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Clinical and genetic characteristics of Multiple Endocrine Neoplasia type 1 (MEN 1) syndrome in a small cohort of Swiss patients

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Abstract

Multiple endocrine neoplasia type 1 (MEN1) syndrome is a polyglandular autosomal dominant transmitted disease characterized by the combined occurrence of several endocrine gland tumors. The main features of the syndrome include parathyroid (95%), enteropancreatic (40-70%), and anterior pituitary (30-40%) tumors. Tumors in MEN1 syndrome display a more aggressive behavior than sporadic tumors and are more resistant to treatment. The disease's prevalence has been estimated between 1:10'000 – 1:100'000, affecting all age groups and demonstrating a very high penetrance with clinical and biochemical manifestations having developed in respectively 80 % and more than 98 % of MEN1 patients by the fifth decade. Primary hyperparathyroidism has been shown to be the first manifestation of the syndrome amongst more than 85% of patients. *MEN1*, the gene responsible for the disease, is located on chromosome 11q13. It consists of 10 exons and is translated into a 610-amino acid protein named menin that behaves as a tumor suppressor in endocrine organs. Menin has an important role in cell division and proliferation, transcription, DNA replication and repair, apoptosis and genome stability. More than 450 different mutations, scattered through the whole sequence and mostly inactivating or leading to a missing or truncated protein have been described.

We studied the MEN1 patients that were treated or followed-up at CHUV between 1995-2015. The objective was to review and analyze clinical and genetic characteristics of MEN1 syndrome among these patients as well as the inter- and intra-familial variability of expression and to search for possible genotype-phenotype correlations. A large amount of data was collected for each patient and entered into a database. Epidemiological data such as age ratio, mean age at first symptoms and at diagnosis, prevalence of each tumor and proportion of patients who underwent *MEN1* mutational analysis was calculated. The data was then analyzed and compared to the literature.

21 patients being part of 11 different pedigrees and 80% of whom having developed clinical, radiological or biological signs of MEN1 at the time of the study were identified. Among 17 out of 21 patients displaying signs or symptoms of MEN1, 82% were affected by primary hyperparathyroidism, 76% had enteropancreatic NET, 18% pituitary tumors and 47% extended spectrum tumors, such as lipomas, carcinoids or adrenal tumors. Mutations of the *MEN1* gene were found in only four out of eleven pedigrees and consisted of two large deletions, one missense and one nonsense. Mutational analysis was either not performed or not documented in the other pedigrees.

Pedigree 1 being well documented, particularly interesting and displaying some unusual features, we focused our research and analysis on it. We report here the case of the proband, a 40 years old patient with a metastatic pituitary carcinoma, and his family.

Several patients whose records were reviewed had benefited from a suboptimal care with no genetic testing being made and what seemed to be an insufficient screening and follow-up. We recommend that MEN1 patients should be identified and gathered in qualified centers. Follow-up should be coordinated by an endocrinologist with expertise in the subject.

Key words

MEN 1 – Primary hyperparathyroidism – Enteropancreatic NETs – Pituitary NETs – Menin

Abbreviations

CHUV:	Centre Hospitalier Universitaire Vaudois
GEP:	Gastroenteropancreatic
MEN 1:	Multiple Endocrine Neoplasia type 1
MLPA:	Multiplex Ligation-dependent Probe Amplification
NET:	Neuroendocrine Tumor
NF:	Non-functioning
NFPT:	Non-functioning Pancreatic Tumor
PHPT:	Primary Hyperparathyroidism
PP:	Pancreatic Polypeptide
PPoma:	PP-secreting adenoma
QMPSF:	Quantitative Multiplex PCR of Short Fluorescent fragments
VIP:	Vasoactive Intestinal Peptide
VIPoma:	VIP-secreting adenoma
y/o:	years old
ZES:	Zollinger-Ellison Syndrome

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Background

Multiple endocrine neoplasia type 1 (MEN1) syndrome, also called Wermer's syndrome (2), is a polyglandular genetic disease that is characterized by the combined occurrence of several endocrine gland tumors (1). The main features include parathyroid (95%), enteropancreatic (40-70%), and anterior pituitary (30-40%) tumors (1,4). Enteropancreatic tumors include gastrinomas, insulinomas, non-functioning adenomas and PPomas, as well as glucagonomas and VIPomas. Pituitary adenomas are mainly prolactinomas, but somatotropinomas, corticotropinomas and non-functioning adenomas can also be found (1). Patients may also less frequently present with adrenal cortical tumors, thymic, bronchopulmonary or gastric and duodenal carcinoids, skin tumors (facial angiofibromas, collagenomas, lipomas), meningiomas and rarely pheochromocytomas (1). Clinical manifestations of MEN1 syndrome are related to the location of the neoplasm, its size and its products of secretion (2).

The prevalence of the disease has been estimated between 1:10'000 – 1:100'000, with possible geographical clustering due to founder effect (1,5,6). The disease can affect all age groups and demonstrates a high penetrance with clinical and biochemical manifestations having developed in respectively 80 % and more than 98 % of MEN1 patients by the fifth decade (1–3). However, first symptoms have been reported in patients as young as age 5 (2). Primary hyperparathyroidism caused by parathyroid tumors has been shown to be the first manifestation of the syndrome in more than 85% of patients, whereas insulinomas and prolactinomas account for the remaining 15% (2).

MEN1 can either be inherited as an autosomal dominant disease, which has important implications for family members of MEN1 patients, or occur sporadically (2,4).

It is well known that MEN1-associated tumors have a different behavior than sporadic tumors in non-MEN1 patients, which can be due to inherited germline mutation in all cells (7). They are, with the exception of pituitary tumors, usually multiple and their locations may vary from the expected locations for sporadic tumors. Additionally, they usually develop earlier than sporadic tumors, and can be larger and more aggressive (1,2,7). Studies have also proven that the prevalence of metastases, especially occult metastatic disease, is higher in MEN1 patients than in patients with sporadic endocrine tumors. MEN1-associated tumors are also more resistant to treatment (1). MEN1 patients thus have both a reduced quality of life and life expectancy (6). The mortality associated with tumors of the MEN1 spectrum is mostly due to enteropancreatic malignancies, particularly non-functioning pancreatic NET that are correlated with a worse prognosis than gastrinomas and insulinomas (1,3,8), but also to thymic NETs (9).

MEN1, the gene responsible for the disease, has been mapped to chromosome 11q13 by linkage analysis (5). This gene consists of 10 exons, 9 of which translated into a 610-amino acid ubiquitously expressed protein named menin (2,10). Figure 1A displays a schematic representation of the gene. Menin, a predominantly nuclear protein in non-dividing cells, behaves as a tumor suppressor and negative regulation factor or proliferation in endocrine organs (2,11,12). Functioning as an adapter protein, it plays an important role in cell division and proliferation, transcription, DNA replication and repair, apoptosis and genome stability, interacting with numerous transcriptions factors, cytoskeletal proteins and cytoplasmic cell signaling mediators (6,11,12). For instance, menin has been show to inhibit JunD-activated transcription, and it has been proposed that JunD switches to an oncongen upon menin inactivation (5,7). Furthermore, knockout

MEN1 $-/-$ mice showing important developmental delays and embryogenic abnormalities demonstrate the important role of *MEN1* in the embryonic development of several organs (12).

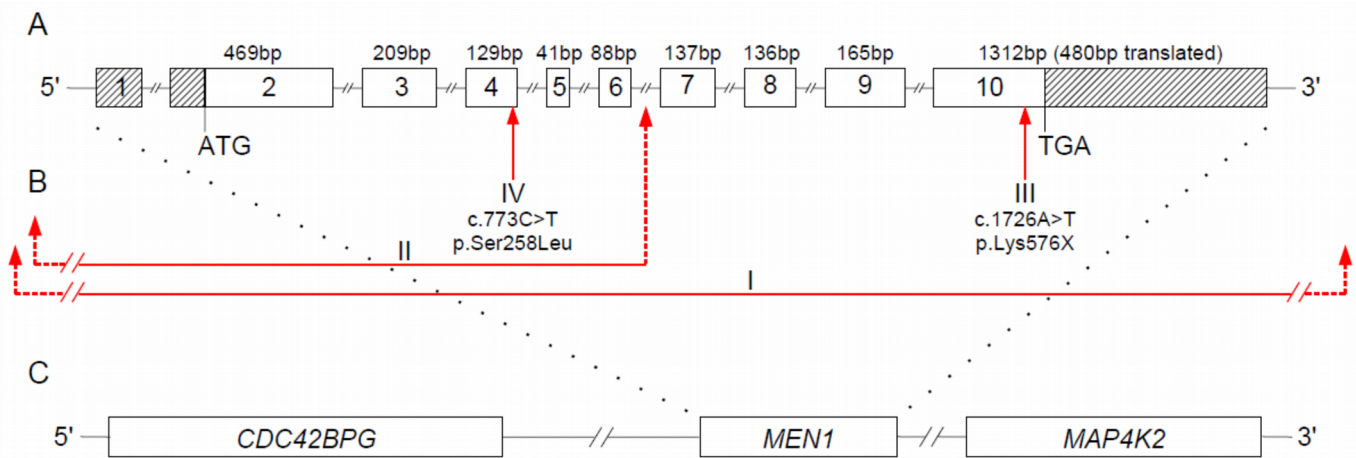


Figure 1. Schematic representation of the *MEN1* gene and adjacent genes on chromosome 11q.

A : The *MEN1* gene consists of 10 exons (boxes, sizes are indicated) and 9 introns (lines, not to scale) that spans >9kb of genomic DNA. Exon 1, part of exon 2 and part of exon 10 are untranslated (indicated by shaded region). The start (ATG) codon in exon 2 and stop (TGA) codon in exon 10 are indicated. **B :** Mutations found in Pedigrees 1, 2, 9 and 11 are represented. Red arrows indicate their position in the gene. Mutations I and II are large deletions. Arrows with broken line indicate that the exact position of the beginning and the end of the mutations is unknown. I : Pedigree 1. Deletion of exons 1-10 (whole gene deletion), c.1-?_1833+?del. Deletion of other genes adjacent to *MEN1* in the 5' direction is not excluded. II : Pedigree 2. Deletion of exons 1-6, c.1-?_912+?del. Deletion of other genes adjacent to *MEN1* in the 5' direction is not excluded. III : Pedigree 9. Nonsense mutation in exon 10, c.1726A>T. IV : Pedigree 11. Missense mutation in exon 4, c.773C>T. **C :** Genes adjacent to *MEN1* in the 5' (*CDC42BPG*) and the 3' (*MAP4K2*) direction. Genes size and intervals not to scale.

Inspired and adapted from **1)** Lemmens I, Van de Ven WJ, Kas K, Zhang CX, Giraud S, Wautot V, et al. Identification of the multiple endocrine neoplasia type 1 (*MEN1*) gene. *The European Consortium on MEN1. Hum Mol Genet.* Juill 1997;6(7):117783. **2)** Lemos MC, Thakker RV. Multiple endocrine neoplasia type 1 (*MEN1*): analysis of 1336 mutations reported in the first decade following identification of the gene. *Hum Mutat.* janv 2008;29(1):2232.

More than a 450 different mutations of *MEN1* have been described since the identification of the gene in 1997 (2,12). The fact that these mutations are scattered through the whole sequence of the gene, coding regions and splice sites (2,6,12) and that most of the mutations are inactivating or lead to a missing or truncated protein is consistent with the tumor suppressor gene role of *MEN1* and the Knudson's two hits hypothesis (1,2,5,10,12). The first hit is a heterozygous germline mutation either inherited from a parent in familial cases, or acquired in early embryonic stage in sporadic cases and thus affects numerous if not all cells at birth (13). It occurs typically as a single-base DNA change, whereas large chromosomal rearrangements or deletions leading to loss of heterozygosity are more frequent for the second hit, however any of the mutational mechanisms can be involved (5,12,14,15). Finally, most of the *MEN1* tumors as well as many sporadic tumors harboring a *MEN1* mutation demonstrate a loss of heterozygosity at locus 11q13 (14,16). However, the precise mechanism leading to *MEN1* in the absence of a correctly functioning menin remains to be clarified (6).

We studied *MEN1* patients that were treated or followed-up at CHUV between 1995-2015. The objective was to review and analyze clinical and genetic characteristics of *MEN1* syndrome among these patients as well as the inter- and intra-familial variability of expression with the hope of identifying possible genotype-phenotype correlations.

Material and methods

Source

Patients were identified via the center for medical archives in CHUV. We included all patients with a diagnosis or suspicion of MEN1 syndrome attested by a specialist (i.e. an endocrinologist) and that had been followed at CHUV between 1995-2015. There were no particular exclusion criteria.

Definition

MEN1 syndrome was defined as meeting at least one of the following diagnostic criteria (1) :

- Clinical : the presence of ≥ 2 of the primary MEN1-associated tumors (parathyroid, pancreas, pituitary)
- Familial : the presence of one of the primary feature of the MEN1 syndrome, plus a first degree relative with MEN1
- Genetic : The presence of mutations in the *MEN1* gene

Database

A large amount of data was collected for each patient, including estimated age of beginning of the symptoms, age of the first biochemical abnormality (if available), age at diagnosis, symptoms, screening strategy, radiological and laboratory finding, tumor pattern, treatment, outcome and, if available, genetic analysis. A database gathering this data was then created. Obtained data was then represented in family trees and several figures and tables using LibreOffice Impress and Calc. All pedigrees were summarized in a table. This study being retrospective, no new clinical, biochemical or radiological examinations were performed.

Statistics

Epidemiological data such as age ratio, mean age at first symptoms and at diagnosis, prevalence of each tumor and proportion of patients who underwent *MEN1* mutational analysis was calculated using LibreOffice Calc.

Genetics

Genetic analysis was performed prior to this study in pedigrees 1, 2, 9 and 11 using conventional PCR sequence analysis of peripheral blood DNA. As the probands from pedigrees 1 and 2 initially tested negative, a second analysis was performed using multiplex ligation-dependent probe amplification (MLPA) in pedigree 1 and quantitative multiplex PCR of short fluorescent fragments (QMPSF) in pedigree 2. The *MEN1* transcript NM_130799 was used for numbering of the mutations.

Mutations are named in this document according to the HGVS recommendations regarding the nomenclature of sequence variants.

Results

Patients

Twenty-one patients from of 11 different pedigrees were identified. Among them, 43% were women and 57% were men. 80% had developed clinical, radiological or biological signs of MEN1 at the time of the study. The appearance of first signs was identified in 17/21 patients at the mean age of 24 years (range 7-53 years). Mean age at diagnosis was 32 years (range 1-65 years) for the whole cohort, 41 years (range 15-65 years) for the probands (i.e. the index case from each family) and 22 years (range 1-64 years) for the affected family members.

Among 17/21 patients displaying signs or symptoms of MEN1, 82% were affected by primary hyperparathyroidism, 76% had enteropancreatic NET, 18% pituitary tumors and 47% extended spectrum tumors, such as lipomas, carcinoids or adrenal tumors. 35% also had a tumor not in the MEN1 spectrum, indicating a potentially increased overall tumor risk in MEN1 patients. Table 1 shows the prevalence of lesions in our cohort of patients and compares it to published data.

Table 1. Prevalence of MEN1 lesions in our cohort, compared with the average prevalence found in a review article and average prevalence in a cohort with the prolactinoma variant of MEN1.
 Note : 4 out of the 21 patients from our cohort that had not developed any manifestation of MEN1 at the time of the study were excluded.
 * incl. NFPT, ** incl. PPoma

	Present report		<i>Thakker RV (1)</i> Review	<i>Hao et al. (49)</i> Burin variant
Number of patients	17			30
	n	%	%	%
PHPT	14	82.4	90	93.3
Pancreatic tumors	13	76.5	30-70	26.7
Gastrinoma	6	35.3	40	10.0
Insulinoma	4	23.5	10	10.0
Glucagonoma	3	17.6	< 1	
VIPoma	1	5.9	< 1	
PPoma	3	17.6	20-55 *	
Somatostatinoma	0	0.0		
Nonfunctioning	0	0.0	20-55 **	6.7
Pituitary tumors	3	17.6	30-40	40.0
Prolactinoma	2	11.8	20	40.0
Somatotrophinoma	0	0.0	10	
Corticotrophinoma	0	0.0	< 5	
Thyrotrophinoma	0	0.0		
Nonfunctioning	2	11.8	< 5	
Other tumors	8	47.1		30.0
Adrenal	1	5.9	40	13.3
Thymic NET	1	5.9	2	0.0
Bronchial NET	3	17.6	2	10.0
Gastric NET	1	5.9	10	3.3
Lipoma	4	23.5	30	
Angiofibromas	0	0.0	85	
Collagenomas	0	0.0	70	
Meningioma	0	0.0	8	
Pheochromocytoma	0	0.0	< 1	
Non MEN1 tumors	6	35.3		

Table 2. Summary of clinical and genetic characteristics of probands from pedigrees 1-11. Tumors listed without an age of onset occurred simultaneously as the last one for which the age is mentioned. G = genetic, F = familial, C = clinical, B = biological, Rx = radiological, H = histopathological, * = first hit, ND = not documented, y/o = years old

Pedigree	Sex	Age at diagnosis / first symptoms	Diagnostic criteria	Parathyroid tumors	Enteropancreatic tumors	Pituitary tumors	Associated tumors	Non-MEN1 tumors	Familial vs sporadic disease	Familial history	Tumors in the family	Main symptoms	Mutation type
1	M	35 / 18	1) C, 2) G	Adenoma (30 & 37 y/o, B, Rx)	-	-	* Invasive macroprolactinoma, then metastatic pituitary carcinoma (19, 29, 30, 37 & 39 y/o, C, B, Rx, H)	Adenomucinous pancreatic cyst (30 y/o)	Familial	Father, brother, daughter affected, son asymptomatic carrier, nephews affected/carriers	Father: parathyroid adenomas, thymic carcinoma; Daughter: lipomas, PHPT; Brother: PHPT; Nephew: insulinoma	Decreased visual acuity, bitemporal hemianopsia, panhypopituitarism	Large deletion
2	F	15 / 13	1) C 2) G	Diffuse hyperplasia, then adenoma (15 & 17 y/o, C, B, Rx, H)	* Insulinoma (14 y/o, C, B, Rx, H)	Nonfunctioning microadenoma (15 y/o, Rx)	-	-	Familial	Father, sister, grandmother, uncle and cousin affected, brother healthy	Sister: microprolactinoma, adrenal adenoma, PHPT	Osteopenia, spontaneous hypoglycemia, improvement after glucose administration	Large deletion
3	M	30 / 28	1) C	* Diffuse and nodular hyperplasia (36 y/o, B, H)	* Multiple gastrinomas (36 & 43 y/o, C, B, Rx, H)	-	-	-	Indefinite	Clinical suspicion in the mother and an aunt	Mother: breast carcinoma, gastric carcinoma, ZES	Diffuse abdominal pain, gastric hemorrhage, bloody diarrhea, rectorrhagia, ZES	ND
4	F	58 / < 53	1) C	* Diffuse hyperplasia (52 y/o, C, B, Rx, H)	Multiple gastrinomas (58 y/o, C, B), PPomas (B, H), glucagonomas, insulinomas (H), indefinite lesions (Rx)	-	-	Mildly differentiated, partly keratinizing epidermoid carcinoma of the proximal esophagus	Probably sporadic	No known endocrine or tumoral disease	-	Severe osteoporosis with pathological fractures, ulcers, diarrhea, melena, hematemesis, multiple enteral perforations with acute peritonitis, ZES	ND
5	M	65 / < 62	1) F 2) C	* Probable adenomas (< 62 & 65 y/o, C, B)	Multiple gastrinomas (65 y/o, C, H)	-	-	Proximal jejunal leiomyoma	Probably familial	A brother affected, unknown details	ND	Repeated renal colic, diarrhea, perforated duodenal ulcer with purulent acute peritonitis, ZES	ND
6	F	65 / < 50	1) C	* Multiple adenomas (65 y/o, C, B, Rx, H)	* Gastrinoma (65 y/o, C, B), occult PPoma (B), indefinite lesions (Rx) Bone and liver, metastases (Rx)	Suspicion of microprolactinoma (67 y/o, B)	Atypical bronchial carcinoid (68 y/o, Rx, H)	Thoracic wall elastofibroma	Probably sporadic	Cancer history in both parents. Daughter and grandson healthy.	Father: gastric cancer with generalized metastasis; Mother: leukemia	Kidney stones, osteoporosis, ulcers, diarrhea, ZES, nausea	ND
7	M	19 / 15	1) C	Nodular hyperplasia (19 & 20 y/o, C, B, Rx, H)	* Insulinoma (15 y/o, C, B, Rx, H)	-	-	-	Probably sporadic	No known endocrine disease	-	Spontaneous hypoglycemia, repeated malaises, improvement after glucose administration	ND
8	M	25 / 18	1) C	* Multiple adenomas (21 & 24 y/o, C, B, Rx)	Gastrinomas (24 y/o, C, B), VIPomas (C, B), PPomas (B, H), glucagonomas (H), indefinite lesions (Rx)	-	-	-	Indefinite	No known endocrine disease	-	Polyuria, polydipsia, kidney stones, gastric ulcers, chronic diarrhea, pyrosis, hematemesis	ND
9	M	32 / 31	1) C, 2) G	Adenoma and hyperplasia (32 y/o, B, Rx)	* Duodenal gastrinomas (31 & 48 y/o, C, B, H), Non gastrin secreting islet cell tumors expressing glucagon and PP (H), indefinite lesions (Rx)	-	Thymic carcinoma, recurring in lymph nodes (41 & 42 y/o, Rx, H); lipomas (43 et 48 y/o, C, H)	-	Familial	Father and sister affected, nephew carrier. Suspicion in paternal grandfather and grand-uncle	Father: gastrinomas, GEP carcinoid; Sister: duodenal gastrinomas, parathyroid adenomas, uterine fibromyomatosis (Non MEN1)	Ulcers, diarrhea, ZES, upper and lower gastrointestinal bleeding with melena and rectorrhagia	Nonsense
10	F	51 / 38	1) C	* Adenomas (39, 51 & 57 y/o, C, B, Rx, H)	Multiple gastrinomas, diffuse islet cell hyperplasia, liver, abdominal and pulmonary metastasis (44, 45, 58 & 75 y/o, C, B, Rx, H)	-	Lipomas (51 y/o, C, H); Stage 1A papillary thyroid carcinoma (51 y/o, H)	-	Indefinite	No known endocrine disease, not enough informations about familial history	-	Kidney stones, osteoporosis, ulcers, diarrhea, anorexia, gastrointestinal bleeding, ZES	ND
11	F	58 / 52	1) G	Adenomas (54 & 55 y/o, C, B, Rx, H)	-	-	* Bronchial carcinoid (49 & 53 y/o, Rx, H); lipoma (53 y/o, C, H)	Uterine myxoid leiomyosarcoma with locoregional recurrence and pulmonary metastases	Probably sporadic	ENT and breast cancers in family	Uncle: ENT cancer; Aunt: breast cancer	Kidney stones, diffuse osteoarticular pain, asthenia, pyrosis, diarrhea	Missense

Table 2 represents the probands from pedigrees 1-11, and summarizes genetic and clinical features of their disease. The 10 out of 21 remaining patients were included as family members. Over those 11 pedigrees, three represented MEN1 families, one a probable familial MEN1 syndrome and four probable sporadic cases. The last three cases were indeterminate. Figure 2 shows the course of the disease over time in probands from each pedigree.

Mutations of the *MEN1* gene were found in only four out of eleven pedigrees. Members of pedigree 1 have a total deletion of exons 1-10, c.[1-?_1833+?del];[=], pedigree 2 a large deletion of exons 1-6, c.[1-?_912+?del];[=] and pedigree 9 a nonsense mutation in exon 10, c.[1726A>T];[=], p.[Lys576X];[=], leading to either a truncated protein or the absence of the *MEN1* transcript via nonsense mediated decay. Finally, patients from pedigree 11 display a missense mutation in exon 4, c.[773C>T];[=], p.[Ser258Leu];[=], which is predicted to have a deleterious effect and has been reported in *MEN1* patients by Tham et al. (17). Figure 1B shows the position of the mutations on the gene. The other seven pedigrees were composed of the proband only and mutational analysis was either not performed or not documented.

Documentation was rare in all pedigrees, in exception of pedigree 1. In pedigree 2, only two patients were followed up at CHUV. In most of the cases, particularly when no mutation was documented and/or there was no familial history for *MEN1*, the patients were followed up on an irregular basis.

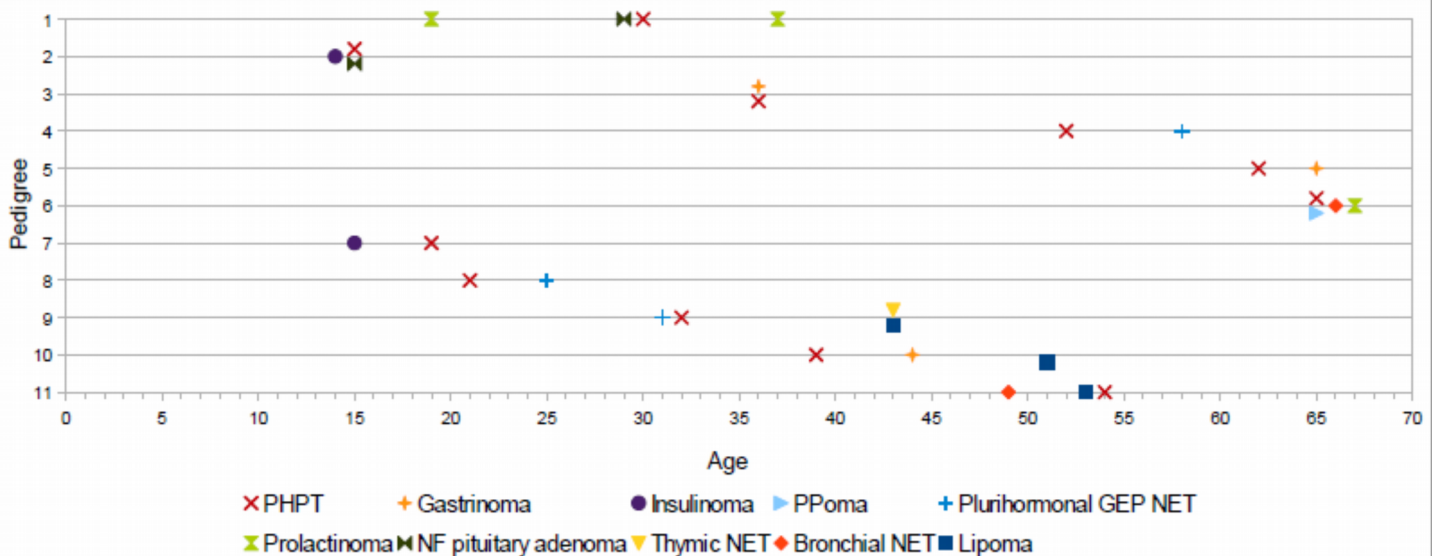


Figure 2. Chronological representation of the age at diagnosis of the first occurrence of each lesion in probands of pedigrees 1-11. Eventual recurrences are not shown. In pedigree 1, the second prolactinoma marks the probable first occurrence of the pituitary carcinoma. Plurihormonal NETs were secreting gastrin, PP, glucagon and insulin in pedigree 4, gastrin, glucagon, PP and VIP in pedigree 8 and gastrin, PP and glucagon in pedigree 9. Proband from pedigree 4 died at age 63 years from causes unrelated to the syndrome. Proband from pedigree 8 died at age 25 years from a hemorrhagic shock following an aorto-jejunal fistula.

Pedigree 1 being well documented, particularly interesting and displaying some unusual features, we focused our research and analysis on it.

Case report, Pedigree 1

We report the case of a male patient, born in 1975 and further referred to as “the proband” or “the patient”, and his family. The proband has MEN1 syndrome with a history of recurring invasive pituitary adenomas since age 18 and recurring primary hyperparathyroidism. At age 39, he was diagnosed with a metastatic prolactin-secreting pituitary carcinoma.

Clinical data

The patient was initially found to have an invasive macroprolactinoma at age 18, when he presented with bi-temporal hemianopsia, progressive loss of visual acuity and retro-ocular pain, without any signs of pituitary failure. Prolactin was 3890 µg/l (N: 12-20 µg/l for males), salivary and plasma testosterone were both lowered but LH and FSH were within the normal range. MRI showed a massive expansive process (70×55×52 mm) within the sella region, invading the optochiasmatic cistern, the sphenoid sinus and the ethmoidal cells along with the carotid siphons. Bromocriptin treatment was introduced with disappearance of the symptoms, a decrease of prolactin up to two times the upper range limit, normalization of the sexual hormones and reduction of the mass along with cystic transformation of most of the macroprolactinoma on a subsequent MRI, confirming the response to the treatment. Mild hyperprolactinemia (30-130 µg/l) persisted, and the patient developed hypogonadotropic hypogonadism, presenting with eunuchoid phenotype, no facial and thoracic body hair, and mild galactorrhea, without any sexual dysfunction. Over the years, Bromocriptin was replaced by Quinagolid then Cabergolin for digestive intolerance.

At age 29 the pituitary adenoma was shown to recur when the patient presented with a novel episode of bi-temporal hemianopsia and a decrease of visual acuity, along with occipital cephalgia. Prolactin was within normal limits, but IGF-1 and testosterone were low, indicating somatotrophic and gonadotrophic axis insufficiency. MRI showed a tissular transformation of the invasive macroadenoma, which was partially transsphenoidally resected. The symptoms subsequently disappeared, with the exception of a right supero-external quadrantsy, a hypogonadotropic hypogonadism that was supplemented and a hypocorticism transiently supplemented. Histology showed no signs of atypia and confirmed the absence of prolactin secretion, which allowed the discontinuation of Cabergolin treatment. The patient then benefited from a complementary radiotherapy, during which a cystic formation developed on the tumor leading to global left anopsy, and gonadotrophic, corticotrophic and somatotrophic axis failure. Emergency craniotomy and optical nerve decompression was performed with a favorable postoperative outcome, although partial atrophy of the left optical nerve led to permanent decreased visual acuity and temporal hemianopsy. Sequellar panhypopituitarism required supplementation of the corticotrophic, thyrotrophic and gonadotrophic axis. Somatotrophic axis, although insufficient as well, was not supplemented.

The pituitary macroadenoma recurred again at age 37, with sudden left anopsy and right temporal hemianopsy. Prolactin level were elevated (385 µg/l) and MRI confirmed the tumor recurrence with extension into the left optic canal and partial extension into the left orbit. Cabergolin was reintroduced and the macroprolactinoma was resected by craniotomy. Total atrophy of the left optical nerve led to permanent left eye blindness. Histology showed diffuse and strong expression of prolactin but not of any other pituitary hormones. The treatment was completed with Gamma Knife radioneurosurgery.

At age 39 the patient developed right temporal hemianopsy again. Brain and pituitary MRI

showed a new progression of the tumor in the zone that had not been treated with Gamma Knife, along with new lesions in the supraorbital dura mater of the left frontal lobe and right gyrus rectus suspect for metastases. Lesion of the right gyrus rectus is shown on Figure 3A. Prolactin was high at 100 µg/l. The pituitary tumor was treated by Gamma Knife and the left frontal lesion was surgically resected. Histopathology showed a pituitary carcinoma with diffuse and strong expression of prolactin, confirming the diagnosis. Vertebral MRI was performed and showed lesions in the right L5 roots and dural sac at level S1-S2, which are shown on Figure 3B and C. Cerebrospinal fluid analysis showed no malignant cells. The decision was then made to treat the patient with chemotherapy with temozolomide and concomitant radiotherapy.

MEN1 syndrome was suspected at age 30 when the patient was found to have an asymptomatic hypercalcemia with total calcium raised to 3.05 mmol/l (N: 2.15-2.55 mmol/l), high calciuria and high PTH (205 pg/ml, N 10-70). Parathyroid scintigraphy highlighted a parathyroid adenoma next to the right inferior thyroid lobe. The adenoma was resected and calcium levels stabilized.

Mutational analysis of the *MEN1* gene was performed, along with biological and radiological evaluation. No mutations were found and the evaluation revealed only an adeno-mucinous pancreatic cyst that was not suspect of an endocrine tumor.

At age 37 primary hyperparathyroidism recurred with both total calcium, corrected calcium and PTH being elevated. The patient was asymptomatic, therefore the treatment was conservative.

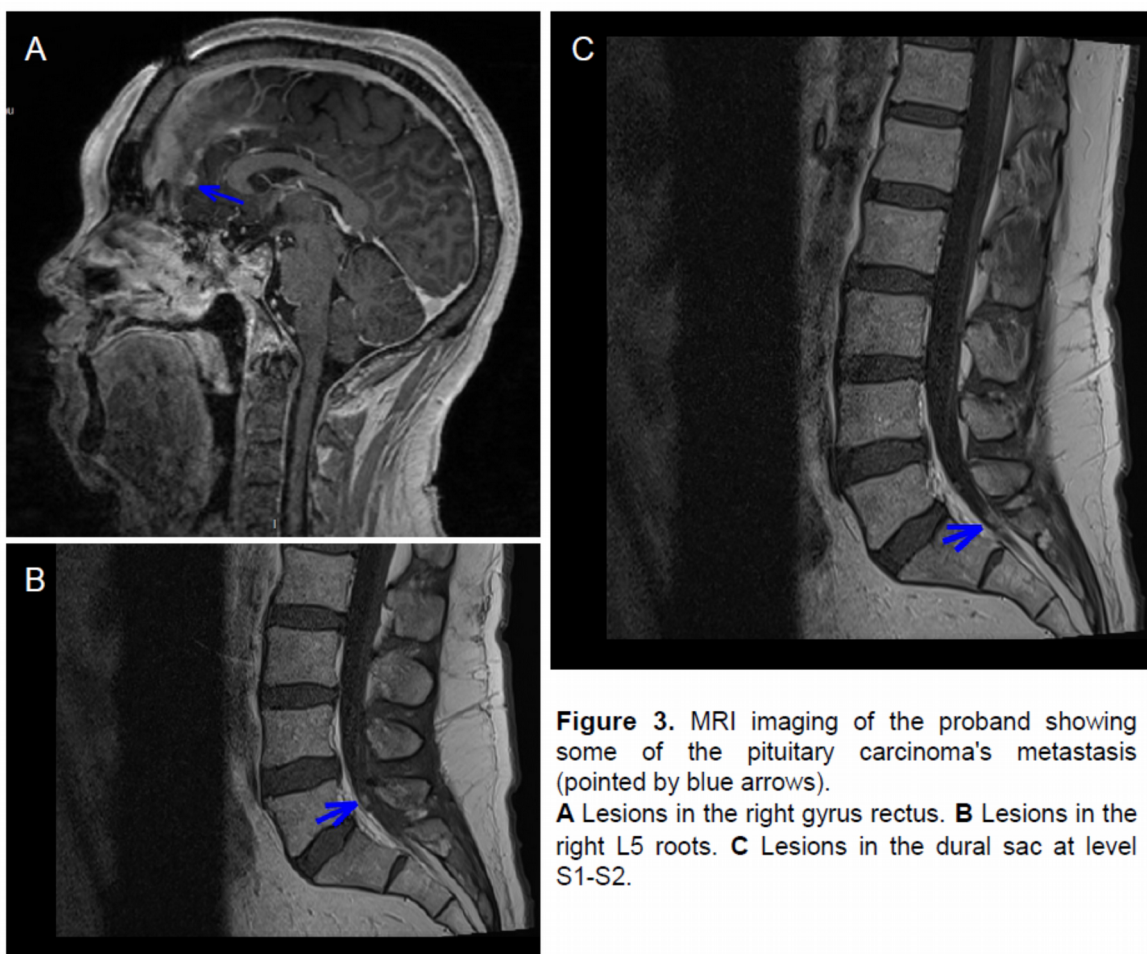


Figure 3. MRI imaging of the proband showing some of the pituitary carcinoma's metastasis (pointed by blue arrows). **A** Lesions in the right gyrus rectus. **B** Lesions in the right L5 roots. **C** Lesions in the dural sac at level S1-S2.

Mutational analysis of the MEN1 gene

A first mutational analysis was performed in the proband in 2005 at age 30, using conventional PCR sequence analysis of peripheral blood DNA. No mutation was detected. In 2010, an additional analysis was performed, using multiplex ligation-dependent probe amplification (MLPA), a method that allows the detection of large deletions across the gene. The MLPA analysis discovered a large deletion of exons 1-10, c.[1-?_1833+?del];[=]. After having found the deletion in the proband, his family was tested as well. His two children (IV.6 & 7), his brother (III.2) and three of his nephews and nieces (IV.1, 2 & 4) tested positive for the deletion. His father (II.2) initially tested negative for mutations in the *MEN1* gene. However, he had been found to have primary hyperparathyroidism with two parathyroid adenomas and an atypical thymic carcinoid that had been resected. Given the family history, the father's presentation met the familial diagnosis criteria of MEN1 syndrome. A second blood sample was obtained and analyzed, along with a saliva sample. Both were negative for the familial mutation. Tissue samples from the thymic carcinoid were then analyzed, revealing the presence of the familial deletion in the tumor cells. This indicates somatic mosaicism and potential germline mosaicism, which would make patient II.2 the founder of the familial mutation. Further explanations regarding mosaicism in patient II.2 are to be found in the discussion.

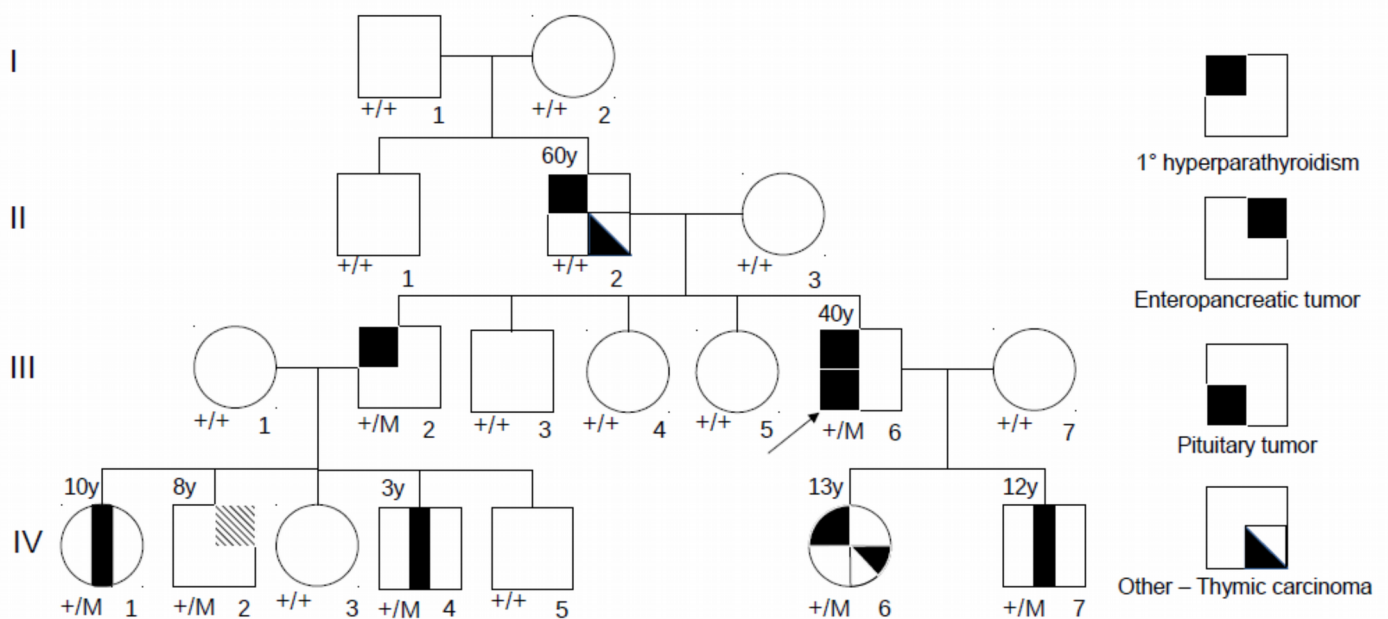


Figure 4. Family tree of pedigree 1. Proband is designated by an arrow and named III.6. +/+ indicates mutation-free family members, and +/M those who have been found to carry the mutation. II.2 carries the mutation at a mosaic state. Age of affected is indicated.

III.6 :
Familial history : Father, brother, daughter affected. Son asymptomatic carrier, nephews affected/carriers.
Disease pattern : Invasive prolactin-secreting pituitary macroadenoma (19 y/o), multi-recurring (nonsecreting at 29 and 30 y/o, prolactin-secreting at 37 and 39 y/o) with malignant transformation in metastatic pituitary carcinoma (39 y/o), parathyroid adenoma with PHPT (30 y/o), recurrent (37 y/o)
Treatments : dopaminergic agonists and prolactin inhibitors (19-30 y/o, 37 y/o –), partial endoscopic transsphenoidal resection of the pituitary adenoma (29 y/o), complementary radiotherapy, emergency craniotomy and extirpation of the lesion (30 y/o), resection of a right inferior parathyroid adenoma (30 y/o), inter- and intradural resection of a macroprolactinoma (37 y/o), radioneurosurgical Gamma Knife treatment of a recurring pituitary macroprolactinoma (37, 39 y/o), excision of an expansive lesion of the left frontal lobe, suspicious of pituitary macroadenoma metastasis, by frontopterional craniotomy (39 y/o), concomitant radiochemotherapy (temozolomide) (39 y/o –)
Mutation : Exons 1-10 deletion, c.[1- ?_1833+?del];[=] detected in III.2, III.6, IV.1, IV.2, IV.4, IV.6 and IV.7. No mutation detected in blood in II.2, but deletion detected in his thymic carcinoid. Somatic and germinal mosaicism, patient in which the mutation first occurred.

Familial history

The full pedigree's family tree is represented in Figure 4. Several family members are affected by the disease or asymptomatic carriers of the mutation. The proband's father (II.2) was diagnosed in 2009 at age 54 with asymptomatic primary hyperparathyroidism while being investigated for poorly controlled arterial hypertension. Parathyroid scintigraphy revealed two parathyroid adenomas. Exploratory cervicotomy and resection of the adenomas was performed, along with median sternotomy and thymectomy. Histology revealed a thymic carcinoid. The proband's daughter (IV.6), was found to have lipomas in 2012 at 10 years old, which were resected. In 2014, she developed primary hyperparathyroidism with total hypercalcemia and high PTH, but no clinical symptoms. The proband's brother (III.2) suffered from primary hyperparathyroidism and benefited from a parathyroidectomy. However, information regarding age of the onset and the treatment that he underwent was not available which led to his exclusion from the study. One of the proband's nephew (IV.2), aged 7 years old, is currently under investigations for a probable insulinoma after several episodes of malaise, asthenia and irritability with spontaneous recovery after glucose administration. In some of those episodes he was found to have low blood glucose levels and non suppressed insulin levels. Both children of the proband and three out of his five nephews and nieces carry the mutation. However, the proband's son (IV.7), niece (IV.1) and nephew (IV.4) were healthy carriers at ages 12, 10 and 3 years old, respectively.

Discussion

We reviewed clinical and genetic features of the syndrome in 21 patients among 11 pedigrees. One of the pedigrees being particularly well documented and followed-up in a specialized center as recommended by the guidelines, we chose to focus on patients from this family.

Clinical features of MEN1 in our cohort

Parathyroid tumors and pancreatic NETs represent most of the tumors in our cohort as represented in Table 1, which is comparable with the literature. The higher prevalence of insulinomas, glucagonomas, VIPomas and PPomas might be due to the fact that plurihormonal secreting tumors were considered as the sum of individual tumors, each secreting one hormone. For instance, multiple pancreatic NETs secreting gastrin, insulin, glucagon and PP were treated as a gastrinoma, an insulinoma, a glucagonoma and a PPoma. Prevalence of non-functioning pancreatic tumors, pituitary, adrenal, cutaneous tumors and meningiomas is significantly lower than reported in the literature. This is probably due to the data and patients records being incomplete, or the absence of screening or detection of those tumors or their relation to the syndrome. Most of the patients with sporadic disease were not followed-up at CHUV on a regular basis and screening was either incomplete or fragmented between CHUV and other institutions.

The study of the different pedigrees from our cohort highlighted the important variability in phenotypical presentation, age of onset and severity of the disease that has been reported, even between members of the same family (2,7). This is particularly well illustrated by the reported case of pedigree 1. We could also observe the great morbidity and early mortality caused by the syndrome (6). For example, probands from pedigree 8 and 10 both had multiple gastrinomas. Proband from pedigree 8 developed PHPT at age

16 and started suffering from pyrosis and diffuse abdominal pain at age 24. Gastrin levels were more than three times above normal range and multiple gastric ulcerous lesions were detected. Despite receiving proton-pump inhibitory treatment, he died one year later from a hemorrhagic shock caused by an aorto-jejunal fistula. The proband from pedigree 10 presented with recurrent nephrolithiasis with ureteral colic at age 36. Three years later, she was diagnosed with parathyroid adenomas. At age 44, she complained from abdominal pain and melena, and was diagnosed with multiple gastrinomas. After several interventions for perforated ulcers, she underwent a total gastrectomy which relieved her from her symptoms for several years. Over the years, hepatic, abdominal and pulmonary gastrinoma metastases were discovered and she suffered from recurrent ulcers, weight loss, nausea, anorexia and asthenia along with chronic diarrhea despite proton-pump inhibitory treatment, requiring multiple surgical interventions with multiple complications. Although still alive and more than 80 years old at the time of the study, she suffered from great morbidity and a reduced quality of life for over half of her life.

Interestingly and as reported in Table 2, clinical, biological and histopathological features of the syndrome sometimes differed. The proband from pedigree 5 suffered from multiple ulcers, one of which perforated and provoked a purulent acute peritonitis, diarrhea and steatorrhea. However, his gastrin levels were always within the normal range. Histology following Whipple duodeno-pancreatectomy showed three submucosal duodenal gastrinomas, raising the point that gastrinomas are difficult to diagnose. The proband from pedigree 8 had elevated gastrin, PP and VIP levels with clinical manifestations of ZES and gastrinomas. A CT scan showed both a pancreatic tumor and thickening of gastric, duodenal and jejunal walls. A hemipancreatectomy along with jejunectomy and partial colectomy was performed, and histological analysis showed surprisingly only PPomas and glucagonomas. This is probably due to the multiplicity and dissemination of tumors in MEN1, which makes comprehensive histological analysis difficult.

Six out of the twenty-one affected members from the eleven families that we studied presented with tumors that are not part of the MEN1 spectrum as described in the literature (1,2). Those were a benign pancreatic adenomucinous cyst (proband, pedigree 1), one mildly differentiated, partially keratinizing epidermoid carcinoma of the esophagus (proband, pedigree 4), one jejunal leiomyoma (pedigree 5), one thoracic wall elasto-fibroma (proband, pedigree 6), uterine fibromyomatosis (pedigree 9) and one uterine leiomyosarcoma with local recurrence and pulmonary metastases (proband, pedigree 11). This might indicate that *MEN1* mutations lead to an increased overall tumor risk, with wider manifestations of tumoral disease than MEN1 syndrome. A study reported leiomyomata of the esophagus and uterus that developed through inactivation of the *MEN1* gene. Advice was given that such tumors should be considered as MEN1 features when multiple, while *MEN1* did not seem to be involved in sporadic leiomyomas (18). This however appears not to be universally accepted, since it was not mentioned in recent guidelines and reviews articles (1,2). We searched the literature for reports of leiomyosarcoma in MEN1 patients, but none were found. Further studies of MEN1 patients with aggressive tumors that are not part of the disease spectrum are necessary to determine if *MEN1* mutations are involved in their pathogenesis. Given the multiple and not yet fully understood roles of menin in transcription and cell division and proliferation amongst others (11,12) and its multiple partners, the possibility of an increased risk of tumors in MEN1 can not be ruled out.

Genetic features

The causative gene for MEN1 was identified in 1997 and hence allowed genetic diagnostic for the disease. Amongst our cohort, only four out of eleven pedigrees had a genetic analysis with a documented mutation. Those pedigrees encompassed 66% (n=14/21) of the patients. Among those four reported mutations, one was missense (25%), one was nonsense (25%) and the remaining two were large deletions (50%), which were initially not detected and required further analysis with Multiplex Ligand-dependent Probe Amplification (MLPA) technology to be identified. According to Lemos and Thakker, large deletions involving at least one exon account for 1% of mutations (12) and for a small part only of the patients with clinical MEN1 in whom no mutations can be identified with non quantitative analysis. Those large deletions require MLPA or an analogous technology to be detected (12). It is interesting to note that two out of the four mutations that we identified were large deletions, although this is biased by the fact that one third of the patients in our cohort did not undergo mutational analysis. However, in our small study this finding is not statistically significant.

The fact that several families were not screened for *MEN1* mutations has impact on their clinical care. Genetic counseling should be offered to every patient meeting diagnostic criteria of MEN1 (suspected or confirmed) and to family members of MEN1 patients. Indeed, genetic testing allows the confirmation of the clinical diagnosis, identifies family members harboring the mutation and thus provides the opportunity to begin screening early for MEN1 symptoms and manifestations. Finally, it allows to identify family members who do not carry the mutation and therefore should not be subjected to the additional burden of screening (1).

Genetic mosaicism

Although MEN1 is thought to display a pure Mendelian inheritance pattern, mosaicism occurs. As reported above, patient II.2 from pedigree 1 has been determined to be the founder, as he was mosaic for the familial mutation. It is known that variable *de novo* mutations, such as aneuploidies, large chromosomal rearrangements, insertions, deletions or point mutations may arise at anytime during development, due to errors in chromosome segregation or DNA replication (19–21). Generation of genetically distinct cell populations from a single common ancestor requires such mutational event to occur postzygotic (22). The time at which they occur is important to determine the amount of cells that will be affected by the mutation. Research on genetic mosaics strongly indicates that the germ line and soma diverge early in development, at approximately 15 divisions, and that multiple cells in the embryo give birth to the germ line, as shown by simultaneous somatic and germ line mosaicism (19,21). Should a postzygotic *de novo* mutation occur in an exon of a protein-coding gene, and given that the mutation is not deleterious at the cellular level, it can lead to a genetic disorder that will have clinical manifestations. Such mutations occur randomly and therefore manifest as sporadic disease in individuals with unaffected parents. The proportion of cells that bear the mutation in each tissue depends on when and where the mutation occurred, and the severity of the phenotype is possibly linked to the degree of mosaicism (19,22,23). Mosaic forms of mutations leading to an autosomal dominant disorder are a major class of mosaic mutations. In such disorder, the mutation is usually transmitted constitutionally through the germ line to the descendance and are thus compatible with viability (22). Mosaicism is also suspected to contribute to cancer genetics (24).

Although reports about mosaicism in MEN1 are rare, combined somatic and germ line

mosaicism, also called gonosomal mosaicism (22), has been shown or suspected to play a role in numerous genetic disorders, including McCune-Albright syndrome, osteogenesis imperfecta, Duchenne muscular dystrophy, haemophilia, neurofibromatosis and numerous other neurodevelopmental and neuropsychiatric disorders (19,20). Several research groups have tested the parents of apparently *de novo* affected children for the mutation causing their disease, and found some of them to have the mutation in a mosaic state. In a study of children affected by Dravet syndrome, an epileptic encephalopathy, parents with a high level of mosaicism were found to have presented with seizures. The clinical status of the mosaic parent was thus related to the amount of mutation in their blood. Unrecognized mosaicism for mutations causing several genetic disorders could even partially explain their variable expressivity and incomplete penetrance (24,25). Other studies showed that some pathogenic alleles are found exclusively in the mosaic state, being lethal at the constitutional state (24). Germ line mosaicism can also be suspected when apparently healthy parents have several children affected by the same sporadic disease (25).

In patient II.2, the mutation occurred postzygotic as it could not be found in his blood leukocyte's DNA and is therefore not present in all his cells, although genome analysis only reflects the average genome of the examined cells (20). Having developed the MEN1-associated tumors himself and transmitted the mutation to his descendants, the mutated cell was probably ancestral to both somatic and germ line cells. In most of the cases, the mosaic parent does not develop the disease and mosaicism may be hard to identify (19). Nevertheless, MEN1 having a very high penetrance, the patient developed tumors. It has been reported that in some diseases, a mosaic form can result in a less severe presentation than a germinal form (23). Although patient II.2 was found to have PHPT with parathyroid adenomas and a thymic carcinoid, this discovery was made at age 54 which is significantly older than the age at which his son (the proband) was diagnosed, although age of onset of the first biochemical alterations is not known. As ascertained by Frank, « It seems almost certain that, for cancer, the frequency of cells that carry a somatic mutation will strongly influence the age of onset » (26). Besides, the diagnose was an incidental discovery as patient II.2 did not show any symptoms of MEN1 syndrome at the time. This might reflect a milder variant of the syndrome due to mosaicism, or manifestation of the well-known variability of penetrance and expression. As observed by Biesecker and Spinner, « Mosaic disorders pose a new challenge for genotype-phenotype correlations and prediction of disease manifestations and severity » (22). The advent of new generation screening will allow to more easily detect and diagnose mosaicism in MEN1 syndrome.

Unusual features of the syndrome in pedigree 1

As reported above, the proband (patient III.6) and his family display several unusual and interesting characteristics of MEN1 syndrome besides mosaicism. First, pituitary disease is the presenting feature of the syndrome in only about 17% of cases, which leads to a significantly longer delay to diagnose MEN1. This was the case in patient III.6 who was diagnosed 11 years after his first symptoms. This might be explained by the younger age at which patients may present with sporadic pituitary adenoma compared to parathyroid adenoma or enteropancreatic tumor, leading to a less frequent consideration of the MEN1 diagnosis in patients presenting with pituitary disease (27). Furthermore, patient III.6's pituitary tumors displayed an aggressive behavior and a tendency to recur. Pituitary adenomas are known to be larger in size and more often invasive in MEN1 patients than in sporadic cases. They demonstrate a greater resistance to treatment and a lesser

sensitivity to dopamine agonist therapy of prolactinomas has been well described (27–29). Studies showed that, compared to the general population, patients harboring MEN1 mutations tend to have more macroadenomas and to be younger at diagnosis, the youngest MEN1 patient with macroprolactinoma having been reported at age 5 (30). They also tend to have more multi-hormonal secreting adenomas than patients with sporadic pituitary disease (28). Despite this, aggressive pituitary tumors or pituitary carcinomas, defined by craniospinal or systemic metastatic spread of the primitive tumor, are extremely rare as they account for only 0.1% of pituitary tumors (31). No evidence has been found so far to support a higher risk of pituitary carcinoma in MEN1 patients compared with the general population (32). Nevertheless, abnormalities of chromosome 11 are suspected to play a role in the aggressiveness of pituitary lesions as, according to Mete et al., “Loss of chromosome 11p and/or 11q, the region known to harbor the MEN1 locus, could be a critical step in prolactinoma progression to aggressive behavior” (32,33).

Only about 150 cases of pituitary carcinoma have been reported in the literature. Diagnosis of such carcinomas is difficult, with metastatic spread being the only criteria differentiating them from adenomas. Hence, their diagnosis is typically delayed from 7-10 years (31). Once these tumors metastasize, their prognosis is poor (34,35). In patient III.6, the diagnosis was delayed by 20 years. This emphasizes the need for diagnostic clues. Several signs, such as early recurrence, partial antisecretory efficacy of dopamine agonists and tumor growth despite this treatment or radiotherapy might indicate a more aggressive pituitary tumor. This does not, however, allow the distinction between a pituitary carcinoma and an aggressive adenoma, although with close to 100% of antitumor efficacy in the series described in literature for non-secreting pituitary adenomas, regrowth of the tumor is highly unusual (36,37). In patient III.6 development of the tumor during radiotherapy occurred at age 30, along with drastic progression next to the zone treated by Gamma Knife.

We searched the literature and found 3 cases of pituitary carcinomas that had been linked to MEN1. Scheithauer et al. reported the case of a thyrotrophin cell carcinoma consisting solely of thyrotroph cells and secreting thyrotrophin, α -subunit and prolactin in a MEN1 patient who developed both craniospinal and systemic metastases (35). Their finding spoke against an adenoma-carcinoma sequence for the development of the tumor, rather indicating a *de novo* pituitary carcinoma development (35). A case of a MEN1 patient with prolactin-secreting pituitary carcinoma initially presenting with pituitary apoplexia and harboring a heterozygous missense mutation in exon 3 (p.C165R) was reported by Philippon et al. The patient had received surgery, radiotherapy and dopamine agonist treatment that allowed control of the pituitary tumor. When the metastatic spread was detected, the patient responded to temozolomide chemotherapy and his tumor was still controlled long-term after temozolomide withdrawal (36). Finally, another case of non-secreting pituitary carcinoma responding to temozolomide treatment in a MEN1 patient was described by Morokuma et al. (38).

The mutation in this family may represent a possible explanation for these unusual features, although correlation between genotype and tumor aggressiveness has not been determined (7). Large deletions have rarely been reported and account for approximately 1% of MEN1 germline mutations (39,40). However, several reports have been published regarding a possible correlation between mutation type and phenotype, but have failed to point out an association between large deletions and a more aggressive phenotype (17,39,41). In a report of 200 unrelated cases from Sweden that included patients with large germline deletions, no correlation was found between mutation type and the severity of the disease (17). Furthermore, they pointed out the considerable variability of

expression within families (17). Moreover, mutations of the *MEN1* gene are usually scattered throughout the whole gene and do not perturb the interaction with one particular partner of menin (17,42). The hypothesis proposed to explain this was that all menin mutations might result in a rapid degradation of the truncated protein via the ubiquitin-proteasome pathway (17,43).

Nevertheless, the limits of the deletion found in pedigree 1 are not known. MLPA showed a total deletion of all 10 exons of the gene, but the boundaries were not delimited. The deletion may thus affect one or numerous other genes or gene promoters, which could account for the aggressiveness of some of the tumors expressed in the family, particularly the pituitary carcinoma. Raef et al. recently studied a large MEN1 family who displayed an aggressive phenotype with malignant pancreatic endocrine tumors being present in five subjects. MLPA and aCGH gene copy number analysis showed a large deletion including the *MEN1* promoter and the two first exons of the gene. LOH analysis showed somatic deletion of maternal chromosome 11 that included *MEN1* locus and imprinting control regions located at 11p15. The consequences were up-regulation of the paternally expressed *IGF-2* and loss of maternally expressed *CDKN1C/p57KIP2* in the pancreatic tumors. They concluded that disruption of other genes, such as *CDKN1/p57KIP2* and *IGF-2* in this case, might play a role in tumor progression and aggressive phenotype (39). We searched the literature for reports of MEN1 patients with a large deletion of the *MEN1* gene and at least one of the adjacent genes on chromosome 11, *MAP4K2* and *CDC42BPG* (Figure 1C), also named *KAPPA-200*. We found a single report about a Japanese family with a 68kbp deletion flanked by a 3-base pair repeat that included *MEN1*, *MAP4K2* and *KAPPA-200* genes (44). Although having a large deletion that included two other genes, members of the family presented with a typical MEN1 syndrome and did not demonstrate unusually aggressive clinical features, leading Kikuchi et al. to assume that heterozygous deletion of those genes does not cause obvious pathological effect (44). However, further genetic analysis aiming to map the boundaries of the deletion should be done in pedigree 1 members, as other genes or imprinting control regions further away on the chromosome might be affected.

We found other studies that had searched for an additional role of other genes in patients displaying unusual features of MEN1 syndrome. In a report about a patient that had presented with a mammosomatotroph pituitary adenoma at age 5 years, the role of somatic point mutations in the *GNAS1* gene has been questioned. No such mutations were found, but different changes in *GNAS1* or changes in other genes have not been excluded (30). Other genes such as *GNAS1* have already been shown to play a role in the etiology of sporadic endocrine tumors (12) and may influence development of tumors in MEN1 syndrome as well. Similarly, the role of environmental and epigenetic factors has been proposed as menin, functioning as a scaffold protein, might modulate gene expression via histone methylation or acetylation. Thus, menin might both repress or activate transcription (2,7,45), which would influence the clinical expression of the syndrome.

The aggressiveness of pituitary tumors in the proband contrasts with the indolence of his parathyroid adenomas, being asymptomatic albeit recurring. Besides patient III.6, patients IV.2 and IV.6 demonstrated early expression of the syndrome with a probable insulinoma at age 7 years and averred PHPT at age 11 years, respectively. However, even if the penetrance at age <10 years is estimated to be 7%, such tumors have already been reported in younger patients (2). Indeed, guidelines regarding screening of mutation carriers recommend to begin screening for insulinomas and PHPT at ages 5 years and 8 years, respectively (1). Patient IV.6 was also found to have multiple lipomas at age 10

years, but we did not find data regarding age-related penetrance of those lesions in MEN1 patients. Patient II.2 was found with atypical thymic carcinoid and PHPT due to two parathyroid adenomas. This type of tumor is rather infrequent, with a reported prevalence of 2% (2). As shown by Goudet et al., thymic NETs increase the risk of death by almost four times compared to patients without those lesions (8). In comparison, gastrinomas and non-functioning pancreatic tumors only multiply the risk of death by a factor 1.9 and 3.4, respectively (8). Despite certain family members demonstrating early onset of tumors, III.6's pituitary carcinoma stands out. Interestingly, none of the other family members displayed a pituitary tumor. If a factor besides the MEN1 deletion influences the phenotype, it might be implicated solely in pathogenesis of pituitary tumors.

Genotype – phenotype correlation

Numerous papers investigating a potential genotype-phenotype correlation in MEN1 have been published, although no correlations have been found. For instance, multiple studies have been conducted on a multicenter cohort based in France and Belgium. No significant differences regarding prevalence, histology and secretion pattern of pituitary lesions have been found between patients with different mutation types (27,28). A case-report of a patient that had presented with insulinoma at age 8 years and his family also speaks against a correlation between the mutation and clinical presentation. Although sharing the same mutation, the patient, his father, and his three brothers (two of them being monozygotic twins), all showed different disease patterns (46) which illustrates well phenotypic variability. Other studies report monozygotic twins with the same *MEN1* mutation and different phenotypes (6,47) or unrelated families sharing the same mutation and expressing large inter- and intra-familial variability (4). It has been assumed that random chance for the second mutation might account for this phenotype variability (48). Alternatively, mosaicism, allele-specific expression, modifier genes and epigenetic or environmental effects may play a role regarding the poor genotype-phenotype correlations. Nevertheless, several elements challenge these conclusions and remain incompletely explained. First, several variants of MEN1 have been reported in patients carrying *MEN1* mutations. Familial isolated hyperparathyroidism (FIHP) defined by the sole occurrence of parathyroid tumors has been diagnosed in patients with *MEN1* mutations, with a predominance of missense mutations. However, those mutations are not clustered but rather spread through the whole gene as in MEN1 and the some mutations have been shown to occur both in MEN1 and FIHP, as a 4bp deletion at codons 83-84 for example (1,2,12). Moreover, a so-called Burin or prolactinoma variant of MEN1 with frequent prolactinoma (40 vs. 22% prevalence) and rare gastrinoma (10 vs 42% prevalence) has been reported in a large kindred and two further families with no shared mutations, although they were found to harbor nonsense mutations (Tyr312X, Arg460X) (1,49,50). Finally, although recently confirming the absence of direct genotype-phenotype correlations, a multicenter study on over 800 patients conducted in France and Belgium reported a higher risk of death due to MEN1 tumors in patients with mutations affecting the JunD interacting domain (51). As our understanding of menin and the different pathways in which it is involved expands, explanations to these phenomenons and genotype-phenotype connections, if not correlations, may be discovered.

Limitations

Several limitations may affect our study. First, most of the files lacked documentation and complete information about the patient, including the treatment and the course of the

disease. For example, the lack of information or documentation regarding certain patients prevented us from determining whether the syndrome was familial or sporadic in three out of eleven pedigrees. Additionally, most of the patients have a long history of disease. A comprehensive and precise collection of the data is therefore difficult, particularly when patients are followed by several centers and general practitioners. Furthermore, gathering of different family members affected by MEN1 is sometimes difficult or impossible. Such situations may lead to incomplete patient records, adding a new bias to retrospective studies based on those files. To minimize this, we had to exclude one patient for whom we could not gather enough information. Moreover, screening guidelines and diagnostic criteria as well as genetic testing availability and methods have evolved over the years, which has likely influenced the diagnosis of certain lesions, their treatment or the outcome in different patients. Besides, a bias regarding the age of patients related with the increasing penetrance of the syndrome with the age exists. Hence, the tumor pattern depends on the age. To minimize this bias, we excluded the asymptomatic patients from our statistics regarding prevalence of the different lesions. Finally and as stated before, seven out of eleven families did not undergo genetic testing and the MEN1 diagnosis in these families is based solely on the clinical criteria. An effort is being made to gather DNA for the patients that were not tested.

Conclusion

Follow-up in Multiple Endocrine Neoplasia type 1 is challenging, as it requires regular blood tests and imaging to detect the evolution of the disease and the occurrence of new lesions. Such intensive care can be exhausting for the patients, particularly when they are suffering from multiple and recurring tumors. Moreover, such follow-up is also demanding for physicians, as it requires coordination between the multiple medical specialties involved. According to the latest guidelines (1), such follow-up should be done in a center with expertise in MEN1 management and experienced endocrinologists, endocrine surgeons, radiologists and geneticists. Such an organization would improve the chances of an exhaustive, up to date and high-quality follow-up and screening. Said screening is particularly important in the context of familial syndrome, as it can greatly influence the age at diagnosis and early treatment of tumors in order to minimize the repercussions of the disease.

As mentioned before, genetic counseling and testing is important for the patient's care. Genetic testing of patients not only confirms the clinical diagnosis, but also has important implications for the entire family. Indeed, it enables the possibility to determine whether descendants are affected by the disease or not and thus helps establishing an efficient screening program for patients, as well as reassuring non affected family members and alleviating them from the burden of the screening (1).

Several patients whose records were reviewed experienced suboptimal care with no genetic testing and what seemed to be an insufficient screening and follow-up. As mentioned previously, genetic analysis of the *MEN1* gene is critical for the care of MEN1 patients, especially when family members are suspected to be affected.

We recommend that MEN1 patients should be identified and gathered in qualified centers and that a multicenter database should be constituted. Such organization would provide a global view of the patient's care and reveal itself useful for further studies on Swiss MEN1 patients.

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