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Detection rate and clinical predictors of MEN1 pathogenic variants in early-onset and multiglandular primary hyperparathyroidism

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Abstract

Purpose

To assess the detection rate of germline *MEN1* pathogenic or likely pathogenic (P/LP) variants in patients with primary hyperparathyroidism (PHPT) presenting at a young age or with multiglandular disease, and to identify features associated with *MEN1* positivity.

Methods

This retrospective single-center study included 30 of 93 eligible patients (2022–2025): 15 with early-onset (≤ 35 years) and 15 with multiglandular PHPT. *MEN1* was analyzed by Sanger sequencing of coding exons 2–10 and multiplex ligation-dependent probe amplification (MLPA). Associated tumors were compared by *MEN1* status using Fisher's exact test, exact odds ratios (OR), Firth logistic regression, and diagnostic accuracy measures.

Results

MEN1 P/LP variants were found in 11/30 patients (36.7%): 5/15 (33.3%) with early-onset and 6/15 (40.0%) with multiglandular PHPT, including 4/18 (22.2%) without a second major MEN1 manifestation. Neuroendocrine tumors (NETs) were most strongly associated with *MEN1* positivity (8/11 vs. 1/19; OR 39, 95% CI 4.3–1100; $p < 0.001$), the only association surviving multiple-comparison correction. Adrenal hyperplasia (9/11 vs. 6/19; OR 8.9, 95% CI 1.3–74) was the only independent predictor in the pre-specified multivariable model, and lipomas were associated with *MEN1* status in the multiglandular group ($p = 0.041$). A NET or adrenal hyperplasia was present in all variant carriers (sensitivity 100%, specificity 63.2%). Pituitary lesions, biochemical parameters, and renal or skeletal involvement did not differ by *MEN1* status.

Conclusion

Early-onset and multiglandular PHPT showed similar *MEN1* detection rates. NETs and adrenal hyperplasia, rather than biochemical severity, best identified variant carriers. These hypothesis-generating findings require validation in larger cohorts.

Introduction

Multiple Endocrine Neoplasia Type 1 (MEN1) is characterized by three cardinal clinical manifestations, namely primary hyperparathyroidism (PHPT), anterior pituitary tumors, and duodenopancreatic neuroendocrine tumors (DP-NETs), together with more than 20 other associated endocrine and non-endocrine tumors[1]. PHPT is a common endocrine disorder that is only rarely associated with inherited syndromes such as MEN1[2].

MEN1 is a rare (estimated prevalence 1/20,000–40,000), age-related, autosomal dominant disorder caused by germline pathogenic or likely pathogenic (P/LP) variants in the *MEN1* tumor suppressor gene on chromosome 11q13; it may also arise from a de novo variant. The gene comprises 10 exons, with the coding sequence spanning exons 2–10, and encodes menin, a nuclear scaffold protein that regulates gene transcription. More than 1,700 pathogenic variants have been reported. Germline variants are detected in about 90% of patients with the classic phenotype; most are point variants, whereas large deletions or duplications account for about 1–4%. The remaining cases may reflect non-coding or deep intronic variants, somatic mosaicism, or phenocopies.[3–5]

In patients with MEN1, PHPT is typically the first clinical manifestation. PHPT is usually asymptomatic in children with MEN1, but may become symptomatic by the third decade[6] The penetrance of PHPT is 100% by age 50 years. PHPT in MEN1 is a multiglandular disease, although glands are often affected asymmetrically and asynchronously[7] Ectopic glands (thymus, mediastinum, pericardium) may also occur, so preoperative functional imaging (99mTc-sestamibi SPECT/CT or 18F-fluorocholine PET/CT) is essential for surgical planning[8–10]

Uncontrolled PHPT causes end-organ damage, mainly nephrolithiasis and reduced bone mineral density (BMD); the timing and extent of surgery require individual assessment of biochemical severity and organ involvement, balancing cure against the risk of postoperative hypoparathyroidism[11, 12]

A clinical diagnosis of MEN1 is established when at least two of the three major tumor manifestations are identified in a single patient, or when one manifestation is present in a first-degree relative of a confirmed case[13]. Approximately 10%–30% of individuals with clinically diagnosed MEN1 do not carry an identifiable germline *MEN1* P/LP variant [14, 15]. In apparently sporadic cases, the detection rate is lower, with pathogenic variants found in only 6%–25% of patients presenting with two associated manifestations[16, 17]

Building on current recommendations for *MEN1* genetic testing, the objective of this study was to assess the detection rate of germline *MEN1* P/LP variants, and the clinical features associated with them, in patients who developed PHPT at or before 35 years of age or who presented with multiglandular involvement at initial evaluation[14, 18]

Materials and Methods

Subjects

We retrospectively identified 93 patients with PHPT diagnosed between January 2022 and December 2025 at the C. I. Parhon National Institute of Endocrinology (Bucharest, Romania), a tertiary referral center, who were aged ≤ 35 years or had multiglandular involvement at diagnosis. The age threshold of 35 years, higher than the 30-year cut-off commonly recommended for genetic testing [13], was chosen because many early-onset patients had elevated serum calcium or imaging-documented nephrolithiasis several years before the final diagnosis of PHPT, indicating an earlier onset of disease. Of the 93 patients, 30 completed the full biochemical and genetic evaluation and consented to participate, forming the study cohort. Patients diagnosed at ≤ 35 years of age formed Group 1 (early-onset PHPT), regardless of the number of affected

glands; patients older than 35 years with multiglandular involvement formed Group 2 (multiglandular PHPT). Clinical, imaging, densitometric, biochemical, and genetic data were extracted from the institutional database. Cervical ultrasound was the first-line imaging method; 99mTc-sestamibi scintigraphy and/or SPECT/CT were added when ultrasound was inconclusive or discordant with the biochemical findings, and were used to localize ectopic glands. Affected glands were identified by these imaging methods or, when imaging was negative, at surgery; multiglandular involvement was defined as ≥ 2 affected glands. Nephrolithiasis and nephrocalcinosis were assessed by renal ultrasound. Bone involvement was defined as a Z-score ≤ − 2.0 (low bone mineral density for age) in Group 1 and as osteopenia or osteoporosis by T-score (WHO criteria) in Group 2. Screening for associated tumors was performed in all patients and included pituitary imaging (CT or MRI) with hormonal evaluation (prolactin, IGF-1, ACTH, TSH, FSH, and LH); adrenal CT with assessment of cortisol secretion, aldosterone and renin, and plasma metanephrines and normetanephrines; and, for neuroendocrine tumors (NETs), chromogranin A, serotonin, 5-hydroxyindoleacetic acid (5-HIAA), neuron-specific enolase (NSE), and fasting glucose and insulin, with imaging performed case by case, at the treating physician's discretion: MRI, CT, or endoscopic ultrasound for pancreatic NETs, CT for ileal NETs, and upper gastrointestinal endoscopy for gastric NETs. Biochemical screening was performed concomitantly with genetic testing, before the genetic result was known.

Genetic testing

The decision to perform genetic testing was based on clinical data, age at diagnosis, and family history. Written informed consent was obtained from each patient before genetic testing.

Genomic DNA was extracted from peripheral blood leukocytes using the Promega Wizard Genomic DNA Purification Kit. DNA concentration was measured with the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific). Coding exons 2–10 and adjacent intronic regions of *MEN1* were amplified with gene-specific primers and sequenced with the GenomeLab DTCS QuickStart Kit (Beckman Coulter), according to an in-house protocol. Sequencing products were analyzed on the CEQ 8000 Genetic Analysis System (Beckman Coulter) with DNA Baser Sequence Assembler v4 software (Heracle BioSoft, 2013; https://www.DnaBaser.com). Chromatograms were inspected visually, and variants were identified against the human reference genome GRCh38 (hg38) and annotated on the *MEN1* MANE Select transcript NM_001370259.2 using the UCSC Genome Browser. All variants were confirmed by bidirectional sequencing.

Because Sanger sequencing does not detect exon-level copy-number changes, multiplex ligation-dependent probe amplification (MLPA) was performed to identify deletions or duplications of *MEN1*, using the SALSA® MLPA® P244 AIP-MEN1-CDKN1B kit (MRC Holland), according to the manufacturer's instructions. Variants were classified as pathogenic, likely pathogenic, of uncertain significance, likely benign, or benign according to the American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG/AMP) guidelines, using ClinVar and the Franklin by Genoox platform. Patients carrying a P/LP variant were considered *MEN1*-positive.

Statistical analysis

Statistical analyses were performed using SPSS version 26 and Python 3.11 (SciPy 1.13). Continuous variables are presented as mean ± SD or median (IQR) and were compared using Student's t-test or the Mann–Whitney U test, according to normality (Shapiro–Wilk test). Categorical variables were compared using Fisher's exact test, with odds ratios and exact 95% confidence intervals consistent with this test. A two-sided $p < 0.05$ was considered significant.

Predictors of *MEN1*-positive status were assessed by Firth's penalized logistic regression, given the small number of events (n = 11) and quasi-complete separation, with profile-likelihood confidence intervals[19]. Pre-specified predictors were age, multiglandular disease, pituitary tumor, and adrenal hyperplasia; a model including NETs was fitted as an exploratory post-hoc analysis.

Sensitivity, specificity, predictive values, and positive likelihood ratio (LR+) were calculated for each feature, with Wilson score (proportions) and log-method (LR+) confidence intervals. Bonferroni, Holm, and Benjamini–Hochberg corrections were applied as a sensitivity analysis for multiple comparisons.

Results

Study population

Each group included 15 patients: Group 1 (early-onset PHPT; mean age 30.7 ± 4.8 years, range 18–35; 3 men) and Group 2 (multiglandular PHPT; mean age 55.2 ± 8.9 years, range 41–71; 4 men). Baseline characteristics are summarized in Table 1.

Table 1
Baseline clinical characteristics of the two study groups

Variable	Group 1 Early-onset (≤ 35 years) (n = 15)	Group 2 Multiglandular (n = 15)	Total (n = 30)
Demographics			
Age at diagnosis, mean ± SD (range)	30.7 ± 4.8 (18–35)	55.2 ± 8.9 (41–71)	—
Sex, male	3/15 (20.0%)	4/15 (26.7%)	7/30 (23.3%)
MEN1 pathogenic/likely pathogenic variant	5/15 (33.3%)	6/15 (40.0%)	11/30 (36.7%)
Biochemical Parameters			
Serum calcium, mg/dL, median (IQR)	10.80 (10.70–11.00)	11.40 (11.10–12.00)	11.05 (10.70–11.72)
PTH, pg/mL, median (IQR)	125.0 (91.0–204.3); n = 14	169.0 (100.0–258.0)	158.0 (99.5–246.5); n = 29
Vitamin D, ng/mL, median (IQR)	19.7 (15.7–24.5); n = 12	24.4 (17.4–31.0)	21.0 (17.4–29.8); n = 27
Parathyroid involvement			
Parathyroid glands affected, median (range)	1 (1–4); n = 14	2 (2–3)	—
Ectopic parathyroid gland	2/15 (13.3%)	2/15 (13.3%)	4/30 (13.3%)
Organ Involvement			
Renal involvement	9/15 (60.0%)	10/15 (66.7%)	19/30 (63.3%)
Bone involvement (any)	4/15 (26.7%)	10/15 (66.7%)	14/30 (46.7%)
Osteoporosis	—	7/15 (46.7%)	—
MEN1-Associated Tumors			
Pituitary tumor (any)	13/15 (86.7%)	10/15 (66.7%)	23/30 (76.7%)
Macroadenoma	2/15 (13.3%)	3/15 (20.0%)	5/30 (16.7%)
Functioning pituitary adenoma	4/15 (26.7%)	3/15 (20.0%)	7/30 (23.3%)
Neuroendocrine tumor (NET)	4/15 (26.7%)	5/15 (33.3%)	9/30 (30.0%)
Adrenal hyperplasia	6/15 (40.0%)	9/15 (60.0%)	15/30 (50.0%)
Lipomas	0/15 (0.0%)	7/15 (46.7%)	7/30 (23.3%)

Data are presented as mean ± SD (range), median (IQR), or n/N (%); n = patients with available data, when fewer than the group size. Renal involvement = nephrolithiasis or nephrocalcinosis on renal ultrasound. Affected glands were identified by imaging or, when imaging was negative, at surgery. PTH = parathyroid hormone. Vitamin D = 25-hydroxyvitamin D.

Variable	Group 1 Early-onset (≤ 35 years) (n = 15)	Group 2 Multiglandular (n = 15)	Total (n = 30)
Demographics			
Other malignancies	0/15 (0.0%)	6/15 (40.0%)	6/30 (20.0%)

Data are presented as mean ± SD (range), median (IQR), or n/N (%); n = patients with available data, when fewer than the group size. Renal involvement = nephrolithiasis or nephrocalcinosis on renal ultrasound. Affected glands were identified by imaging or, when imaging was negative, at surgery. PTH = parathyroid hormone. Vitamin D = 25-hydroxyvitamin D.

Genetic testing

A P/LP *MEN1* variant was identified in 5/15 patients (33.3%) in Group 1 (4 pathogenic, 1 likely pathogenic; benign variants in 7 and no variant in 3 of the remaining 10) and in 6/15 (40.0%) in Group 2 (1 pathogenic, 5 likely pathogenic), giving an overall detection rate of 11/30 (36.7%), with no difference between groups (p = 1.000). No deletions or duplications were detected by MLPA. The 11 carriers had 10 distinct variants (the two relatives shared one): seven frameshift, one in-frame deletion, one missense, and one synonymous variant (Table 2). Applying the 2025 guideline criteria [14], 12 patients (40.0%; 6 per group) fulfilled a clinical diagnosis of MEN1 (PHPT plus a pancreatic or gastric NET, or a significant pituitary lesion). A P/LP variant was found in 7/12 (58.3%) of these patients and in 4/18 (22.2%) of those without a second major manifestation (p = 0.063). Associations between *MEN1* status and each manifestation are shown in Table 3. The patients belonged to 29 families: two *MEN1*-positive patients in Group 2 were relatives carrying the same variant; both met the inclusion criteria independently and were retained. After excluding one of them, the associations with NETs (p = 0.001) and adrenal hyperplasia (p = 0.021) remained significant, whereas the association with lipomas in Group 2 did not (p = 0.091).

Table 2

MEN1 pathogenic and likely pathogenic variants and associated manifestations in the 11 carriers

Patient	Age, sex	*MEN1* variant (HGVS) (NM_001370259.2)	Variant type	ACMG	Glands	Associated manifestations
Group 1 (early-onset PHPT)						
5	35, F	c.734del (p.Pro245Leufs*36)	Frameshift	P	1	NET
12	31, M	c.252del (p.Ile85Serfs*34)	Frameshift	LP	1	NET; adrenal hyperplasia; NF pituitary microadenoma
13	33, F	c.1546del (p.Arg516Glyfs*43)	Frameshift	P	4	NET; adrenal hyperplasia; macroprolactinoma
14	35, F	c.1546dup (p.Arg516Profs*15)	Frameshift	P	3	Adrenal hyperplasia; NF pituitary microadenoma
15	23, M	c.841G > A (p.Gly281Arg)	Missense	P	1	NET; NF pituitary microadenoma
Group 2 (multiglandular PHPT)						
24	43, F	c.1700_1701insTTGGTGGC (p.Thr568Trpfs*23)	Frameshift	LP	2	Adrenal hyperplasia; microprolactinoma; lipoma
25	63, F	c.1670_1672del (p.Lys557del)	In-frame deletion	P	2	NET; adrenal hyperplasia
26	47, M	c.1152_1162dup (p.Glu388Glyfs*61)	Frameshift	LP	3	Adrenal hyperplasia; NF pituitary microadenoma; lipoma
27	50, F	c.639C > A (p.Ala213=)	Synonymous	LP	2	NET; adrenal hyperplasia; NF pituitary microadenoma; lipoma

Variants are listed as reported by the genetic laboratory. NM_001370259.2 is the MANE Select MEN1 transcript. Variant type was inferred from the HGVS description. Relatives carrying the same variant. Ectopic gland. Exon 2; not reported in the databases consulted at the time of testing (2021). Age at diagnosis in years. Glands = number of affected parathyroid glands. ACMG = American College of Medical Genetics and Genomics; P = pathogenic; LP = likely pathogenic; F = female; M = male; NET = neuroendocrine tumor; NF = non-functioning.

Patient	Age, sex	*MEN1* variant (HGVS) (NM_001370259.2)	Variant type	ACMG	Glands	Associated manifestations
Group 1 (early-onset PHPT)						
28	41, M	c.520_521insCA (p.His174Profs*12)	Frameshift	LP	3	NET; adrenal hyperplasia; NF pituitary macroadenoma; lipoma
30	56, M	c.639C > A (p.Ala213=)	Synonymous	LP	2	NET; adrenal hyperplasia; lipoma

Variants are listed as reported by the genetic laboratory. NM_001370259.2 is the MANE Select MEN1 transcript. Variant type was inferred from the HGVS description. Relatives carrying the same variant. Ectopic gland. Exon 2; not reported in the databases consulted at the time of testing (2021). Age at diagnosis in years. Glands = number of affected parathyroid glands. ACMG = American College of Medical Genetics and Genomics; P = pathogenic; LP = likely pathogenic; F = female; M = male; NET = neuroendocrine tumor; NF = non-functioning.

Table 3
Associated features according to *MEN1* status in the whole cohort and in each indication group

Associated feature	*MEN1*+, n (%)	*MEN1*−, n (%)	OR (95% CI)	p
A. Whole cohort (N = 30): *MEN1*+ (n = 11) vs *MEN1*− (n = 19)				
Neuroendocrine tumor (NET)	**8 (72.7)**	**1 (5.3)**	**39 (4.3–1100)**	**< 0.001**
Adrenal hyperplasia	**9 (81.8)**	**6 (31.6)**	**8.9 (1.3–74)**	**0.021**
Lipomas	5 (45.5)	2 (10.5)	6.6 (0.92–58)	0.068
Pituitary tumor (any)	8 (72.7)	15 (78.9)	0.72 (0.12–4.5)	1.000
Other malignancies	3 (27.3)	3 (15.8)	2.0 (0.29–13)	0.641
Renal involvement	7 (63.6)	12 (63.2)	1.02 (0.21–5.0)	1.000
Bone involvement	4 (36.4)	10 (52.6)	0.53 (0.11–2.7)	0.466
Ectopic parathyroid gland	1 (9.1)	3 (15.8)	0.54 (0.02–5.7)	1.000
B. Early-onset group (n = 15): MEN1+ (n = 5) vs MEN1− (n = 10)				
Neuroendocrine tumor (NET)	**4 (80.0)**	**0 (0.0)**	**∞ (2.6–∞)**	**0.004**
Adrenal hyperplasia	3 (60.0)	3 (30.0)	3.2 (0.33–39)	0.329
Lipomas	0 (0.0)	0 (0.0)	NE	1.000
Pituitary tumor	4 (80.0)	9 (90.0)	0.47 (0.01–21)	1.000
Other malignancies	0 (0.0)	0 (0.0)	NE	1.000
C. Multiglandular group (n = 15): MEN1+ (n = 6) vs MEN1− (n = 9)				
Neuroendocrine tumor (NET)	4 (66.7)	1 (11.1)	12 (0.99–430)	0.089
Adrenal hyperplasia	**6 (100)**	**3 (33.3)**	**∞ (1.6–∞)**	**0.028**
Lipomas	**5 (83.3)**	**2 (22.2)**	**14 (1.1–460)**	**0.041**
Pituitary tumor	4 (66.7)	6 (66.7)	1.00 (0.10–11)	1.000
Other malignancies	3 (50.0)	3 (33.3)	1.9 (0.21–18)	0.622

OR = conditional maximum-likelihood odds ratio with exact 95% confidence interval obtained by inverting Fisher's exact test (consistent with the p-values). ∞ = odds ratio not estimable because one cell is zero (no MEN1-negative patient had the feature, as for NETs in the early-onset group, or all MEN1-positive patients had it, as for adrenal hyperplasia in the multiglandular group); the lower 95% confidence bound is shown. NE = not estimable (feature absent in both subgroups). p-values from Fisher's exact test.

Number of parathyroid glands affected

In Group 1 (gland localization available in 14/15 patients), 10 patients (71.4%) had single-gland disease, 2 had two, 1 had three, and 1 had four affected glands (median 1). In Group 2, 12 patients (80.0%) had two and 3 (20.0%) had three affected glands (median 2). In Group 1, both patients with three or more affected glands

were *MEN1*-positive, although median gland counts did not differ by *MEN1* status (p = 0.357); in Group 2, gland count was similar in carriers and non-carriers (p = 0.351).

Ectopic glands (five in total) were found in 2 patients in each group (overall 4/30, 13.3%), all localized preoperatively by SPECT/CT. In Group 1, ectopic glands were located in the anterior mediastinum (*MEN1*-negative patient) and in a paravertebral position at C7–T1 (*MEN1*-positive patient). In Group 2, both cases were *MEN1*-negative: one patient had two mediastinal glands and the other a paraesophageal gland. Overall, ectopic glands were found in 1/11 *MEN1*-positive and 3/19 *MEN1*-negative patients, without significant differences (p = 1.000 in Group 1; p = 0.486 in Group 2).

Biochemical parameters

Serum calcium, parathyroid hormone (PTH), and vitamin D levels are shown in Table 1 and did not differ between *MEN1*-positive and *MEN1*-negative patients in either group (Group 1: calcium p = 0.951, PTH p = 0.386, vitamin D p = 0.645; Group 2: p = 0.768, p = 0.955, and p = 0.111, respectively).

Organ complications

Nephrolithiasis or nephrocalcinosis was present in 9/15 patients (60.0%) in Group 1 and 10/15 (66.7%) in Group 2, without difference by *MEN1* status in either group (p = 0.580 and p = 0.329, respectively).

Skeletal involvement was more frequent in Group 2 (10/15, 66.7%, including 7 patients with osteoporosis) than in Group 1 (4/15, 26.7%; p = 0.066), although the different densitometric criteria and ages preclude a direct comparison. It did not differ by *MEN1* status in either group (Group 1: p = 1.000; Group 2: p = 0.329).

MEN1- Associated Tumors

Pituitary tumors were found in 13/15 patients (86.7%) in Group 1 and 10/15 (66.7%) in Group 2, including macroadenomas in 2 and 3 and functioning adenomas in 4 and 3 patients, respectively, and were not associated with *MEN1* status. Following the 2025 MEN1 guidelines, only functioning adenomas (excluding microprolactinomas in women) and non-functioning macroadenomas (> 1 cm) were considered significant MEN1-related lesions.[14] These lesions (4/15 in Group 1; 3/15 in Group 2) did not discriminate carriers from non-carriers in either group or in the whole cohort (2/11 vs. 5/19; p = 1.000 for all comparisons) and were numerically more frequent in *MEN1*-negative patients.

NETs showed the strongest association with *MEN1* status. In Group 1, all 4 NETs occurred in *MEN1*-positive patients (p = 0.004), the only significant finding in this group; in Group 2, NETs were more frequent in carriers (4/6 vs. 1/9; p = 0.089). In the whole cohort, 8/11 carriers (72.7%) and 1/19 non-carriers (5.3%) had a NET (p < 0.001). All NETs were non-functioning: two pancreatic, one ileal, and one gastric in Group 1; three pancreatic, one ileal, and one combined pancreatic and gastric in Group 2.

Adrenal hyperplasia was present in 6/15 patients (40.0%) in Group 1, without difference by *MEN1* status (3/5 vs. 3/10; p = 0.329), and in 9/15 (60.0%) in Group 2, including all 6 carriers versus 3/9 non-carriers (p = 0.028);

the association was confirmed in the whole cohort (9/11 vs. 6/19; p = 0.021). One *MEN1*-positive patient in Group 1 had ACTH-independent cortisol secretion, the only functioning adrenal lesion in the cohort.

Lipomas were absent in Group 1 and present in 7/15 patients (46.7%) in Group 2, more often in carriers (5/6 vs. 2/9; p = 0.041).

Other associated malignancies

No non-*MEN1* malignancies were recorded in Group 1. In Group 2, 6 of 15 patients (40.0%) presented with associated malignancies, including papillary thyroid carcinoma (3 cases), biliary tract adenocarcinoma (1 case), breast carcinoma (1 case), and fibrosarcoma (1 case). These occurred in 3/6 *MEN1*-positive and 3/9 *MEN1*-negative patients (p = 0.622).

Odds ratios are shown in Table 3. In the whole cohort, NETs (OR 39, 95% CI 4.3–1100) and adrenal hyperplasia (OR 8.9, 95% CI 1.3–74) were associated with *MEN1*-positive status, lipomas showed a non-significant trend (OR 6.6, 95% CI 0.92–58), and pituitary tumors, renal and skeletal involvement, and ectopic glands were not associated. The direction of these associations was preserved in both subgroups.

In the pre-specified multivariable model (age, multiglandular disease, pituitary tumor, and adrenal hyperplasia), adrenal hyperplasia was the only independent predictor of *MEN1*-positive status (adjusted OR 11, 95% CI 2.0–99; p = 0.005); age (p = 0.055), multiglandular disease (p = 0.17), and pituitary tumor (p = 0.42) were not significant. Because age and multiglandular disease largely reflect the indication group (r = 0.62), they should be read as adjustment for group. In an exploratory post-hoc model, NETs (adjusted OR ≈ 54, 95% CI 5.0–7700; p < 0.001) and adrenal hyperplasia (adjusted OR ≈ 17, 95% CI 1.7–2300; p = 0.014) remained independently associated with *MEN1* status; given the wide intervals, this analysis is hypothesis-generating only.

Diagnostic performance is shown in Table 4. NETs had the highest specificity (94.7%) and LR+ (13.8), adrenal hyperplasia the highest sensitivity (81.8%), and pituitary tumors did not discriminate *MEN1* status (LR + 0.92). A NET or adrenal hyperplasia was present in all 11 variant carriers (sensitivity and negative predictive value 100%), with a specificity of 63.2%. Because all patients already had an indication for testing, these estimates reflect the added value of each feature in an enriched cohort (*MEN1* prevalence 36.7%).

Table 4
Diagnostic performance of associated features for identifying *MEN1*-positive patients (whole cohort, n = 30)

Associated feature	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	LR+ (95% CI)
Single features					
Neuroendocrine tumor (NET)	72.7 (43.4–90.3)	94.7 (75.4–99.1)	88.9 (56.5–98.0)	85.7 (65.4–95.0)	13.8 (2.0–96)
Adrenal hyperplasia	81.8 (52.3–94.9)	68.4 (46.0–84.6)	60.0 (35.7–80.2)	86.7 (62.1–96.3)	2.6 (1.3–5.3)
Lipomas	45.5 (21.3–72.0)	89.5 (68.6–97.1)	71.4 (35.9–91.8)	73.9 (53.5–87.5)	4.3 (1.00–18.6)
Pituitary tumor (any)	72.7 (43.4–90.3)	21.1 (8.5–43.3)	34.8 (18.8–55.1)	57.1 (25.0–84.2)	0.92 (0.60–1.42)
Combined criterion					
NET or adrenal hyperplasia	100 (74.1–100)	63.2 (41.0–80.9)	61.1 (38.6–79.7)	100 (75.8–100)	2.7 (1.5–4.9)

PPV = positive predictive value; NPV = negative predictive value; LR + = positive likelihood ratio. Confidence intervals: Wilson score method for proportions, log method for LR+. Predictive values depend on the MEN1 prevalence of this enriched cohort (36.7%). Age and multiglandular disease were not evaluated because they define the inclusion criteria. Counts (TP/FP/FN/TN): NET 8/1/3/18; adrenal hyperplasia 9/6/2/13; lipomas 5/2/6/17; pituitary tumor 8/15/3/4; NET or adrenal hyperplasia 11/7/0/12.

Discussion

In this study, *MEN1* P/LP variants were identified in approximately one third of patients in both indication groups (33.3% and 40.0%). Consistent with previous reports, the pattern of associated tumors, rather than biochemical severity, was the strongest predictor of *MEN1* positivity.[20, 21]. Twelve patients already fulfilled clinical criteria for *MEN1* at testing, with a detection rate of 58.3%; P/LP variants were also found in 4 of 18 patients (22.2%) whose only major manifestation was PHPT, supporting early-onset and multiglandular PHPT as indications for genetic testing even in the absence of a second major tumor. In addition, 4 of the 5 *MEN1*-positive patients in Group 1 were aged 31–35 years (4/11 vs. 1/4 among those aged ≤ 30 years), suggesting that a strict 30-year threshold could miss carriers; this observation requires confirmation in larger cohorts.

NETs showed the strongest association with *MEN1* status (Group 1, $p = 0.004$; whole cohort, $p < 0.001$), the only one that remained significant after correction for multiple comparisons. In Group 1, NETs occurred exclusively in *MEN1*-positive patients, affecting 4 of the 5 carriers. Malignant NETs, particularly non-functioning pancreatic NETs, gastrinomas, and thymic NETs, are the leading cause of MEN1-related mortality. [22–24]. MEN1-related morbidity and mortality derive mainly from gastroenteropancreatic NETs[25]. Long-

term follow-up shows that most small non-functioning pancreatic NETs grow slowly, although a subset progresses and requires surgery[26, 27].

Our data support the view that the incidental identification of any NET in a patient with PHPT, regardless of age, should prompt genetic counseling and *MEN1* testing, even in the absence of a family history. This is consistent with guideline recommendations for lifelong multiorgan surveillance once *MEN1* is confirmed.[14]

Pituitary lesions were the most frequent associated finding (23/30, 76.7%), but most were smaller than 1 cm (18/23) and they did not discriminate *MEN1* variant carriers, even when the analysis was restricted to lesions considered significant by the 2025 guidelines. Incidental pituitary microadenomas are reported in about 10% of the general population; systematic imaging in our cohort likely detected many sporadic lesions. The two significant lesions in carriers were a macroprolactinoma and a non-functioning macroadenoma, consistent with prolactinomas and non-functioning adenomas being the most common pituitary tumors in MEN1[14, 21]. In contrast, all four growth hormone-secreting adenomas occurred in *MEN1*-negative patients. Although somatotroph adenomas belong to the MEN1 spectrum, their coexistence with PHPT in the absence of a *MEN1* variant may reflect sporadic coincidence or phenocopies, including *AIP*- or *CDKN1B*-related disease, which our protocol did not assess. A pituitary microlesion in a patient with PHPT should therefore not, by itself, be regarded as evidence of MEN1.

Adrenal hyperplasia was associated with *MEN1* status in Group 2 ($p = 0.028$) and in the whole cohort ($p = 0.021$), although not after correction for multiple comparisons. Adrenal lesions are part of the broader MEN1 phenotype, but their reported prevalence varies widely (9–73%) depending on imaging modality, follow-up duration, and diagnostic criteria. Clemente-Gutierrez et al., in a review of 382 MEN1 patients, documented adrenal lesions in 23.3%, with a mean detection age of 47 years, while other cohorts have reported adrenal involvement in more than half of patients at diagnosis [28, 29]. The 100% prevalence among *MEN1*-positive Group 2 patients exceeds most published estimates, possibly reflecting systematic screening or referral bias. The absence of a similar finding in Group 1 may reflect its younger age and the age-dependent accumulation of adrenal manifestations, as adrenal lesions are typically diagnosed several years after the initial MEN1 diagnosis[30]

Lipomas were associated with *MEN1* status in Group 2 ($p = 0.041$), although not after excluding one of the two related carriers. They are a well-recognized non-endocrine manifestation of MEN1, alongside facial angiofibromas and collagenomas. Their reported prevalence ranges from 5% to 34%. Prior series have shown a significantly higher prevalence of lipomas in *MEN1* variant-positive than variant-negative patients ($OR \approx 2.7$; $p = 0.02$), consistent with our findings[31] The complete absence of lipomas in Group 1 is consistent with the age-dependent nature of non-endocrine manifestations (mean age 30.7 vs. 55.2 years). Although the combined-cohort analysis showed only a trend ($p = 0.068$), the within-Group 2 finding supports systematic skin and soft-tissue examination during MEN1 evaluation. Angiofibromas and collagenomas were not recorded, which may underestimate the non-endocrine burden.

Consistent with existing literature, we found no significant differences in serum calcium, PTH, or vitamin D levels between *MEN1*-positive and *MEN1*-negative patients in either group or overall [32] Current guidelines state that no genotype–phenotype correlation exists for PHPT severity; the decision to test for *MEN1* should therefore rest on the clinical and tumor profile rather than on biochemical thresholds[14]

Bone involvement was more prevalent in Group 2 (66.7% vs. 26.7%; p = 0.066), with osteoporosis in 46.7% of multiglandular patients, consistent with registry data showing early, progressive, and extensive demineralization in MEN1-PHPT [33]. This likely reflects a multifactorial etiology. First, the mean age of Group 2 (55.2 years) includes postmenopausal women, in whom estrogen deficiency accelerates bone turnover and amplifies the skeletal effects of PTH excess.[34, 35] Second, renal involvement was present in 10/15 patients (66.7%) in Group 2, including nephrocalcinosis (n = 2) and chronic kidney disease (n = 1). Renal impairment reduces calcitriol synthesis and adds a secondary hyperparathyroid stimulus, compounding bone resorption[36, 37]. Third, adrenal hyperplasia was present in 9/15 patients (60.0%); although MEN1 adrenal lesions are mostly non-functioning, ACTH-independent cortisol secretion in one *MEN1*-positive patient supports biochemical adrenal screening even without Cushingoid features.[29, 38]

The lack of difference in bone involvement by *MEN1* status suggests that organ damage reflects accumulated multifactorial risk rather than genotype.

Limitations

This study has several limitations. First, the retrospective design and small sample (30 patients, 11 *MEN1*-positive) reduced statistical power and produced very wide confidence intervals (e.g., OR 39, 95% CI 4.3–1100 for NETs); the effect sizes are therefore imprecise, which limits their immediate clinical use. Some clinically relevant differences, such as the higher prevalence of NETs in Group 2 (66.7% vs. 11.1%) and of lipomas in the whole cohort (45.5% vs. 10.5%), may not have reached significance despite reflecting true associations. Second, referral to a tertiary center enriched the cohort for hereditary disease (*MEN1* positivity 36.7%), limiting generalizability. Third, only 30 of the 93 eligible patients (32.3%) completed the full biochemical and genetic evaluation and consented to participate; because the characteristics of non-participants were not analyzed, selection bias cannot be excluded. Fourth, genetic analysis was limited to *MEN1*, and other hereditary causes of multiglandular PHPT (*CDKN1B*/MEN4, *CDC73*, *CASR*, *GCM2*, *RET*) were not excluded. Multigene panels in *MEN1*-negative patients could refine the interpretation of negative results. Fifth, given the exploratory nature of this study and the rarity of *MEN1*, p-values were not adjusted for multiple comparisons. In a sensitivity analysis applying Bonferroni, Holm, and Benjamini–Hochberg corrections to the 16 testable associations in Table 3, the association between NETs and *MEN1* status in the whole cohort remained significant under all three methods (adjusted p = 0.004), and the corresponding association in the early-onset subgroup remained significant only under false discovery rate control (q = 0.029); the associations with adrenal hyperplasia and lipomas did not survive formal correction and are hypothesis-generating. Finally, the 100% sensitivity and negative predictive value of the combined NET or adrenal hyperplasia criterion rest on only 11 variant carriers. Nevertheless, this simple combination could serve as a screening tool to prioritize genetic testing in PHPT, provided it is confirmed in larger prospective cohorts.

Conclusions

In this single-center cohort, *MEN1* P/LP variants were identified in approximately one third of PHPT patients meeting genetic testing criteria, with similar detection rates for early age at onset and for multiglandular involvement, supporting both indications, even in the absence of a second major MEN1 manifestation. NETs showed the strongest association with *MEN1* positivity and were the only feature whose association

withstood correction for multiple comparisons. Adrenal hyperplasia was additionally associated with *MEN1* status in the multiglandular group and in the whole cohort. Lipomas, associated with *MEN1* status in the multiglandular group, may be an accessible clinical clue. Collectively, these findings suggest that the tumor profile accompanying PHPT, particularly NETs, adrenal hyperplasia, and lipomas, may help guide the decision to pursue genetic testing independently of biochemical severity. Given the small single-center sample, these results should be regarded as hypothesis-generating and require validation in larger multicenter cohorts.

Declarations

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Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the C.I.Parhon National Institute of Endocrinology.

Consent to participate: Informed consent was obtained from all individual participants included in the study.

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Data Availability: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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