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Multiple Endocrine Neoplasia Type 1 in Children and Adolescents: Clinical Features and Treatment Outcomes

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Abstract:	Background: Clinical manifestations and treatment outcomes in children and adolescents with multiple endocrine neoplasia type 1 are not well characterized. Methods: A retrospective cohort study of 80 patients with multiple endocrine neoplasia type 1 who commenced tumor surveillance at ≤18 years of age. Results:

	Fifty-six patients (70%) developed an endocrine tumor by age ≤18 years (median age = 14 years, range = 6-18 years). Primary hyperparathyroidism occurred in >80% of patients, with >70% undergoing parathyroidectomy, in which less-than-subtotal (<3-gland) resection resulted in decreased disease-free outcomes vs subtotal (3-3.5-gland) or total (4-gland) parathyroidectomy (median 27 months vs not reached, P = .005). Pancreaticoduodenal neuroendocrine tumors developed in ~35% of patients, of whom >70% had non-functioning tumors, >35% had insulinomas, and <5% had gastrinomas, with ~15% having metastases, and >55% undergoing surgery. Pituitary tumors developed in >30% of patients and ~35% were macroprolactinomas. Tumor occurrence in males and females was not significantly different. Genetic analyses revealed 38 germline MEN1 mutations, of which 3 were novel. Conclusions: Seventy percent of children aged ≤18 years with multiple endocrine neoplasia type 1 develop endocrine tumors, which include: parathyroid tumors for which less-than-subtotal parathyroidectomy should be avoided; pancreaticoduodenal neuroendocrine tumors that may metastasize; and pituitary macroprolactinomas.

Multiple Endocrine Neoplasia Type 1 in Children and Adolescents: Clinical Features and Treatment Outcomes

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ABSTRACT (Word count: 200; Limit: 200)

Background:

Clinical manifestations and treatment outcomes in children and adolescents with multiple endocrine neoplasia type 1 are not well characterized.

Methods:

A retrospective cohort study of 80 patients with multiple endocrine neoplasia type 1 who commenced tumor surveillance at ≤18 years of age.

Results:

Fifty-six patients (70%) developed an endocrine tumor by age ≤18 years (median age = 14 years, range = 6-18 years). Primary hyperparathyroidism occurred in >80% of patients, with >70% undergoing parathyroidectomy, in which less-than-subtotal (<3-gland) resection resulted in decreased disease-free outcomes *vs* subtotal (3-3.5-gland) or total (4-gland) parathyroidectomy (median 27 months *vs* not reached, $P = .005$). Pancreaticoduodenal neuroendocrine tumors developed in ~35% of patients, of whom >70% had non-functioning tumors, >35% had insulinomas, and <5% had gastrinomas, with ~15% having metastases, and >55% undergoing surgery. Pituitary tumors developed in >30% of patients and ~35% were macroprolactinomas. Tumor occurrence in males and females was not significantly different. Genetic analyses revealed 38 germline *MEN1* mutations, of which 3 were novel.

Conclusions:

Seventy percent of children aged ≤18 years with multiple endocrine neoplasia type 1 develop endocrine tumors, which include: parathyroid tumors for which less-than-subtotal parathyroidectomy should be avoided; pancreaticoduodenal neuroendocrine tumors that may metastasize; and pituitary macroprolactinomas.

INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by the combined occurrence of primary hyperparathyroidism (PHPT), pituitary tumors, and pancreaticoduodenal neuroendocrine tumors (PD-NETs).[1] Patients may also develop adrenocortical neoplasms, bronchial and thymic carcinoids, and non-endocrine tumors such as lipomas, facial angiofibromas, and collagenomas.[1] Over 90% of patients with MEN1 have germline pathogenic variants affecting the coding region of the *MEN1* gene, which is located on chromosome 11q13, and encodes a ubiquitously expressed 610-amino acid tumor suppressor protein, menin. Over 90% of individuals with *MEN1* pathogenic variants will have developed at least 1 tumor by the fifth decade,[2] and MEN1-related tumors have been reported to occur as early as 5 years of age.[1] Thus, current guidelines recommend that patients with MEN1 undergo comprehensive screening for endocrine neoplasms from childhood, to enable early tumor recognition and ensure timely intervention before the development of adverse sequelae, such as metastatic disease.[1] However, there is no consensus regarding the age at which screening should start, as the benefits are offset by concerns about burden of testing, radiation exposure, and cost.[3] Moreover, some studies have indicated that clinically-significant or symptomatic MEN1 manifestations are rare before 16 years of age,[4] although it is of note that these reports consist of either small numbers of pediatric and adolescent patients from single institutions,[5-8] or in the case of larger multi-center series,[9,10] include historical cohorts of patients seen prior to the introduction of contemporary screening methods or in whom screening began relatively late in childhood (Table 1). Thus, the incidence of MEN1-related manifestations in this age group is not well-characterized, and may have been underestimated.[4-10] Furthermore, there is also a lack of consensus regarding the optimal management of MEN1 manifestations, such as PHPT and non-functioning pancreatic neuroendocrine tumors (NF-PNETs), in children and adolescents. Our aims were therefore to evaluate the occurrence of MEN1-related manifestations by ≤18 years of age, their treatment outcomes, and genotype-phenotype correlations.

METHODS

Study population and surveillance screening

A retrospective analysis of patients with MEN1, who underwent evaluation and treatment at 5 different European and North American centers, was undertaken following approval by local institutional review boards. Diagnosis of MEN1 was based on established criteria that included the presence of a: 1) pathogenic germline *MEN1* variant; 2) ≥2 MEN1-associated endocrine tumors; or 3) at least 1 MEN1-related tumor in a patient with a first-degree relative with MEN1.[1] Patients ≤35 years of age were selected to maximise the likelihood of obtaining childhood data from medical records that would contain information from annual surveillance programs of combined clinical, biochemical, and radiological screening, in accordance with current clinical practice guidelines (Table 2).[1]

Data collection

Baseline demographic data, age of MEN1 diagnosis, and results of genetic testing (if performed) were ascertained. For each MEN1 manifestation, the age of diagnosis, results of biochemical testing, imaging, histopathology (if applicable), treatments received, and disease status at last follow-up were obtained. Data collection was compliant with the Health Insurance Portability and Accountability Act (HIPAA).

Statistical analysis

Values are reported as: mean ± standard deviation (SD) or median (range), when appropriate, for continuous data, which were analysed by Student's t-test or the Wilcoxon rank-sum test; and as percentages for categorical data, that were analysed by chi-squared test or Fisher's exact test. Kaplan–Meier curves were generated to estimate: the cumulative age-specific penetrance of each MEN1 manifestation and disease-free outcome after parathyroidectomy. Comparisons between Kaplan–Meier curves were made using the log-rank test. *MEN1* genotype-phenotype correlations were investigated, as described.[11-13] Statistical significance was set at $P \leq .05$ with all analyses performed using GraphPad Prism (La Jolla, CA).

RESULTS

Clinical features of cohort and tumor development

A total of 118 patients with MEN1 (median age = 25 years, range = 6-35 years) were ascertained, and 38 patients were excluded because: screening was not commenced until after 18 years of age (n = 34); follow-up was not continued after their initial visit (n = 3); and surveillance abdominal imaging had not been performed at the time of last follow-up (n = 1) (Fig 1). The remaining 80 patients, from 60 unrelated families, consisted of 50 females (63%) and 30 males (38%), who had MEN1 diagnosed at a median age of 11.5 years (range = 0.8-18 years), with the median duration of follow-up being 92.5 months (range = 7-306 months) (Supplementary Table 1 available online only). Eighteen percent (14/80) of patients had not reached 18 years of age at the time of inclusion.

Seventy percent (56/80) of patients (37 females and 19 males) developed at least 1 MEN1-related manifestation at ≤18 years of age, with the first manifestation detected at a median age of 14 years (range = 6-18 years) (Table 3). Of these 56 patients, 68% (38/56) had prospective screening that preceded the development of the first MEN1 manifestation, whereas 32% (18/56) had commenced screening only after the first manifestation was detected and, of these, 12 had a known family history and had either not been referred for screening or were referred and declined further participation, while the remaining 6 were index cases. Out of the overall cohort of 80 patients, by the age of 18 years ~70% had PHPT, ~35% had PD-NETs (by subtype, ~25% had NF-PNETs and ~15% had insulinomas), ~30% had pituitary tumors, 1% had adrenal adenomas (Figs 2A and 2B), and 0% had developed bronchial or thymic carcinoids. Further features of these tumors and genetic analyses are detailed below.

Primary hyperparathyroidism (PHPT)

PHPT at ≤18 years of age was diagnosed in >80% (46/56) of patients who had an MEN1 manifestation in childhood or adolescence, and was the first manifestation of MEN1 disease (either alone or synchronously with another manifestation) in ~60% (33/56) of patients (Table 3). The median age of PHPT diagnosis was 15 years (range = 6-18 years), with 3 patients diagnosed before 10 years of age. PHPT occurrence was similar in males and females (89% males *vs* 78% females, $P = .467$). Symptomatic disease or end-organ

manifestations of PHPT were present in 20% (9/46) of patients) and these comprised: nephrolithiasis (n = 3), bone pain (n = 2), gastrointestinal dysfunction (n = 2), muscle weakness (n = 1), and ischemic stroke (n = 1), which occurred in an index case who, at 14 years of age, presented with a generalized seizure, with MRI revealing a right-sided cortical infarct.[14]

Over 70% (33/46) of patients with PHPT had parathyroidectomy, which was performed at ≤18 years in ~70% (23/33) of cases (median age = 15 years, range = 6-18 years), with the remaining ~30% (10/33) of patients having surgery as adults (median age = 20.5 years, range = 19-28 years). Less than 10% (3/33) of patients had total parathyroidectomy, TPTX, (i.e., resection of 4 glands) with auto-transplantation of parathyroid tissue into the non-dominant forearm or sternocleidomastoid muscle; >40% (14/33) of patients had subtotal parathyroidectomy, SPTX, (i.e., resection of 3-3.5 glands, leaving a remnant *in situ*); and >45% (16/33) of patients had less than SPTX, <SPTX (i.e., resection of <3 glands) (additional information provided in the Supplementary Text (available online only)). Transcervical thymectomy was performed in >65% (22/33) of patients. Post-operatively (median follow-up = 62 months, range = 1-290 months), >50% (17/33) of patients developed persistent or recurrent PHPT, and these comprised >85% (14/16), >20% (3/13), and 0% (0/13) of patients having <SPTX, SPTX, and TPTX, respectively ($P < .001$). Thus, patients who underwent <SPTX had significantly worse disease-free outcome compared with those who underwent SPTX/TPTX (median 27 months *vs* not reached, log-rank hazard ratio 4.3; 95% confidence interval 1.8 – 12.6; $P = .005$) (Fig 2C). Eleven of 17 (~65%) patients with persistent or recurrent PHPT following SPTX (n = 2) and <SPTX (n = 9) had further surgery (median time following the first procedure = 61 months, range = 3-262 months), which resulted in normocalcemia in only 4 patients, with the remaining 7 patients continuing to have persistent/recurrent disease. Two patients underwent a third operation (leading to completion TPTX) which failed to restore normocalcemia. Less than 10% (3/33) of patients have developed post-surgical permanent hypoparathyroidism, and these comprise a patient who had TPTX as the only surgery, and 2 patients who initially had <SPTX followed by completion TPTX.

Pancreaticoduodenal neuroendocrine tumors (PD-NETs)

PD-NETs were identified at ≤18 years of age in ~40% (21/56) of patients, with similar occurrences in males and females (42% males *vs* 35% females, *P* = .772) (Table 3). Synchronous or metachronous NF-PNETs and functioning PD-NETs occurred in ~10% (2/21) of patients, with NF-PNETs only occurring in ~60% (13/21) of patients, and insulinomas only occurring in ~30% (6/21) of patients (Fig 2B). One patient had gastrinomas, and no patients had glucagonomas or vasoactive intestinal peptidomas (VIPomas). MRI, CT, and EUS were used as the initial imaging modality for diagnosis in ~50% (11/21), ~25% (5/21), and ~25% (5/21) of patients, respectively.

Insulinomas, which were found at a median age of 15.5 years (range = 6-18 years, n = 8), and were the first manifestation of MEN1 in >10% (6/56) of patients, were associated with neuroglycopenic symptoms, which included loss of consciousness in 1 patient and seizures in another patient (Table 4). Diagnosis was established by demonstrating hypoglycemia and hyperinsulinemia during a 72-hour fast in 75% (6/8) of patients. Insulinoma occurrence was similar in males and females (17% males *vs* 14% females, *P* = .99). In >85% (7/8) of patients, the insulinomas were located in the pancreatic body/tail, while the remaining patient had multifocal insulinomas in the pancreatic head. Surgical resection by distal pancreatectomy (n = 7) or completion pancreatic head resection (n = 1), at median age 16 years (range = 6-19 years) resulted in successful resolution of hypoglycemia without recurrence in all patients (median follow-up = 101 months, range = 14-293 months) (additional information provided in the Supplementary Text (available online only)).

NF-PNETs, which were detected at a median age of 15 years (range = 10-18 years, n = 15) and were the first detected MEN1 manifestation in >15% (9/56) of patients (Table 5), occurred as solitary lesions in ~55% (8/15) of patients (median size = 9 mm, range = 4-38 mm) and as multifocal lesions in ~45% (7 /15) of patients (median size of the largest tumor 10 mm, range = 7-17 mm). NF-PNET occurrence was similar in males and females (27.8% males *vs* 25% females, *P* = .99). In ~75% (11/15) of patients, NF-PNETs were located in the pancreatic body/tail, and in the remaining ~25% (4/15) of patients they were located in the head/uncinate process. Approximately 75% (8/11) of patients had tumor growth ≥2 mm, by a median follow-up of 81 months (range = 13-125 months) (Fig 2D). Distal pancreatectomy was performed

in 33% (5/15) of patients, all of whom had NF-PNETs that were >20 mm in diameter at diagnosis or during follow-up (median age at surgery = 16 years, range = 12-20 years). The remaining 10/15 patients had tumors that did not exceed the 20 mm threshold during follow-up**.** Surgery was not followed by recurrence in 3 patients, but 2 patients developed new tumors in the pancreatic remnant (median follow-up = 72.5 months, range = 40-135 months). Three patients developed metastases, with lymph node metastasis occurring in 1 patient with insulinoma, and hepatic metastases occurring in 2 patients with NF-PNETs, both of whom also had metachronous functioning PD-NETs (Table 4) (additional information provided in the Supplementary Text (available online only)).

Pituitary tumors

Pituitary tumors occurred in >30% (18/56) of patients at ≤18 years of age and were the first detected MEN1 lesion in ~30% (16/56) of patients (median age of pituitary adenoma diagnosis = 14 years, range = 9-18 years) (Table 6). Pituitary tumor occurrence was similar in males and females (26% males *vs* 35% females, $P = .560$). Clinical symptoms included amenorrhea/galactorrhea in >45% (6/13) of female patients, and headaches in 17% (3/18), visual field defects in >10% (2/18), and delayed puberty in >5% (1/18) of all patients, with ~45% (8/18) remaining asymptomatic. Prolactinomas were found in ~85% (15/18) of patients and non-functioning adenomas were found in ~15% (3/18) of patients. Somatotrophinomas or corticotrophinomas were not found in any patients. Over 66% (12/18) of patients had microadenomas, while the remaining ~35% (6/18) of patients (1 male and 5 females) had macroadenomas (median size = 15 mm, range = 12-55 mm), all of which were prolactinomas.

Treatment was required in ~70% (12/18) of patients and of the remaining ~30% (6/18) who did not require treatment, all had microadenomas (3 prolactinomas and 3 non-functional adenomas) that remained stable during a median follow-up of 61 months (range = 23-135 months). In 80% (12/15) of patients with prolactinomas, dopamine agonists were used as first-line therapy, and were successful in >80% (10/12). The 2 patients who failed dopamine agonist therapy had macroprolactinomas and comprised: a male diagnosed with a 55 mm prolactinoma at age 11 years,[15] that initially responded to cabergoline, but

subsequently developed resistance and required 2 transcranial debulking procedures (at 12 years of age) and proton beam irradiation (at 13 years of age); and a female diagnosed with a 13 mm prolactinoma at 16 years of age, who experienced persistent amenorrhea and galactorrhea despite cabergoline treatment and underwent successful transsphenoidal resection at 26 years of age. In another female patient, who presented with bitemporal hemianopia and amenorrhea at 14 years of age due to a macroprolactinoma (33 mm), transsphenoidal surgery was used as first-line therapy; she remained disease-free at 12 months follow-up.

Adrenal tumors

A 20 mm non-functioning left adrenal adenoma was detected as an incidentaloma on MRI in 1 female patient at 10 years of age, who was being investigated for an insulinoma (Table 4). Repeat MRI 6 months later showed the mass to have increased in size to 25 mm, and open adrenalectomy was therefore performed at the same time that the patient underwent a laparotomy and hepatic wedge resection for a metastatic NF-PNET. Pathological assessment showed a benign adrenal adenoma.

***MEN1* genetic analysis and genotype-phenotype correlations**

Genetic analysis, which was performed in 96% (77/80) of the overall cohort, revealed 38 different pathogenic germline *MEN1* variants, which occurred throughout the length of the *MEN1* gene, with >70% (27/38) of these occurring in patients who developed an MEN1 manifestation by ≤18 years of age (Fig 3 and Supplementary Table 1 (available online only)). The majority of these pathogenic variants (~70%, 26/38) are predicted to result in a loss of function through premature truncation of the encoded menin protein (i.e. nonsense variants (>25%, 10/38); frameshift variants (>30%, 12/38) with predicted premature truncations; splice site variants (~8%, 3/38); and exonic deletion (~3%, 1/38)). The remaining ~30%, which are predicted in-frame variants (~5%, 2/38) and missense variants (>25%, 10/38), are also likely to disrupt menin function. Three of these pathogenic variants have not been previously reported (Fig 3). Ten variants occurred in members of ≥2 unrelated families, and the remaining 28 variants each occurred in only 1 family, 6 of which had multiple affected members (Supplementary Table 1 (available online only)). Menin, which

is a scaffold protein, has roles in regulating transcription, genome stability, cell division, and proliferation through direct associations with interacting protein partners, such as the transcription factor JunD and checkpoint kinase 1 (CHES1).[12,13] Moreover, *MEN1* variants affecting the menin-JunD interacting domains (i.e. codons 1-40, 139-242, and 323-428) are reported to be associated with an increased risk of premature death,[12] while those involving the menin-CHES1 interaction domain (i.e. codons 428-610) are reported to be associated with a higher frequency of aggressive PD-NETs and death.[13] An examination for such correlations revealed a higher occurrence of *MEN1* variants affecting the menin-JunD interaction domain in patients developing PD-NETs at ≤18 years of age, compared to those without PD-NETs (80% *vs* 51.9%; *P* =.046) (Table 7). Other statistically significant genotype-phenotype correlations were not observed.

DISCUSSION

Our study demonstrates that morbidity from MEN1-related manifestations occured during childhood and adolescence in 70% of patients, and that PD-NETs, which developed in ~35% of patients at ≤18 years of age, may be metastatic in ~15% of cases. Furthermore, pituitary tumors occurred in >30% of these children and adolescents with MEN1 and ~35% of these were macroprolactinomas. These findings are of clinical importance because enteropancreatic NETs are the leading cause of mortality and reduced life-expectancy in patients with MEN1;[16] and pituitary macroadenomas are an important cause of morbidity due to local compressive effects, such as visual loss, and systemic effects from alterations in hormone secretion. Thus, our study underscores the importance of establishing an early genetic diagnosis of MEN1 with timely initiation of surveillance screening in childhood. Other studies have reported that screening in children with MEN1 is associated with diminished health-related quality of life (HR-QOL) because of the burden of frequent physician visits and life-long surveillance,[17] and that screening tests could be postponed until the age of 16 years.[4] However ~50% of the 80 patients in our study developed manifestations at ≤16 years of age, some of which were life-threatening, metastases, and/or required surgical intervention, and we would therefore caution against delaying screening until the end of adolescence.

Our observed ~35% penetrance of PD-NETs at ≤18 years of age is comparable to a study that showed a penetrance of >40% by 20 years of age in 19 patients with MEN1 who also underwent screening using a combination of MRI/CT and/or EUS,[6] but contrasts with the <5%[9] and <15%[10] reported from 2 other studies of patients aged <21 and <22 years, respectively (Table 1). The lower penetrances in the latter 2 studies may be partly explained by the limitations of the imaging modalities that were used, as ~66% of patients in one study had been screened prior to 1999, and before the advent of modern MRI technology and high-resolution EUS,[9] and in the other study, patients were screened only with transabdominal US, which has limited sensitivity for the detection of pancreatic lesions, when compared to MRI, CT, and EUS.[18]

Our finding that a third of pituitary adenomas were macroadenomas at diagnosis is consistent with other studies describing MEN1-related pituitary tumors in patients of all ages.[19,20] All macroadenomas in our study were prolactinomas, and this differs from findings in adults with MEN1, in whom microprolactinomas are more likely.[19] Moreover, >80% of patients with macroadenomas in our study were female, and this contrasts with the occurrences of sporadic macroprolactinomas in children and adolescents, which are more common in males.[21] Furthermore, ~50% of patients with macroadenomas did not respond to medical therapy and required surgical treatment. This is consistent with a report of pituitary adenomas in MEN1 patients, compared to non-MEN1 pituitary tumors, being less responsive to medical therapy and more aggressive,[22] although another study, which excluded pediatric patients, reported that MEN1-related prolactinomas rarely required surgery.[20] Further studies are required to determine the underlying differences in the biological behavior of MEN1-related pituitary tumors in children and adolescents.

PHPT occurred in >80% of patients who had an MEN1 manifestation at ≤18 years of age, with >70% having parathyroidectomy, in which <SPTX was associated with significantly worse disease-free outcome and a higher incidence of reoperations compared to SPTX or TPTX. Although current clinical practice guidelines generally recommend SPTX or TPTX (with or without auto-transplantation),[1] some groups recommend <SPTX in selected MEN1 patients, e.g. those with localized disease on preoperative imaging, as a treatment that provides an acceptable outcome while obviating the risk of permanent hypoparathyroidism.[23] However, the proposed benefits of <SPTX are negated by the increased incidence

of persistent or recurrent disease and reoperations, which pose a higher risk of complications.[23] Notably, of the 3 patients who developed permanent postoperative hypoparathyroidism in our cohort, 2 underwent <SPTX as their initial procedure, followed by reoperative parathyroidectomy due to recurrent disease, and 1 patient underwent TPTX as their only procedure. In contrast, none of the 14 patients who underwent SPTX developed hypoparathyroidism and only 3 of these patients had PHPT persistence or recurrence. Thus, our findings suggest that <SPTX should be avoided in children and adolescents with MEN1-related PHPT. However, the small number of patients who underwent TPTX (n = 3) may have led to an underestimation of the risk of hypoparathyroidism in the SPTX/TPTX group, and precluded statistical comparisons between subgroups.

Our genetic analyses identified 38 pathogenic *MEN1* germline variants, of which 3 are previously unreported, and a correlation between variants located at the menin domains that interact with JunD and the occurrence of PD-NETs at ≤18 years of age. An association between pathogenic variants affecting the menin-JunD interaction domain and a higher risk of death secondary to MEN1-related tumors, the majority of which were PD-NETs, has been reported by one study,[12] although another study of patients with PD-NETs did not find such an association.[13] Further such studies may help to elucidate the roles, if any, of such genotype-phenotype correlations in patients with MEN1.

Our study has several limitations, which include: its retrospective nature, which may have introduced bias because of ascertaining historical data that may be incomplete; selecting ≤35 years of age for study inclusion, that may have missed identifying some patients who had documented MEN1 manifestations in childhood or adolescence; and having ~33% of patients who did not have prospective screening prior to the development of their first manifestation. Nevertheless, our study provides a detailed overview of MEN1 manifestations and treatment outcomes in children and adolescents.

In conclusion, 70% of children and adolescents with MEN1 develop parathyroid, pancreatic, and pituitary tumors. These findings have implications for the counselling of young MEN1 patients and their families, and underscore the importance of initiating surveillance screening early in childhood.

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CONFLICTS OF INTEREST/DISCLOSURES

The authors have no related conflicts of interest to declare.

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FIGURE LEGENDS

Figure 1. Flow chart of the study population. [a]14 patients had not yet reached 18 years of age at the time of study inclusion.

MEN1, multiple endocrine neoplasia type 1

Figure 2. (A) Age-related penetrance of MEN1 manifestations in the overall cohort of 80 patients. The dashed vertical line indicates the age threshold of 18 years for pediatric and adolescent patients in this study. **(B)** Age-related penetrance of NF-PNETs, insulinomas, and gastrinomas. New development of insulinomas was not observed in patients above 18 years of age, whereas NF-PNETs continued to develop in patients above 18 years of age, and gastrinomas developed only in those who were 18 years of age and older. **(C)** Kaplan-Meier curves showing disease-free outcome and time to recurrence in 33 patients with PHPT who underwent <SPTX compared to those who had SPTX or TPTX. *P = .005 indicating a statistically significant difference between the survival curves on log-rank test. **(D)** Tumor growth in 11/15 MEN1 patients with NF-PNETs that developed during childhood/adolescence. Approximately 75% (8/11) of patients had tumor growth ≥2 mm, by a median follow-up of 81 months (range = 13-125 months). The horizontal dashed line indicates the 20 mm size threshold that was used as an indication[1] for surgical resection at centers included in this study. In patients with multiple NF-PNETs, the maximal diameter of the largest tumor was plotted. Four out of 15 patients were excluded from this analysis: 2 patients (cases 43 and 24) who underwent surgery at the time of NF-PNET diagnosis; 1 patient (case 44) who had hepatic metastases at the time of diagnosis; and 1 patient (case 79) who had <2 abdominal imaging scans (Supplementary Table 1 (available online only)). At a median follow-up of 81 months (range: 6-137 months), there were no deaths in the group of 15 patients with NF-PNETs. AA, adrenal adenoma; NF-PNET, non-functioning pancreatic neuroendocrine tumor; PA, pituitary adenoma; PD-NET, duodenopancreatic neuroendocrine tumor; PHPT, primary hyperparathyroidism; <SPTX, less than subtotal parathyroidectomy; SPTX, subtotal parathyroidectomy; TPTX, total parathyroidectomy.

Figure 3. Schematic representation of the genomic organisation of the *MEN1* gene.

The human *MEN1* gene consists of 10 exons that span more than 9 kilobases (kb) of genomic DNA and encodes a 610–amino-acid protein, menin. The 1.83-kb coding region (open boxes) is organized into 9 exons (exons 2 to 10) and 8 introns (indicated by a line but not to scale). The sizes of the exons (boxes) range from 41 to 1297 base pairs (bp), and that of the introns range from 80 to 1564 bp. The start (ATG) and stop (TGA) codons in exons 2 and 10, respectively, are indicated. Exon 1, the 5' part of exon 2, and the 3' part of exon 10 are untranslated (grey boxes). The sites of 38 germline variants which were found in the 60 unrelated families (Supplementary Table 1 available online only) are represented with the affected codon number(s) shown. The locations of the predicted mutations are reported with reference to the transcript encoding the 610 amino acid menin protein (*MEN1* reference sequence NM_130799 using Human Genome Variation Society (HGVS) nomenclature). These 38 germline variants are predicted to be pathogenic and comprise: 10 nonsense mutations; 12 frameshift variants with predicted premature truncations that resulted from 3 intragenic duplications (dup), 8 deletions (del), or 1 insertion of nucleotides; 2 donor and 1 acceptor splice site mutations; 1 large deletion of exon 2; 1 in-frame intragenic duplication (dup) or 1 in-frame deletion (del) of nucleotides; and 10 missense variants. Three of these variants have not been previously reported, and these novel variants are indicated by an asterisk. Menin, a scaffold protein, interacts with a large number of proteins and 2 of these are the transcription factor JunD and checkpoint kinase 1 (CHES1), and the domains of menin that are involved in interactions with these proteins are shown in the lower-most past of the figure. A correlation between pathogenic variants located at the menin domains that interact with JunD and the occurrence of PD-NETs at ≤18 years of age was found, but other genotype-phenotype correlations were not observed (Table 7). Variants that occurred: in patients who developed an MEN1 manifestation by ≤18 years of age, are shown in italics; and in more than one patient are indicated in superscript ([a] = variant occurred in 1 patient, [b] = variant occurred in 2 patients; [c] = variant occurred in 3 patients; [d] = variant occurred in 4 patients, and [e] = variant occurred in 7 patients). †The splice site variant c.784-9G>A has been shown experimentally to result in a frame shift (p.Lys262fsX10) and menin protein

truncation.[24] ‡The splice site variant c.912+1G>A has been shown experimentally to result in a frameshift (p.Tyr276fsX63) and menin protein truncation (Supplementary Table 1 available online only).[25]

Table 1. Studies in the literature (including the current study) that describe pediatric and adolescent MEN1 cohorts

Study (reference in parenthesis)	Newey et al. (2009)[5]	Gonçalvez et al. (2014)[6]	Goudet et al. (2015)[9]	Manoharan et al. (2017)[4]	Vannucci et al. (2018)[8]	Marini et al. (2018)[7]	Herath et al. (2019)[10]	Current study (2021)
Country/countries	UK	Brazil	France, Belgium	Germany	Italy	Italy	Australia	UK, USA, Austria
Patients (n=)[a]	12	8	924	166	22	19	180	80
Upper age limit for pediatric and adolescent group	19 yrs	19 yrs	20 yrs	18 yrs	31 yrs	19 yrs	21 yrs	18 yrs
Patients with MEN1 manifestation below upper age limit (n=)[b]	5 (42%)	8[c]	160 (17%)	20 (12%)	12 (55%)	19[e]	53 (29%)	56 (70%)
Patients with PHPT (n=)[c]	4 (80%)	N/A	122 (76%)	9 (45%)	11 (92%)	19[e]	42 (79%)	46 (82%)
Patients with NF-PNET (n=)[c]	2 (40%)	8[d]	14 (9%)	3 (15%)	2 (17%)	N/A	9 (17%)	15 (27%)
Patients with insulinoma (n=)[c]	0	2[d]	20 (13%)	5 (25%)	0	N/A	3 (6%)	8 (14%)
Patients with PA (n=)[c]	1 (20%)	N/A	55 (34%)	6 (30%)	7 (58%)	N/A	13 (25%)	18 (32%)
Patients with other tumors (n=)[c]	0	N/A	Gastrinoma (n = 3, 2%) ACC (n = 2, 1%) Thymic NET (n = 1, <1%)	Bronchial NET (n = 1, 5%)	0	N/A	Bronchial NET (n = 1, 2%)	Gastrinoma (n = 1, 2%) AA (n = 1, 2%)
Patients without any manifestations below upper age limit (n=)[b]	7 (58%)	N/A	764 (83%)	146 (88%)	10 (45%)	N/A	127 (71%)	24 (30%)

[a]Total includes MEN1 patients of all ages.
[b]Percentage refers to total number of patients (with and without manifestations) included in study.
[c]Percentage refers to total number of patients who developed an MEN1 manifestation before the pediatric/adolescent upper age limit.
[d]Study only described patients with NF-PNETs and insulinomas so percentages not reported.
[e]Study only included patients with PHPT so percentages not reported.
AA, adrenal adenoma; ACC, adrenocortical carcinoma; MEN1, multiple endocrine neoplasia type 1; N/A, not applicable; NET, neuroendocrine tumor; NF-PNET, non-functioning pancreatic NET; PA, pituitary adenoma; PHPT, primary hyperparathyroidism; UK = United Kingdom; USA = United States of America

Table 2. Screening protocol and definitions of MEN1 manifestations[a]

Surveillance screening protocol[b]	• Annual measurement of serum calcium, PTH, PD-NET markers (gastrin, glucagon, pancreatic polypeptide, and chromogranin A), and pituitary tumor markers (prolactin and IGF-1) from the ages of 5 to 10 years. • Pituitary-directed head MRI every 2-3 years beginning from the age of 5-10 years old. • Annual abdominal MRI/CT and/or EUS from the age of 10 years old. • Chest CT every 1-2 years, from 15 years of age.
PHPT	• Defined as a concomitant elevation in serum calcium with an elevated or inappropriately normal PTH level. • Persistent PHPT: defined as hypercalcemia with raised or inappropriately normal PTH levels within 6 months of surgery. • Recurrent PHPT: defined as a return of hypercalcemia with raised or inappropriately normal PTH levels after a 6-month period of eucalcemia.
PD-NETs	• Diagnosed based on histology (if available) and/or detection of ≥1 lesion on two more sequential abdominal imaging studies. • Insulinoma: diagnosis established in the setting of an inappropriately high serum insulin or C-peptide concentration during a spontaneous or induced episode of hypoglycemia. • NF-PNET: diagnosed in the absence of a clinical syndrome of hormone hypersecretion. • Gastrinoma: diagnosed when fasting hypergastrinemia was present with confirmed positive immunohistochemical staining for gastrin.

Pituitary adenomas	• Diagnosed if a lesion was detected on pituitary-directed MRI on at least two sequential imaging studies.
	  ○ Macroadenoma: defined as a pituitary adenoma > 1 cm in maximal dimension
	  ○ Microadenoma: defined as a pituitary adenoma < 1 cm in maximimal dimension.
	• Prolactinoma: the presence of an MRI-identifiable pituitary tumor with an elevated serum prolactin (macroprolactin excluded)
	• Non-functioning pituitary adenoma: the presence of an MRI-identifiable pituitary tumor in the absence of biochemical evidence of hormonal hypersecretion.
	• Somatotrophinoma: the presence of an MRI-identifiable pituitary tumor with elevated serum IGF-1 levels and/or an OGTT showing lack of growth hormone suppression.
	• Corticotrophinomas: the presence of an MRI-identifiable pituitary tumor with biochemical evidence of hypercortisolism and suppression of corticotrophin on a high dose dexamethasone suppression test.

Adrenal tumors	• Present on ≥2 consecutive imaging studies and/or on histology (if available). • Functionality was determined by biochemical testing (e.g. plasma renin and aldosterone concentrations, low dose dexamethasone suppression test, urinary catecholamines and/or metanephrines)

Bronchial carcinoids	• Present on ≥2 consecutive imaging studies, and/or on histology (if available).
Thymic carcinoids	• Present on ≥2 consecutive imaging studies, and/or on histology (if available).
Malignancy	• Presence of involved lymph nodes or distant metastasis seen on imaging and/or on histopathology.
Cutaneous manifestations	• Diagnosed based on physical examination for subcutaneous lipomas, facial collagenomas, and andgiofibromas

[a]Adapted from current MEN1 clinical practice guidelines by Thakker *et al.*[1]

[b]Surveillance was initiated from the age of 5-10 years in patients for whom a genetic diagnosis of MEN1 was made prior to the onset of any MEN1 manifestations. All 5 centers followed these surveillance protocols, with minor variations in use of abdominal imaging modalities; thus, 1 center utilized both CT and endoscopic ultrasound EUS, while the other centers used MRI +/- EUS.

CT, computed tomography; EUS, endoscopic ultrasound; IGF-1, insulin-like growth factor 1; MEN1, multiple endocrine neoplasia type 1; MRI, magnetic resonance imaging; NF-PNET; non-functioning pancreatic neuroendocrine tumor; OGTT, oral glucose tolerance test; PD-NET: pancreaticoduodenal neuroendocrine tumor; PHPT, primary hyperparathyroidism; PRL prolactinoma; PTH, parathyroid hormone

Table 3. Details of 56 MEN1 patients with manifestations detected at ≤ 18 years of age

	Overall (n = 56)	**Male (n = 19)**	**Female (n = 37)**
Median age at MEN1 diagnosis, yrs [range]	13 [2 – 18]	11 [6 – 18]	13 [2 – 17]
Median age at first MEN1 manifestation, yrs [range]	14 [6 – 18]	14 [6 – 18]	14.5 [6 – 18]
Median age at last follow-up, years [range][a]	22.5 [6 – 35]	24 [6 – 35]	22 [6 – 34]
Index case	4 (7 %)	3 (16%)	1 (%)
Genetic testing performed	53 (95%)	17 (90%)	36 (97%)
MEN1 screening started before first manifestation detected	38 (68%)	10 (53%)	28 (76%)
Initial MEN1 manifestation(s) detected			
PHPT only	29 (52%)	9 (47%)	20 (54%)
Pituitary adenoma only	12 (21%)	3 (16%)	9 (24%)
NF-PNET only	5 (9%)	1 (6%)	4 (11%)
Insulinoma only	3 (5%)	0	3 (8%)
PHPT + pituitary adenoma	2 (4%)	1 (5%)	1 (3%)
PHPT + NF-PNET	2 (4%)	2 (11%)	0
PHPT + insulinoma	2 (4%)	2 (11%)	0
Pituitary adenoma + NF-PNET	2 (4%)	1 (5%)	1 (3%)
PHPT ≤ 18 years old	46 (82%)	17 (90%)	29 (78%)
PD-NET ≤ 18 years old			
NF-PNET	15 (27%)	5 (28%)	10 (27%)
Insulinoma	8 (14%)	3 (17%)	5 (13%)
Gastrinoma	1 (2%)	0	1 (2%)
PA ≤ 18 years old	18 (32%)	5 (36%)	13 (54%)
Adrenal tumor ≤ 18 years old	1 (2%)	0	1 (2%)

[a]No deaths occurred during the follow-up period. MEN1, multiple endocrine neoplasia type 1; NF-PNET, non-functioning pancreatic neuroendocrine tumor; PA, pituitary adenoma; PD-NET, pancreaticoduodenal neuroendocrine tumor; PHPT, primary hyperparathyroidism

Table 4. Characteristics and treatment outcomes of 8 patients with insulinomas at ≤18 years of age

Case ID	Sex	Germline pathogenic variant[a]	Age at diagnosis of insulinoma	Other MEN1 features ≤ 18 years old (age, years)	Tumors on imaging (n =)	Size of largest tumor on imaging, mm	Location of largest tumor	Disease extent at diagnosis	Type of surgery	Tumors on pathology (n =)	Grade, stage, and size of largest insulin-positive tumor	Status at last F/U	Duration of F/U, months
36	F	c.421C>T; p.Gln141X	15	PHPT (18 yrs)	1	60	Tail	Localised	DP	Unknown	Unknown	No recurrence of hypoglycaemia	158
38	F	Unknown	18	PHPT (18 yrs)	2	11	Body	Localised	DP	9	G2, T1N0M0 (18 mm)	No recurrence of hypoglycaemia	71
43	F	c.1045C>T; p.Gln349X	18	NF-PNET (12 yrs), PRL (12 yrs), GA (18 yrs)	3	5	Head	Localised	CP	7	G1, T1N0M0 (15mm)	No recurrence of hypoglycaemia New liver mets from NF-PNET (age 24)	137
44	F	c.1045C>T; p.Gln349X	10	NF-PNET (10 yrs) PRL (10 yrs) AA (10 yrs)	2	13	Tail	Solitary liver met from NF-PNET	DP	2	G2, T1N0M0[b] (17mm)	No recurrence of hypoglycaemia Progresion of liver mets from NF-PNET (age 13)	45
61	M	c.549G>A; p.Trp183X	10	None	1	10	Tail	Localised	DP	Unknown	Unknown	No recurrence of hypoglycaemia New NF-PNETs (x2) in remnant (age 24): stable at last F/U	293
68	M	c.1204_1208del; p.Ser402fsX5	6	PHPT (6 yrs)	3	4	Body	Localised	DP	25 (micro-adenomatosis)	G1, T1N0M0 (4.1mm)	No recurrence of hypoglycaemia	14
71	M	c.1075_1077del; p.Glu359del	18	PHPT (18 yrs)	1	20	Tail	Localised	DP	1	G1, T1N1M0 (6mm)	No recurrence of hypoglycaemia	106
75	F	Unknown	16	PHPT (17 yrs)	2	21	Tail	Localised	DP	2	G2, T1N0M0 (21mm)	No recurrence of hypoglycaemia	96

[a]Further details of germline variants are provided in Figure 3 and in Supplementary Table 1 (available online only).

[b]Classified as "M0" as liver lesion thought to represent mestastasis from synchronous NF-PNET, not insulinoma.

AA, adrenal adenoma; DP, distal pancreatectomy; CP; completion pancreatectomy; F, female; F/U, follow-up; G, grade; GA, gastrinoma; M, male; MEN1, multiple endocrine neoplasia type 1; NF-PNET, non-functioning pancreatic neuroendocrine tumor; PHPT, primary hyperparathyroidism; PRL prolactinoma;

Table 5. Characteristics and treatment outcomes of 15 patients diagnosed with NF-PNETs at ≤18 years old

	NF-PNET ≤ 18 years (n = 15)
Age at MEN1 diagnosis, years, median [range]	14 [6 – 17]
Sex, female	9 (64%)
Age at last follow-up, median [range]	22 [16 – 27]
Age at NF-PNET diagnosis, years, median [range]	15.5 [12 – 18]
Imaging modality for diagnosis	
CT	2 (13%)
MRI	10 (67%)
EUS	3 (20%)
Multifocality	7 (47%)
Number of tumors on imaging, median [range]	1 [1 – 6]
Size of largest tumor at diagnosis, mm, median [range]	9 [4 - 38]
Location of largest tumor on imaging	
Head/uncinate	4 (27%)
Body/tail	11 (73%)
Metastasis at time of diagnosis	1 (7%)
Surgical resection	5 (36%)
Age at surgery, median [range]	16 [12-20]
Number of tumors on pathology, median [range][a]	2 [1 – 5]
Size of largest tumor on pathology, mm, median [range][a]	22 [18 – 40]
Tumor grade	
G1	1 (20%)
G2	2 (40%)
G3	1 (20%)
Missing	1 (20%)
Positive lymph nodes on pathology	0
Recurrence in pancreatic remnant after surgery	2 (40%)
Development of metastasis during follow-up	1
Duration of follow-up, months, median [range]	79 [6-131]

[a]Pathology data were missing for 1 patient.

CT, computed tomography; EUS, endoscopic ultrasound; G, grade; MRI, magnetic resonance imaging; NF-PNET, non-functioning pancreatic neuroendocrine tumor

Table 6. Characteristics of 18 patients diagnosed with pituitary adenomas at ≤ 18 years of age

Case ID	Sex	Germline pathogenic variant[a]	Age at MEN1 diagnosis, years	Age at PA diagnosis, years	Type of PA	Micro or macro-adenoma at diagnosis	Size at diagnosis, mm	Presenting symptoms	Management
9	F	c.1400_1413del; p.Ala467fsX59	5	9	PRL	Microadenoma	4	None	Observation
46	F	c.1008dup; p.Ala337fsX30	6	10	PRL	Microadenoma	7	None	Cabergoline
16	M	c.658T>C; p.Trp220Arg	7	11	NF	Microadenoma	5	None	Observation
6	F	c.503T>C; p.Leu168Pro	2	11[b]	PRL	Macroadenoma	12	None	Cabergoline
63	M	c.784-9G>A; p.Lys262fsX10	11	11[c]	PRL	Macroadenoma	55	BH, headaches	Cabergoline, debulking surgery x2, adjuvant proton beam irradiation
48	F	c.1204_1208del; p.Ser402fsX5	6	12	PRL	Microadenoma	9	Galactorrhea	Cabergoline
43	F	c.1045C>T; p.Gln349X	8	12	PRL	Microadenoma	6	None	Cabergoline
74	F	c.1546dup; p.Arg516fsX15	15	14[d]	PRL	Macroadenoma	17	Delayed puberty	Cabergoline
22	M	Unknown[e]	14	14	NF	Microadenoma	9	None	Observation
23	F	Unknown[f]	14	14[d]	PRL	Macroadenoma	33	BH, amenorrhoea	Tran-sphenoidal surgery
34	F	c.668T>C; p.Leu223Pro[g]	14	15	PRL	Microadenoma	6	None	Bromocriptine
14	M	c.770_775dup; p.Leu259_Gln260dup	15	15	PRL	Microadenoma	7	Headache	Observation
29	F	Unknown[e]	16	16	PRL	Microadenoma	5	Galactorrhea	Cabergoline
25	F	c.654+3A>G	16	16[d]	PRL	Macroadenoma	13	Amenorrhea	Cabergoline, trans-sphenoidal surgery
51	F	c.668T>C; p.Leu223Pro[g]	15	17[b]	PRL	Macroadenoma	12	None	Cabergoline
31	F	c.1243C>T; p.Arg415X	7	18	PRL	Microadenoma	7	None	Bromocriptine
78	F	Unknown[h]	10	18	NF	Microadenoma	4	None	Obervation
37	M	Unknown[h]	8	18	PRL	Microadenoma	4	None	Observation

[a]Further details of germline variants are provided in Figure 3 and in Supplementary Table 1 (available online only).

[b]Patient was enrolled in an MEN1 surveillance screening program before macroadenoma was detected.

[c]Patient is an index case who had not undergone prior screening for MEN1 manifestations before the diagnosis of macroprolactinoma was made.

[d]Patient had a family history of MEN1, but had not yet commenced screening at the time that the macroadenoma was detected.

[e]Germline variant unknown as family declined genetic testing.

[f]Patient's first-degree relative tested positive for *MEN1* germline pathogenic variant, but specific variant unknown.

[g]Unrelated patients with an identical genotype (e.g. c.668T>C; p.Leu223Pro) noted to have variable aggressiveness of pituitary tumors.

[h]Patient tested positive for *MEN1* germline pathogenic variant but specific variant unknown.

BH, bitemporal hemianopsia; F, female; M, male; NF, non-functioning; PRL, prolactinoma

Table 7. Frequency of MEN1 manifestations at ≤18 years of age according to genotype[a]

	Truncating variant, yes[b]	Nonsense or frameshift variant in exons 2, 9, 10, yes[c]	Variant affecting JunD interacting domain, yes[d]	Variant affecting CHES1 interacting domain, yes[e]
Any manifestation at ≤18 years				
Yes	30 (68%)	11 (24%)	28 (62%)	7 (16%)
No	14 (58%)	6 (25%)	12 (50%)	4 (17%)
P	.438	.590	.234	.579
PHPT at ≤18 years				
Yes	26 (70%)	11 (29%)	22 (60%)	7 (19%)
No	18 (58%)	6 (19%)	28 (67%)	4 (13%)
P	.320	.220	.334	.348
PD-NET at ≤18 years				
Yes	10 (67%)	3 (20%)	12 (80%)	0
No	34 (64%)	14 (2%)	28 (52%)	20 (20%)
P	.999	.461	**.046**	.105
PA at ≤18 years				
Yes	7 (58%)	3 (23%)	8 (62%)	2 (15%)
No	37 (66%)	14 (25%)	32 (57%)	9 (16%)
P	.741	.598	.513	.660

[a]Patients who did not undergo genetic testing (n = 3) or did not have information available regarding their specific *MEN1* germline pathogenic variant (n = 8) were excluded.

[b]Truncating germline variants were defined as nonsense, frame shifts, and gross deletions. Nontruncating variants were defined as missense, in-frame deletions, or insertions. The splice site variants c.784-9G>A (n = 3 patients) and c.912+1G>A (n = 1 patient) have been shown experimentally result in menin protein truncation so these patients were included in the truncating variants group.[24,25] The impact of the splice site variant c.654+3A>G (n = 1 patient) on menin protein structure has not been experimentally validated; therefore, this patient was excluded from this analysis.

[c]All known variants included in analysis.

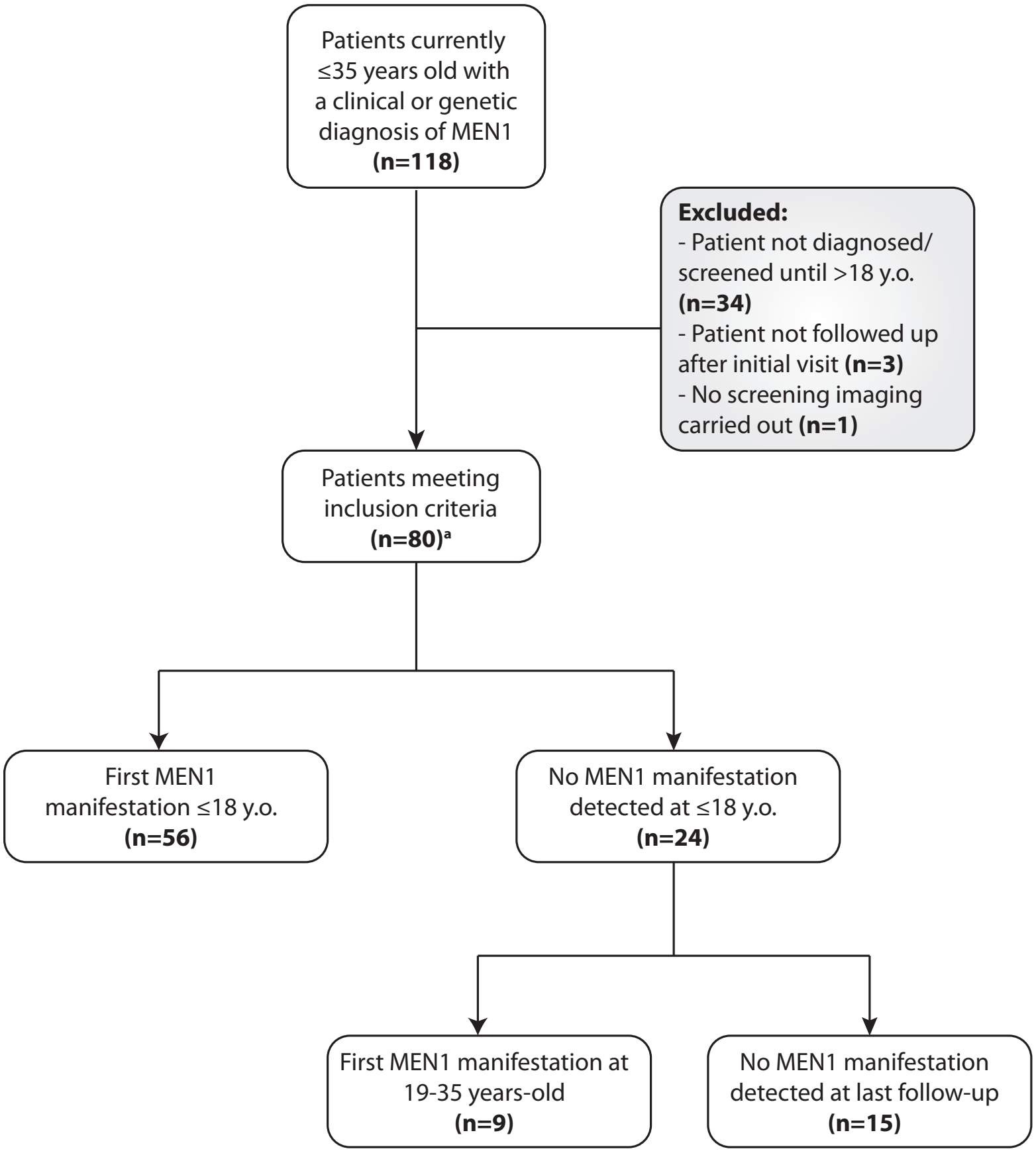
[d]JunD interaction domain: codons 1-40, 139-242, and 323-428

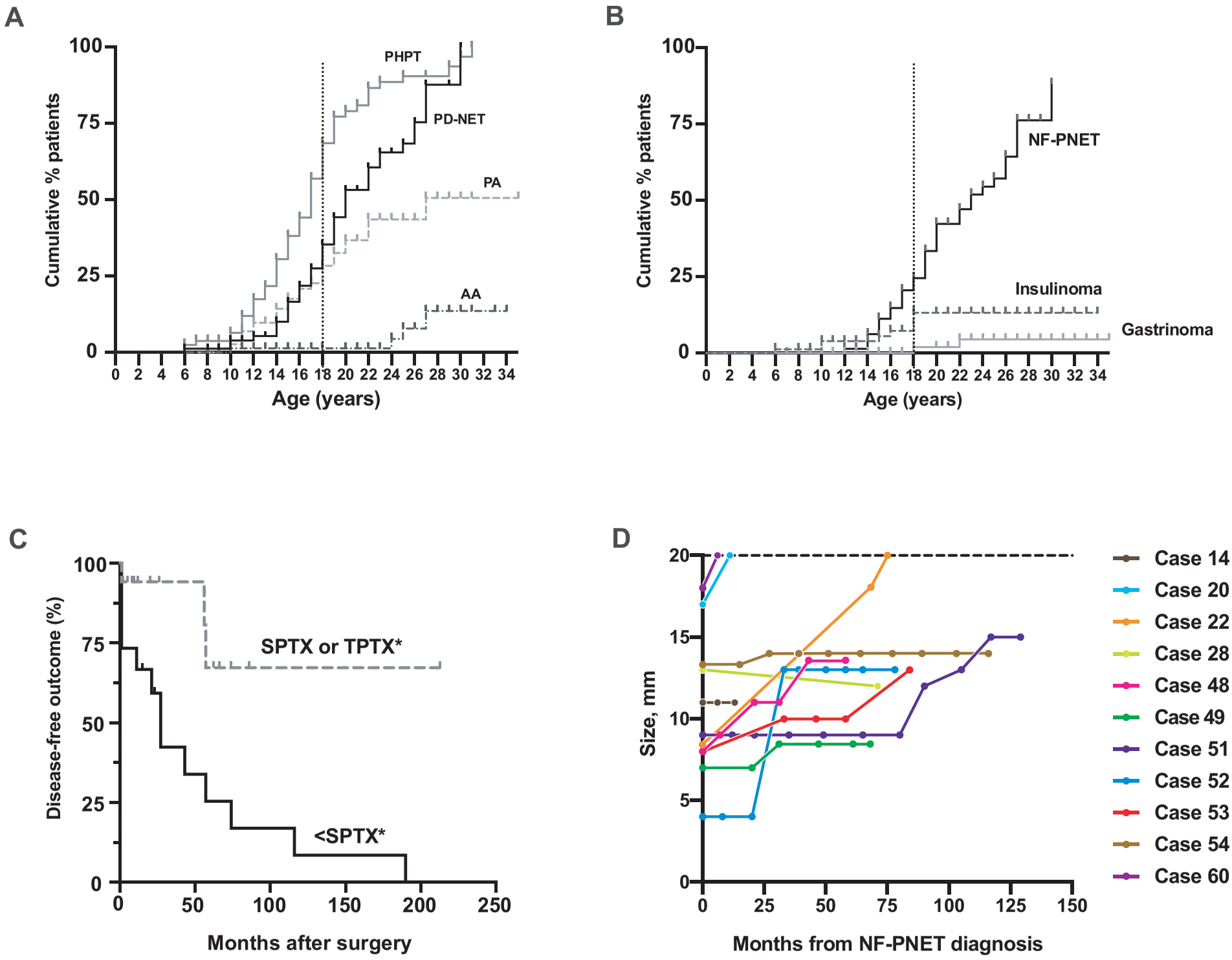
[e]CHES1 interaction domain: codons 428–610

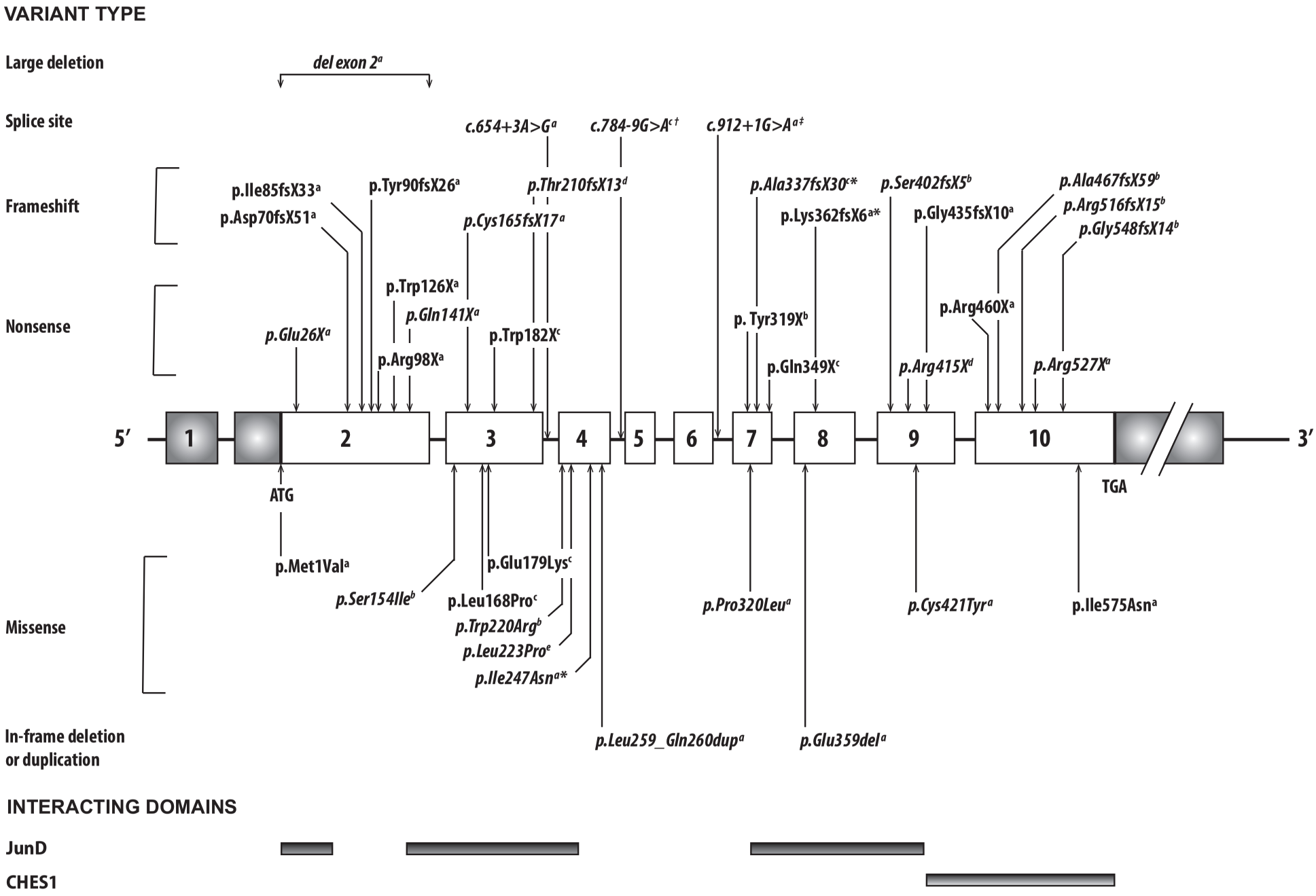
MEN1, multiple endocrine neoplasia type 1; PA, pituitary adenoma; PD-NET, pancreaticoduodenal neuroendocrine tumor; PHPT, primary hyperparathyroidism

Figure 1

Click here to access/download;Figure;Figure 1.eps







SUPPLEMENTARY MATERIAL – Available online only

Multiple Endocrine Neoplasia Type 1 in Children and Adolescents: Clinical Features and Treatment Outcomes

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SUPPLEMENTARY RESULTS

Case histories of 3 patients with metastatic pancreatic neuroendocrine tumors (PNETs)

SUPPLEMENTARY MATERIAL – Available online only

Case 43: hepatic metastases

This patient had a family history of MEN1, and underwent genetic testing at 8 years of age, which revealed a nonsense pathogenic *MEN1* variant (c.1045C>T; p.Gln349X). At 12 years of age, she remained asymptomatic with no biochemical abnormalities. However, MRI of the abdomen revealed a 38 mm mass in the distal body of the pancreas. Distal pancreatectomy was performed, and histopathology revealed this to be a well-differentiated grade 2 (G2) tumor. She remained clinically well until the age of 18 years, when she developed diarrhea, weight loss, and abdominal pain. Biochemical evaluation revealed a fasting serum gastrin level of >500 pmol/L (normal <84 pmol/L), and MRI showed six new lesions (2 mm – 11 mm in diameter) in the duodenal wall which were suspicious for gastrinomas. Despite maximal proton pump inhibitor therapy, her symptoms continued to worsen and she underwent a pancreas-sparing duodenectomy, following which she was eugastrinemic. Histology confirmed multiple well-differentiated G2 lesions (largest 7 mm in diameter) which immunostained for gastrin. Nine months later she presented with symptomatic hypoglycemia, and a 72-hour fast resulted in her developing hypoglycemia with hyperinsulinema, consisitent with a diagnosis of insulinoma. A ^{68}Ga-Exendin-4 PET/CT scan demonstrated intense tracer uptake in the uncinate process, and she underwent a completion pancreatectomy, which resulted in resolution of her symptoms. Histology revealed 7 well-differentiated G1 tumors (6 – 15 mm in diameter) which immunostained for insulin. At 20 years of age, a follow-up MRI scan revealed the prescence of multiple sub-centimeter foci in the right lobe of the liver, some of which were arterially-enhancing. Repeat MRI scans at 21 and 23 years of age showed no change in appearance of these lesions, and no uptake was seen on an ^{111}Octreotide SPECT/CT scan. However, a subsequent MRI at 24 years of age demonstrated an increase in the size and number of the hepatic lesions with new arterial enhancement, thereby raising the suspicion of hepatic metastases, which was confirmed by a DOTATATE PET/MRI that demonstrated heterogeneous uptake. At the most recent follow-up appointment, the patient had been commenced on long-acting somatostatin analog therapy.

Case 44: hepatic metastases

This patient is the sibling of case 43 and was diagnosed with MEN1 at 4 years of age, following predictive genetic testing which showed her to have the same nonsense pathogenic *MEN1* variant (c.1045C>T; p.Gln349X. She presented with hypoglycemic symptoms at 10 years of age and had an abdominal MRI which showed 13 mm and 7 mm lesions in the tail and head of the pancreas, respectively, and also a 13 mm well-circumscribed lesion in hepatic segment VII. Localization tests for the insulinoma were inconclusive, but it was felt that the larger pancreatic tail lesion was the most likely source of insulin hypersecretion, and a distal pancreatectomy was performed, following which hypoglycemic symptoms resolved. The 7 mm pancreatic head lesion was considered to be a non-functioning PNET (NF-PNET) and was not resected, and the 13 mm liver lesion was considered to be a benign hemangioma for which observation was recommended. However, on follow-up MRI 9 months later, the hepatic lesion had increased in size to 20 mm and hepatic wedge resection was performed with histopathology revealing this to be a metastatic G2 well-differentiated NET that had likely originated from the pancreatic head NF-PNET. Four months postoperatively, she remained asymptomatic, but a ^{68}Gallium-DOTATATE PET/CT demonstrated the prescence of new multiple bi-lobar hepatic lesions. She was started on octreotide therapy, but had disease progression and was commenced on peptide receptor radionuclide (PRRT) therapy at 12 years of age. After 4 cycles of PRRT, there appeared to be complete resolution of the lesions; however, at the most recent follow-up DOTATATE scan at 14 years of age there was evidence of disease recurrence with the appearance of four (<1cm) tracer-avid hepatic lesions.

Case 71: lymph node metastasis

This patient was an index MEN1 case, who presented with hypoglycemia at 18 years of age and was found to have a sub-centimeter pancreatic tail insulinoma on abdominal MRI. Genetic testing revealed an in-frame deletion in the *MEN1* gene (c.1075_1077del; p.Glu359del). Distal pancreatectomy was performed and histopathology revealed a well-differentiated G1 tumor with one positive lymph node (T1N1M0). At the most recent follow-up appointment (104 months after surgery) there was no evidence of recurrent or distant metastatic disease.

SUPPLEMENTARY DISCUSSION

Choice of surgical approach in patients with primary hyperparathyroidism (PHPT)

Although current clinical practice guidelines recommend subtotal parathyroidectomy (SPTX) or total parathyroidectomy (TPTX) with autotransplantation as the procedure of choice for MEN1-associated PHPT,[1] some surgeons at centers in our study favored more limited initial resections (either single gland excision or unilateral removal of 2 parathyroid glands; i.e. <SPTX) to mitigate the risk of postoperative hypoparathyroidism. This approach was usually utilized for patients who had preoperative imaging that showed a concordant single or unilateral focus of disease. In total 16 patients had <SPTX, and these comprised: 8 patients who had concordant preoperative sestamibi and ultrasound scans; 3 patients in whom the surgeon was not aware of the diagnosis of MEN1 at the time of surgery; 3 patients who were planned to have a 4-gland exploration, but during which the surgeon made the decision to excise only 1 abnormal-appearing gland while leaving 3 normal-appearing glands in-situ; and 2 patients who had their initial surgery at other centers at which the reasons for <SPTX were not documented."

Choice of surgical approach in patients with insulinomas

MEN1-associated insulinomas, unlike non-MEN1 (sporadic) insulinomas are often multi-centric and difficult to localize preoperatively. Thus, surgical management of MEN1-associated insulinomas is more challenging than that of sporadic insulinomas, which can usually be treated by parenchymal-sparing enucleations. Although enucleation of a small (i.e. <2cm) insulinoma that does not abut the pancreatic duct may be feasible in a MEN1 patient, there is nevertheless a high likelihood of recurrence because multiple tumors may be present. The North American Neuroendocrine Tumor Society (NANETS) surgical consensus guidelines[2] therefore recommend that the decision to perform enucleation versus major pancreatic resection in patients with MEN1 should be made on a case-by-case basis. Based on these guidelines, none of the 8 MEN1 patients with insulinomas in our study had

parenchymal-sparing enucleations beacuse they either had multi-centric tumors (n=4), large (>2cm) tumors (n=2), or a tumor that was in close proximity to the pancreatic duct (n=1).

SUPPLEMENTARY REFERENCES

1. Thakker RV, Newey PJ, Walls GV, et al. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metab.* 2012;97(9):2990-3011.

2. Howe JR, Merchant NB, Conrad C, et al. The North American Neuroendocrine Tumor Society Consensus Paper on the Surgical Management of Pancreatic Neuroendocrine Tumors. *Pancreas.* 2020;49(1):1-33.

Supplementary Table 1. Details of manifestations and germline pathogenic variants in 80 patients with MEN1 from 60 unrelated families

Case ID	Family ID	Sex	Age at MEN1 diagnosis, years	Nucleotide change	Protein change	Variant type	Age at last F/U	Manifestation(s) detected at ≤18 years	Manifestation(s) detected at > 18 years	Duration of F/U (months)
1	1	M	0.9	c.957C>A	p.Tyr319Stop	Nonsense	6	None	N/A	68
2	2	M	0.75	c.503T>C	p.Leu168Pro	Missense	6	None	N/A	71
3	1	M	5	c.957C>A	p.Tyr319Stop	Nonsense	10	None	N/A	65
4	3	F	4	c.668T>C	p.Leu223Pro	Missense	10	None	N/A	70
5	3	F	7	c.668T>C	p.Leu223Pro	Missense	9	None	N/A	14
6	2	F	2	c.503T>C	p.Leu168Pro	Missense	13	Prolactinoma (11 y.o.)	N/A	140
7	4	F	5	c.668T>C	p.Leu223Pro	Missense	13	PHPT (12 y.o.)	N/A	90
8	5	F	5	c.1400_1413del	p.Ala467GlyfsX59	Frameshift	13	None	N/A	93
9	6	F	5	c.1400_1413del	p.Ala467GlyfsX59	Frameshift	11	Prolactinoma (9 y.o.) PHPT (11 y.o.)	N/A	82
10	7	F	13	c.1262G>A	p.Cys421Tyr	Missense	15	PHPT (14 y.o.)	N/A	22
11	8	F	15	c.959C>T	p.Pro320Leu	Missense	16	PHPT (14 y.o.)	N/A	24
12	3	F	6	c.668T>C	p.Leu223Pro	Missense	16	None	N/A	119
13	9	F	10	c.1A>C	p.Met1Val	Missense	12	None	N/A	23
14	10	M	14	c.776_781dup	p.Leu259_Gln260dup	In-frame duplication	17	PHPT (15 y.o.) NF-PNET (15 y.o.) Prolactinoma (15 y.o.)	N/A	23
15	4	F	6	c.668T>C	p.Leu223Pro	Missense	18	PHPT (15 y.o.)	N/A	127
16	11	M	7	c.658T>C	p.Trp220Arg	Missense	18	NFPA (11 y.o.) PHPT (18 y.o.)	N/A	135
17	11	M	14	c.658T>C	p.Trp220Arg	Missense	25	None	None	135
18	12	F	16	c.784-9G>A	p.Lys262fsX10	Acceptor splice site	18	PHPT (15 y.o.)	N/A	15

SUPPLEMENTARY MATERIAL – Available online only

Case ID	Family ID	Sex	Age at MEN1 diagnosis, years	Nucleotide change	Protein change	Variant type	Age at last F/U	Manifestation(s) detected at ≤18 years	Manifestation(s) detected at > 18 years	Duration of F/U (months)
19	13	F	15	c.1724T>A	p.Ile575Asn	Missense	22	None	PHPT (19 y.o.) NF-PNET (19 y.o.) Prolactinoma (22 y.o.)	74
20	14	F	13	Unknown[a]	Unknown	Unknown	16	PHPT (13 y.o.) NF-PNET (15 y.o.)	N/A	37
21	15	M	11	c.1643_1721del	p.Gly548fsX14	Frameshift	23	PHPT (11 y.o.)	NF-PNET (20 y.o.)	150
22	16	M	14	Unknown[b]	Unknown	Unknown	24	PNET (14 y.o.) Prolactinoma (14 y.o.)	None	117
23	17	F	14	Unknown[a]	Unknown	Unknown	15	PHPT (14 y.o.) Prolactioma (14 y.o.)	N/A	12
24	18	F	16	Unknown[a]	Unknown	Unknown	22	PHPT (16 y.o.) NF-PNET (17 y.o.)	Prolactinoma (22 y.o.)	66
25	19	F	16	c.654+3A>G	N/A	Donor splice site	27	Prolactinoma (16 y.o.)	PHPT (19 y.o.) NF-PNET (26 y.o.)	121
26	15	M	13	c.1643_1721del	p.Gly548fsX14	Frameshift	24	PHPT (14 y.o.)	None	124
27	20	F	17	c.1579C>T	p.Arg527X	Nonsense	23	PHPT (18 y.o.)	None	62
28	16	M	17	Unknown[c]	Unknown	Unknown	25	PHPT (17 y.o.) NF-PNET (18 y.o.)	None	92
29	21	F	16	Unknown[c]	Unknown	Unknown	26	Prolactinoma (16 y.o.) PHPT (17 y.o.o)	NFAA (25 y.o.)	109
30	22	F	13	c.495_504del	p.Cys165fsX17	Frameshift	29	PHPT (14 y.o.)	NF-PNET (27 y.o.) ACTH-oma (27 y.o.)	193
31	23	F	18	c.1243C>T	p.Arg415X	Nonsense	31	Prolactinoma (18 y.o.)	PHPT (30 y.o.) NF-PNET (30 y.o.)	144
32	24	M	17	c.912+1G>A	N/A	Donor splice site	30	PHPT (13 y.o.)	GH-oma (19 y.o.) Gastrinoma (22 y.o.) NFAA (30 y.o.)	194
33	25	M	4	c.76G>T	p.Glu26X	Nonsense	32	PHPT (7 y.o.)	NF-PNET (27 y.o.)	306
34	3	F	14	c.668T>C	p.Leu223Pro	Missense	34	Prolactinoma (15 y.o.)	PHPT (19 y.o.) NF-PNET (22 y.o.)	225

SUPPLEMENTARY MATERIAL – Available online only

Case ID	Family ID	Sex	Age at MEN1 diagnosis, years	Nucleotide change	Protein change	Variant type	Age at last F/U	Manifestation(s) detected at ≤18 years	Manifestation(s) detected at > 18 years	Duration of F/U (months)
35	23	M	18	c.1243C>T	p.Arg415X	Nonsense	34	PHPT (10 y.o.)	NF-PNET (19 y.o.) Prolactinoma (20 y.o.)	294
36	26	F	15	c.421C>T	p.Gln141X	Missense	28	Insulinoma (15 y.o.) PHPT (18 y.o.)	None	157
37	27	M	8	Unknown[a]	Unknown	Unknown	20	Prolactinoma (18 y.o.)	None	178
38	28	F	18	Unknown[c]	Unknown	Unknown	25	PHPT (18 y.o.) Insulinoma (19 y.o.)	None	88
39	29	F	14	c.784-9G>A	N/A	Acceptor splice site	25	None	PHPT (20 y.o.) NF-PNET (23 y.o.)	124
40	30	F	11	c.1378C>T	p. Arg460X	Nonsense	27	None	NF-PNET (20 y.o.)	83
41	31	F	17	c. 503T>C	p.Leu168Pro	Missense	33	None	PHPT (19 y.o.) Prolactinoma (20 y.o.) NF-PNET (23 y.o.)	200
42	32	F	17	Unknown[a]	Unknown	Unknown	25	PHPT (18 y.o.)	None	90
43	33	F	8	c.1045C>T	p.Gln349X	Nonsense	23	NF-PNET (12 y.o.) Prolactinoma (12 y.o.) Gastrinoma (18 y.o.) Insulinoma (18 y.o.)	PHPT (19 y.o.)	189
44	33	F	4	c.1045C>T	p.Gln349X	Nonsense	14	Insulinoma (10 y.o.) PHPT (10 y.o.) NFAT (10 y.o.)	N/A	98
45	33	M	5	c.1045C>T	p.Gln349X	Nonsense	8	None	N/A	37
46	34	F	6	c.1008dup	p.Ala337fsX30	Frameshift	13	Prolactinoma (10 y.o.) PHPT (11 y.o.)	N/A	58
47	35	M	2	c.549G>A	p.Trp183X	Nonsense	16	None	N/A	15
48	36	F	6	c.1204_1208del	p.Ser402fsX5	Frameshift	20	Prolactinoma (12 y.o.) PHPT (13 y.o.) NF-PNET (14 y.o.)	None	159
49	37	F	12	c.461G>T	p.Ser154Ile	Missense	22	NF-PNET (15 y.o.) PHPT (16 y.o.)	None	80

SUPPLEMENTARY MATERIAL – Available online only

Case ID	Family ID	Sex	Age at MEN1 diagnosis, years	Nucleotide change	Protein change	Variant type	Age at last F/U	Manifestation(s) detected at ≤18 years	Manifestation(s) detected at > 18 years	Duration of F/U (months)
50	38	F	2	c.202_206dup	p.Asp70fsX51	Frameshift	9	None	N/A	83
51	39	F	15	c.668T>C	p.Leu223Pro	Missense	27	PHPT (15 y.o.) NF-PNET (17 y.o.) Prolactinoma (17 y.o.)	None	146
52	34	M	8	c.1008dup	p.Ala337fsX30	Frameshift	22	NF-PNET (16 y.o.) PHPT (17 y.o.)	None	129
53	34	F	6	c.1008dup	p.Ala337fsX30	Frameshift	21	NF-PNET (14 y.o.) PHPT (16 y.o.)	None	114
54	40	M	17	c.1243C>T	p.Arg415X	Nonsense	26	PHPT (17 y.o.) NF-PNET (17 y.o.)	None	104
55	40	M	17	c.1243C>T	p.Arg415X	Nonsense	28	PHPT (17 y.o.)	NF-PNET 26 y.o.)	126
56	41	F	12	c.549G>A	p.Trp183X	Nonsense	30	PHPT (12 y.o.)	NF-PNET (20 y.o.)	148
57	42	F	18	c.461G>T	p.Ser154Ile	Missense	26	None	PHPT (23 y.o.)	58
58	43	M	18	c.269_270del	p.Tyr90fsX26	Frameshift	29	None	PHPT (29 y.o.)	125
59	44	M	11	c.628_631del	p.Thr210fsX13	Frameshift	21	None	None	103
60	44	F	14	c.628_631del	p.Thr210fsX13	Frameshift	24	NF-PNET (16 y.o.)	PHPT (22 y.o.)	110
61	41	M	10	c.549G>A	p.Trp183X	Nonsense	35	Insulinoma (10 y.o.)	NF-PNET (24 y.o.) NFAA (24 y.o.) PHPT (31 y.o.)	135
62	45	M	13	Deletion exon 2	N/A	Gross deletion	16	PHPT (15 y.o.)	N/A	33
63	46	M	11	c.784-9G>A	N/A	Acceptor splice site	15	Prolactinoma (11 y.o.) PHPT (15 y.o.)	N/A	45
64	47	F	3	c.535G>A	p.Glu179Lys	Missense	14	PHPT (11 y.o.)	N/A	139
65	48	M	7	c.292C>T	p.Arg98X	Nonsense	13	None	N/A	77
66	47	F	5	c.535G>A	p.Glu179Lys	Missense	6	PHPT (6 y.o.)	N/A	7
67	47	F	7	c.535G>A	p.Glu179Lys	Missense	17	None	N/A	115
68	49	M	6	c.1204_1208del	p.Ser402fsX5	Frameshift	7	Insulinoma (6 y.o.) PHPT (6 y.o.)	N/A	14
69	50	M	9	c.378G>A	p.Trp126X	Nonsense	14	None	N/A	67

SUPPLEMENTARY MATERIAL – Available online only

Case ID	Family ID	Sex	Age at MEN1 diagnosis, years	Nucleotide change	Protein change	Variant type	Age at last F/U	Manifestation(s) detected at ≤18 years	Manifestation(s) detected at > 18 years	Duration of F/U (months)
70	51	F	16	c.628_631del	p.Thr210fsX13	Frameshift	18	PHPT (16 y.o.)	N/A	28
71	52	M	18	c.1075_1077del	p.Glu359del	In-frame deletion	26	PHPT (18 y.o.) Insulinoma (18 y.o.)	None	106
72	51	F	13	c.628_631del	p.Thr210fsX13	Frameshift	25	PHPT (18 y.o.)	NF-PA (19 y.o.)	84
73	53	M	18	c.1304del	p.Gly435fsX10	Frameshift	28	None	Prolactinoma (22 y.o.) PHPT (22 y.o.) NF-PNET (25 y.o.)	70
74	54	F	15	c.1546dup	p.Arg516fsX15	Frameshift	21	Prolactinoma (14 y.o.) PHPT (18 y.o.)	None	70
75	55	F	16	Unknown[a]	Unknown	Unknown	25	Insulinoma (16 y.o.) PHPT (17 y.o.)	None	96
76	56	F	18	c.1085del	p.Lys362fsX6	Frameshift	23	None	NF-PNET (22 y.o.) PHPT (22 y.o.)	49
77	57	F	16	c.1546dup	p.Arg516fsX15	Frameshift	26	Hyperparathyroidism (16 y.o.) PHPT (17 y.o.)	None	162
78	58	F	10	Unknown[a]	Unknown	Unknown	30	Prolactinoma (18 y.o.) NF-PNET (19 y.o.)	PHPT (25 y.o.)	198
79	59	F	17	c.740T>A	p.Ile247Asn	Missense	18	Hyperparathyroidism (16 y.o.)	N/A	15
80	60	M	8	c.249_252del	p.Ile85fsX33	Frameshift	12	Insulinoma (12 y.o.)	N/A	46

[a]Patient tested positive for *MEN1* germline pathogenic variant but specific variant unknown.

[b]Germline variant unknown as family declined genetic testing.

[c]Patient's first-degree relative tested positive for *MEN1* germline pathogenic variant, but specific variant unknown.

ACTH-oma, corticotrophin-secreting pituitary adenoma (corticotrophinoma); GH-oma, growth-hormone secreting pituitary adenoma (somatotrophinoma); F, female; F/U, follow-up; M, male; MEN1, multiple endocrine neoplasia type 1; NF-PNET, non-functioning pancreatic neuroendocrine tumor; NFAA, non-functioning adenal adenoma; NF-PA, non-functioning pituitary adenoma; PHPT, primary hyperparathyroidism

Two-sentence article summary for: "Multiple Endocrine Neoplasia Type 1 in Children and Adolescents: Clinical Features and Treatment Outcomes"

Our multi-center cohort study of patients with multiple endocrine neoplasia type 1 demonstrated that 70% of children and adolescents aged ≤18 years developed endocrine tumors, which included: parathyroid tumors, for which less-than-subtotal parathyroidectomy was associated with higher rates of persistent or recurrence disease; pancreaticoduodenal neuroendocrine tumors that may have metastasized; and pituitary adenomas, some of which presented as macroprolactinomas. These findings have implications for the counselling of young patients and families with multiple endocrine neoplasia type 1, and underscores the importance of initiating surveillance screening early in childhood.

1. Please confirm that you have mentioned all organizations that funded your research in the Acknowledgements section of your submission, including grant numbers where appropriate.

   ◉ I confirm that I have mentioned all organizations that funded my research in the Acknowledgements section of my submission, including grant numbers where appropriate.

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   Please provide two sentences; the first sentence should briefly summarize what you did or your findings. The second sentence should start out with "The importance of this (report or finding) is..." and then add your short justification to the end of this sentence. 50-60 WORD MAXIMUM IN TOTAL OR IT WILL NOT BE PUBLISHED WITH ARTICLE. Please make this the same as your article summary in the manuscript, these two sentences should be verbatim in both places.

   Our multi-center cohort study of patients with multiple endocrine neoplasia type 1 demonstrated that 70% of children and adolescents aged ≤18 years developed endocrine tumors, which included: parathyroid tumors, for which less-than-subtotal parathyroidectomy was associated with higher rates of persistent or recurrence disease; pancreaticoduodenal neuroendocrine tumors that may have metastasized; and pituitary adenomas, some of which presented as macroprolactinomas. These findings have implications for the counselling of young patients and families with multiple endocrine neoplasia type 1, and underscores the importance of initiating surveillance screening early in childhood.

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