310</td>
</tr>
<tr>
<td>Relatives</td>
<td>39.4%</td>
<td>32.0 (0-88)</td>
<td>527</td>
<td>52.9</td>
<td>467</td>
<td>557</td>
</tr>
</tbody>
</table>

Table 2: molecular description of the 7 BVs referred in the UMD-MEN1 database and minor allele frequency reported in the gnomAD browser.

<table>
<thead>
<tr>
<th>Genomic change (GRCh37)</th>
<th>Intron/exon</th>
<th>Nucleotide change (NM_130799)</th>
<th>Amino acid change</th>
<th>Molecular event</th>
<th>gnomAD minor allele frequency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr11:g.64577620G&gt;C</td>
<td>intron 1</td>
<td>c.-23-16C&gt;G</td>
<td>mid-intronic</td>
<td>16.65</td>
<td></td>
</tr>
<tr>
<td>Chr11:g.64577147G&gt;A</td>
<td>2</td>
<td>c.435C&gt;T</td>
<td>p.(Ser145Ser)</td>
<td>synonymous</td>
<td>2.86</td>
</tr>
<tr>
<td>Chr11:g.64575505C&gt;T</td>
<td>3</td>
<td>c.512G&gt;A</td>
<td>p.(Arg171Gln)</td>
<td>missense</td>
<td>1.22</td>
</tr>
<tr>
<td>Chr11:g.64572602G&gt;A</td>
<td>9</td>
<td>c.1254C&gt;T</td>
<td>p.(Asp418Asp)</td>
<td>synonymous</td>
<td>38.55</td>
</tr>
<tr>
<td>Chr11:g.64572557A&gt;G</td>
<td>9</td>
<td>c.1299C&gt;T</td>
<td>p.(His433His)</td>
<td>synonymous</td>
<td>0.76</td>
</tr>
<tr>
<td>Chr11:g.64572403G&gt;C</td>
<td>intron 9</td>
<td>c.1350+103G&gt;C</td>
<td>mid-intronic</td>
<td>31.82</td>
<td></td>
</tr>
<tr>
<td>Chr11:g.64572018T&gt;C</td>
<td>10</td>
<td>c.1621G&gt;A</td>
<td>p.(Ala541Thr)</td>
<td>missense</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Mid-intronic: intronic nucleotide variations in the intron center (beyond the -10 or +10 positions); BV: Benign variants.

Table 3: Co-occurrence of variants within the same patient in the UMD-MEN1 database and clinical characteristics of the patients.

<table>
<thead>
<tr>
<th>First variant: PV, LPV or VUS</th>
<th>Second variant: LBV or VUS</th>
<th>Patients' description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide change</td>
<td>Amino acid change</td>
<td>Variant classification</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>1</td>
<td>c.842G&gt;A</td>
<td>p.(Gly281Glu)</td>
</tr>
<tr>
<td>2</td>
<td>c.654+1dup</td>
<td>p.(?)</td>
</tr>
<tr>
<td>3</td>
<td>c.629C&gt;T</td>
<td>p.(Thr210lle)</td>
</tr>
<tr>
<td>4</td>
<td>c.1382_1390dup</td>
<td>p.(Glu461_Glu463dup)</td>
</tr>
<tr>
<td>5</td>
<td>c.628_631del</td>
<td>p.(Thr210Serfs*13)</td>
</tr>
</tbody>
</table>

ADRE: adrenal tumor; PIT: pituitary adenoma; DP-NET: duodeno-pancreatic neuroendocrine tumor; HPTH: primary hyperparathyroidism; PV: pathogenic variant; LPV: likely pathogenic variant; VUS: variant of uncertain significance; LBV: likely benign variant.
Table 4: Type and number of variation events of the MEN1 gene from patients referenced in the UMD-MEN1 database.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Index cases</th>
<th>Relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Total</td>
<td>1,676</td>
<td></td>
<td>680</td>
</tr>
<tr>
<td>Frameshift microRGT</td>
<td>593</td>
<td>35.4%</td>
<td>298</td>
</tr>
<tr>
<td>Missense</td>
<td>375</td>
<td>22.4%</td>
<td>178</td>
</tr>
<tr>
<td>Non-sense</td>
<td>283</td>
<td>16.9%</td>
<td>100</td>
</tr>
<tr>
<td>Splice junction</td>
<td>172</td>
<td>10.3%</td>
<td>67</td>
</tr>
<tr>
<td>Large RGT</td>
<td>100</td>
<td>6%</td>
<td>15</td>
</tr>
<tr>
<td>Mid-Intronic</td>
<td>71</td>
<td>4.2%</td>
<td>64</td>
</tr>
<tr>
<td>Synonymous</td>
<td>46</td>
<td>2.7%</td>
<td>29</td>
</tr>
<tr>
<td>In-frame microRGT</td>
<td>42</td>
<td>2.5%</td>
<td>16</td>
</tr>
<tr>
<td>Start loss</td>
<td>4</td>
<td>0.2%</td>
<td>3</td>
</tr>
</tbody>
</table>

Frameshift microRGT: microdeletion, microduplication, microinsertion, and indels with frameshift of the coding sequence domain; Splice junction: intronic nucleotide variations in extreme position (-10 to +10 nucleotides from the exons); Large RGT: (large rearrangement): deletion or duplication of one or more exons; Mid-intronic: intronic nucleotide variations in the intron center (beyond -10 or +10 nucleotides from the exons); In-frame microRGT: microdeletion, microduplication, microinsertion, and indels not leading to a frameshift of the coding sequence domain.

Table 5: UMD-MEN1 variants with a frequency higher than 1.5% in index cases.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Type of event</th>
<th>Index cases in UMD-MEN1 database</th>
<th>Frequency in MEN1-patients reported in Lemos et al., Hum Mut, 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>c.1546dup</td>
<td>p.(Arg516Profs*15)</td>
<td>Frameshift duplication</td>
<td>31</td>
<td>6%</td>
</tr>
<tr>
<td>2</td>
<td>c.249_252delGTCT</td>
<td>p.(Ile85Serfs*33)</td>
<td>Frameshift deletion</td>
<td>18</td>
<td>3.5%</td>
</tr>
<tr>
<td>10</td>
<td>c.1378C&gt;T</td>
<td>p.(Arg460*)</td>
<td>Non-sense</td>
<td>18</td>
<td>3.5%</td>
</tr>
<tr>
<td>9</td>
<td>c.1252G&gt;A</td>
<td>p.(Asp418Asn)</td>
<td>Missense</td>
<td>14</td>
<td>2.7%</td>
</tr>
<tr>
<td>3</td>
<td>c.628_631delACAG</td>
<td>p.(Thr210Serfs*13)</td>
<td>Frameshift deletion</td>
<td>12</td>
<td>2.3%</td>
</tr>
<tr>
<td>2</td>
<td>c.292C&gt;T</td>
<td>p.(Arg98*)</td>
<td>Non-sense</td>
<td>10</td>
<td>1.9%</td>
</tr>
</tbody>
</table>

The frequencies of variants are expressed regarding the 517 index cases harboring a pathogenic or likely pathogenic variant in the UMD-MEN1 database.

Table 6: MEN1 variants presented in families with FIHP in the UMD-MEN1 database.

<table>
<thead>
<tr>
<th>Family</th>
<th>Familial variant</th>
<th>Type of variant</th>
<th>Class</th>
<th>Age of HPTH (yrs)</th>
<th>Age of last follow-up (yrs)</th>
<th>Age of HPTH (yrs)</th>
<th>Age of last follow-up (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>c.79_88del, p.(Leu27Argfs*89)</td>
<td>Frameshift mRGT</td>
<td>PV</td>
<td>28</td>
<td>42</td>
<td>36</td>
<td>44</td>
</tr>
<tr>
<td>F2</td>
<td>c.202_206dup, p.(Asp20Profs*51)</td>
<td>Frameshift mRGT</td>
<td>PV</td>
<td>43</td>
<td>43</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>F3</td>
<td>c.1069C&gt;T, p.(Asp357His)</td>
<td>Missense</td>
<td>PV</td>
<td>47</td>
<td>63</td>
<td>15.20</td>
<td>21.36</td>
</tr>
<tr>
<td>F4</td>
<td>c.957C&gt;A, p.(Tyr319*)</td>
<td>Non-sense</td>
<td>PV</td>
<td>40</td>
<td>40</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>F5</td>
<td>c.1213C&gt;T, p.(Gln405*)</td>
<td>Non-sense</td>
<td>PV</td>
<td>35</td>
<td>55</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>F6</td>
<td>c.783+1G&gt;A, p.(?)</td>
<td>Splice junction</td>
<td>PV</td>
<td>31</td>
<td>31</td>
<td>14.61</td>
<td>14.61</td>
</tr>
<tr>
<td>F7</td>
<td>c.1050-1G&gt;C, p.(?)</td>
<td>Splice junction</td>
<td>PV</td>
<td>43</td>
<td>43</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>F8</td>
<td>c.1351-1G&gt;T, p.(?)</td>
<td>Splice junction</td>
<td>PV</td>
<td>NA</td>
<td>89</td>
<td>25</td>
<td>63</td>
</tr>
</tbody>
</table>

Frameshift microRGT: microdeletion, microduplication, microinsertion, and indels with frameshift of the coding sequence domain; HPTH: primary hyperparathyroidism Splice junction: intronic nucleotide variations in extreme position (-10 to +10 nucleotides from the exons); NA: not available; PV: pathogenic variant
**Table B**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>All lesions Penetration (%)</th>
<th>HPTH Penetration (%)</th>
<th>PIT Penetration (%)</th>
<th>DP-NET Penetration (%)</th>
<th>ADRE Penetration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>0.9</td>
<td>0.4</td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>20</td>
<td>17.1</td>
<td>12.8</td>
<td>6.9</td>
<td>5.4</td>
<td>0.5</td>
</tr>
<tr>
<td>30</td>
<td>38.3</td>
<td>32.1</td>
<td>15.1</td>
<td>17.2</td>
<td>3.4</td>
</tr>
<tr>
<td>40</td>
<td>59.8</td>
<td>54.8</td>
<td>27.0</td>
<td>32.8</td>
<td>10.1</td>
</tr>
<tr>
<td>50</td>
<td>76.2</td>
<td>72.5</td>
<td>34.3</td>
<td>49.6</td>
<td>20.1</td>
</tr>
<tr>
<td>60</td>
<td>89.3</td>
<td>86.7</td>
<td>40.5</td>
<td>66.7</td>
<td>39.4</td>
</tr>
<tr>
<td>70</td>
<td>96.0</td>
<td>94.5</td>
<td>44.8</td>
<td>78.9</td>
<td>38.6</td>
</tr>
<tr>
<td>80</td>
<td>99.2</td>
<td>98.9</td>
<td>49.1</td>
<td>83.7</td>
<td>42.0</td>
</tr>
</tbody>
</table>
% of total different variants (n=370)

Missense: n=122
Frameshift microRGT: n=108
Non-sense: n=34
Splice Junction: n=34
Mid intronic: n=28
Synonymous: n=19
In-frame microRGT: n=14
Large RGT: n=8
Start loss: n=3
First major manifestation

Large RGTs
Truncating variants
Non-truncating variants

p=0.009
p=0.011
p<0.0001
p=0.0154
p=0.0014
p=0.004
p=0.0003
ns
ns

A

B

C

D

HPTH

PIT

DP-NET

Endocrinology

THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

Downloaded from https://academic.oup.com/jcem/advance-article-abstract/doi/10.1210/jc.2018-01170/5134196 by guest on 09 January 2019