CLINICAL STUDY

Multiple endocrine neoplasia type 1 in Brazil: MEN1 founding mutation, clinical features, and bone mineral density profile

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Abstract

Objective: Only few large families with multiple endocrine neoplasia type 1 (MEN1) have been documented. Here, we aimed to investigate the clinical features of a seven-generation Brazilian pedigree, which included 715 at-risk family members.

Design: Genealogical and geographic analysis was used to identify the MEN1 pedigree. Clinical and genetic approach was applied to characterize the phenotypic and genotypic features of the family members.

Results: Our genetic data indicated that a founding mutation in the MEN1 gene has occurred in this extended Brazilian family. Fifty family members were diagnosed with MEN1. Very high frequencies of functioning and non-functioning MEN1-related tumors were documented and the prevalence of prolactinoma (29.6%) was similar to that previously described in prolactinoma-variant Burin (32%). In addition, bone mineral density analysis revealed severe osteoporosis \((T, K=2.87, G=0.32)\) of compact bone (distal radius) in hyperparathyroidism (HPT)/MEN1 patients, while marked bone mineral loss in the lumbar spine \((T, K=1.95, G=0.39)\), with most cancellous bone, and femoral neck (mixed composition; \(T=1.48, G=0.27\)) were also present.

Conclusions: In this study, we described clinically and genetically the fifth largest MEN1 family in the literature. Our data confirm previous findings suggesting that prevalence of MEN1-related tumors in large families may differ from reports combining cumulative data of small families. Furthermore, we were able to evaluate the bone status in HPT/MEN1 cases, a subject that has been incompletely approached in the literature. We discussed the bone loss pattern found in our MEN1 patients comparing with that of patients with sporadic primary HPT.

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Introduction

Multiple endocrine neoplasia type 1 (MEN1; OMIM 131100) is an autosomal dominant inherited disorder characterized by a predisposition towards the combined occurrence of multiple endocrine tumors, mostly involving the parathyroid and anterior pituitary glands, pancreatic islets, and endocrine duodenal cells (1, 2). More than 20 other endocrine and non-endocrine tumors have been reported in this disease, including bronchopulmonary, gastric and thymic carcinoids, non-functioning adrenal tumors, and dermic tumors (1, 2).

The MEN1 gene codes a protein MENIN. Most MEN1 cases carry a germline mutation in the MEN1 gene, which affects MENIN protein interaction with Jun-D and other factors involved in transcriptional regulation, DNA repair, genome stability, apoptosis regulation, and endocrine cell proliferation (3–5). Still, no important genotype–phenotype correlation in MEN1 has been established so far (6–9).

The prevalence of hyperparathyroidism (HPT) in MEN1 has been found to be high in most studies (82–100%) (10, 11); however, the prevalence of enteropancreatic endocrine tumors (PETs; 10–85%) (6, 12) and pituitary tumors (PITs; 16–65%) vary greatly in the reported MEN1 series (MEN1-S) (11, 13). This variable prevalence combined with different biological behaviors of MEN1-related tumors leads to considerable intra- and inter-familial phenotypic variability, which is typical of MEN1 (2, 14). We reviewed the literature and nine informative MEN1-S were found. These series were characterized mostly by small families, including 343 families with 873 affected cases and a highly variable prevalence of classic
MEN1-related tumors (6, 8, 10, 11, 15–19; Supplemental Table 1, which can be viewed online at http://www.eje-online.org/supplemental/).

In contrast with this disorganized and variable phenotypic pattern, which is common for MEN1, large families have contributed to characterize phenotypic variants with homogeneous, specific, and reproducible patterns: i) MEN1-familial isolated primary HPT variant, which is defined by the presence of HPT without occurrence of the other two classic MEN1-related tumors (20–23) and ii) prolactinoma variant of MEN1 (MEN1_Burin), which is characterized by the predominance of prolactinomas and a low penetrance of gastrinomas (12, 14, 24).

Only a few large families with MEN1 have been described. Trump (1996) and Wautot (2002) studied 62 and 170 families and reported that only 7.2 and 5.9% of them had five or more affected members (7, 17) respectively. We reviewed 527 MEN1 families (OMIM, www.pubmed.com, www.hgmd.org): 12 of them (12/527; 2.3%) had 20 or more MEN1 cases and 5 (5/527; 1%) presented more than 50 MEN1 cases (ranging from 55 to 165) and/or more than 25 affected members were clinically analyzed (29–124) (12, 13, 25, 26) (Supplemental Table 2, which can be viewed online at http://www.eje-online.org/supplemental/).

Studying these five very large MEN1 families (here considered as more than 25 affected cases) has documented phenotypic peculiarities, such as the absence of acromegaly in Tasman 1 family, characterization of clinical variants as in MEN1_Burin, and low prevalence of gastrinoma in a Finnish family (14, 24, 26, 27).

Furthermore, 12 founding MEN1 mutations were reported in MEN1 kindreds from Europe (Finland, France and Sweden), Oceania (Australia), and North America (Canada and USA) (15, 30–35). Notably, all five previously reported very large MEN1 genealogies were associated with founder chromosomes (12, 13, 25, 26). Founder effects were documented in these five very large families by combining genetic studies (same germline MEN1 mutation and common haplotype) and clinical investigations with genealogical data associated with a common geographic origin (12–14, 25–30).

Another aspect of the MEN1 phenotype is bone mineral status. Recently, Burgess et al. (1999) evaluated bone mineral density (BMD) in MEN1 by analyzing two bone sites: the lumbar spine and femoral neck (FN) (31). The proximal one-third of the distal radius (DR), however, which is enriched in compact bone and is preferentially affected in sporadic HPT, has not been studied so far in MEN1.

In this paper, we studied three apparently unrelated Brazilian MEN1 clusters, involving 50 cases, which shared the same MEN1 mutation and common ancestry. The frequency of classic MEN1-related tumors observed in this family was compared with that of the five previously reported very large MEN1 families (333 affected cases), as well as with nine MEN1-S made up of 343 families involving 873 MEN1-affected cases. To further characterize the MEN1 phenotype, we also evaluated BMD, a subject that had been incompletely approached so far.

**Materials and methods**

**Clinical approach**

The diagnosis criteria used for MEN1 and MEN1-related endocrine tumors were those of the MEN Consensus 2001 (1) (See Supplemental material, which can be viewed online at http://www.eje-online.org/supplemental/). Briefly, the diagnosis of MEN1 was based on the presence of at least two of three main MEN1-related endocrine tumors (parathyroid, pituitary, and PETs). Familial MEN1 was defined as one MEN1 case (propositus) plus at least one first-degree relative with at least one of the three classic tumors, as previously established. The main MEN1-related endocrine tumors and other clinical manifestations such as carcinoid and adrenal tumors were systematically evaluated in affected and at-risk relatives (1, 32).

We excluded the asymptomatic MEN1 mutation carrier from the estimation of the prevalence of HPT as well as of other MEN1-related tumors, as this inclusion may cause a bias in this type of analysis (Hao et al. 2004). Further, when the age-related penetrance of the main MEN1 tumors was examined, the asymptomatic MEN1 carrier was appropriately included.

This study was approved by the ethics committee of the Hospital das Clínicas of the University of São Paulo and the Brazilian Federal Committee. Informed consent was obtained from each individual before performing the genetic analysis.

**Clinical data**

We initially identified three apparently unrelated MEN1 families (A, B, and C index cases) living in an area about 300 km apart from each other in Southeast Brazil (Table 1).

**Genetic approach**

Blood samples were collected from the MEN1 index cases, affected patients, and at-risk family members. Genomic DNA extraction, specific primers, PCR conditions, and sequencing reaction protocols were carried out as previously described (33, 34).

**Haplotype analysis**

To verify a common chromosomal ancestry of the three apparently unrelated MEN1 families, the haplotype of the MEN1 gene region was determined.
automatically using fluorescently labeled primers for four microsatellite loci: PYGM, D11S1314, D11S4175, and D11S901.

**BMD analysis**

BMD was assessed using dual-energy X-ray absorptiometry (DEXA; Hologic QDR-4500 S/N 45130, Waltham, MA, USA). Three different bone sites were examined: the proximal one-third of non-dominant DR (1/3 DR), the right FN, and the lumbar spine (L1–L4). The coefficients of variation were lower than 3% for all bone sites. Data were reported as BMD (g/cm²), T-scores (difference from the mean BMD value in a healthy young reference population, in S.D. units), and Z-scores (age-matched comparison in S.D. units). We used the World Health Organization (WHO) criteria for the diagnosis of osteoporosis (T-score ≤ −2.5 S.D.) and osteopenia (T-score ≤ −1.0 S.D.) (35).

**Statistical analysis**

For analysis of the descriptive data, we used the mean (S.E.M. or S.D.) and/or median (range). For comparison of the prevalence of classic MEN1-related tumors in our family to those of the other five very large MEN1 families
previously reported as well as the prevalence of tumors within these families and nine MEN1-S, we used an unpaired t-test and defined $P < 0.05$ as statistically significant. When appropriate, Fisher’s exact test and Mann–Whitney $U$ test were used. Age-related penetrance of the main MEN1-related endocrine tumors was estimated using the Kaplan–Meier life table.

Results

Germline MEN1 mutation analysis

Mutational analysis of three apparently unrelated MEN1 families revealed the 308delC disease-causing frameshift mutation at exon 2 of the MEN1 gene (Fig. 1A). This mutation could also be written as c.198delC considering the MEN1 cDNA reference sequence (9). The deletion is predicted to lead to a truncated protein due to stop codon at position 118 and was not present in 100 control chromosomes (33, 34).

Haplotype analysis

Haplotype analysis with four MEN1-surrounding microsatellites was performed in the propositus of each of the three genealogies, in order to see whether this recurrent mutation resulted from either a founding mutation or independent genetic events. The three index cases shared the same MEN1-flanking haplotype (Fig. 1B), indicating that the 3 clusters had inherited the 308delC mutation from a common founder.

Genealogical and geographic analysis

The genealogical investigation was performed through the analysis of personal official documents, records, and the geographic origin of the three clusters. This allowed us to identify that the three apparently unrelated genealogies came from a common Italian founder couple, who were localized by the Immigrant Museum (Sao Paulo). This couple was born in 1863–1864 in Veneto, Italy and came to Brazil in 1888 (St Giorgio ship; Fig. 2). They had ten children: five of them were obligatory MEN1 mutation carriers and two others had no affected MEN1 descendants (Fig. 3). Two other siblings (with more than 200 descendants) could not be studied, as with the last sibling, who probably died in infancy. This family is presently in its seventh generation, including 715 at-risk members, 81 already deceased (ten of them during the study) members, and 634 members that are still living.

Fifty MEN1 germline mutation carriers were documented using different criteria (Table 1; Fig. 3). Thirty-four patients were clinically identified (cases 1–34). Of them, 30 (88.2%) were also genetically documented as 308delC MEN1 mutation carriers and two others had no affected MEN1 descendants (Fig. 3). Two other siblings (with more than 200 descendants) could not be studied, as with the last sibling, who probably died in infancy. This family is presently in its seventh generation, including 715 at-risk members, 81 already deceased (ten of them during the study) members, and 634 members that are still living.

Clinical findings

Diagnosis and distribution of MEN1-related tumors

In the 28 affected cases that were fully examined clinically, 76 MEN1-related tumors were diagnosed: 25 parathyroid tumors, 13 PPTs, 24 PETs, 2 bronchopulmonary carcinoid tumors (BC), 2 gastric...
carcinoid tumors (GC), and 10 adrenocortical tumors (ATs; Table 1). Only seven tumors (9.2%) had been recognized before admission of the patients to our service. The mean age at the diagnosis of MEN1 was 39.10 ± 16.10 years (14–61). Twenty-five percent of patients (7/28) had combined tumor involvement of the three main target endocrine glands; 39.2% (11/28) presented with tumors of two glands; 32.14% (9/28) of one gland; and only one presented with no tumors (3.6%; case 12: asymptomatic mutation carrier). The most prevalent tumor patterns were HPT only (28.6%), HPT/PIT/PET (25%), and HPT/PET (25%). Less frequent combinations were HPT/PIT (10.7%), PIT/PET (3.6%), and PIT only (3.6%).
Symptomatic versus asymptomatic cases

In the 24 symptomatic MEN1 cases (85.7%), HPT was the first clinical manifestation in 70.8% (17/24), PIT in 25% (6/24), and PET in 4.2% (1/24). Among the four asymptomatic cases (14.3%), three had HPT only or HPT associated with another non-functioning classic MEN1 tumor (Table 1), and the fourth presented dermic tumors (angiofibromas and collagenomas) as the first clinical manifestation (case 12; Table 1).

Clinical pattern of MEN1-related tumors

HPT. The prevalence of HPT in the studied patients was 93% (25/27), excluding the asymptomatic carrier (case 12; Table 1). The mean age at the time of HPT diagnosis was 40.68 ± 14.99 years (range, 14–61), and mean serum values of parathyroid hormone (PTH) and calcium were 115.2 ± 23.1 pg/ml (range, 67.6–163) and 11.02 ± 0.11 mg/dl (range, 10.7–11.2) respectively. Hypercalciuria (greater than 300 mg/vol., 24 h) was present in 57% (12/21). All cases with symptomatic HPT (19/25; 76%) had a clinical history of nephrolithiasis. The mean age at the first episode of renal calculi was 23.1 ± 5.25 years (range, 16–40), and the mean interval between the first renal crisis and the diagnosis of HPT was 22.2 ± 10.3 years (range, 1–38). All (6/25) except one of the asymptomatic HPT cases were younger than 25 years old, and four of them became symptomatic 2 years after the diagnosis: three with nephrolithiasis and one with a pathological forearm fracture (case 19).

HPT and BMD. We analyzed the BMD of 20 MEN1 adult cases with uncontrolled HPT (three with a history of unsuccessful previous parathyroid surgery; Table 2). We excluded five HPT/MEN1 cases from the BMD analysis when patients were less than 20 years old or when bone densitometry could not be performed. The clinical data referring to the individual HPT/MEN1 phenotype of the 20 cases with BMD analysis are shown in Table 3A.

Using the WHO criteria, 75% of these cases had osteoporosis in at least one of the three bone sites (15/20): 50% (10/20) of them in one of the main bone sites, 5% in two bone sites, and 20% in all three bone sites. Additionally, 60% (12/20) of the cases presented with osteopenia in at least one of the three bone sites: 25% in one of the bone sites and 35% in two sites. Only one case (1/20, 5%) had normal BMD at all three bone sites (case 10; Table 3B: full densitometric data of the 20 cases).

Sixty T-score values were obtained when BMD was analyzed at the three bone sites in these 20 patients. Reduced bone mass was noted in 72% (43/60); most cases were compatible with osteoporosis (24/60; 40%); and osteopenia was also common (19/60; 33.4%; Table 3B).

Low BMD occurred preferentially in the proximal one-third of the DR (90%; 1/3R), followed by the FN (65%; FN) and lumbar spine (60%; L1–L4; Fig. 4). Osteoporosis was more prevalent in the 1/3R (55%) compared with...
L1–L4 (40%) and FN (20%). Conversely, osteopenia was more prevalent in the FN (45%) than in the 1/3R (35%) and L1–L4 (20%; Fig. 4). The degree of bone demineralization was more severe in the 1/3R (T = −2.87 ± 0.32) and less severe in L1–L4 (T = −1.92 ± 0.39) and FN (−1.48 ± 0.27; Table 2; Fig. 5).

In 20% of the cases, the 1/3R was the only compromised bone site. In contrast, no case presented bone demineralization occurring only in either the FN or the lumbar spine (Table 3).

We found a direct relationship between serum levels of PTH and serum ionized calcium (iCa), as well as a correlation between the age at the time of HPT diagnosis and the duration of history of renal calculi. There was no correlation between serum levels of PTH and BMD values. We found an inverse relationship between the duration of history of renal calculi and T-score values in both the 1/3R and L1–L4 sites. We also observed an inverse correlation between the duration of history of renal calculi and T-score values in both the 1/3R and L1–L4.

Additionally, we found an inverse correlation between the age at the time of HPT diagnosis and 1/3R and L1–L4 T-score values.

PETs. All 28 cases were submitted to complete hormonal screening for pituitary adenomas. Pituitary MRI studies were performed in 24 of these cases (86%; Table 1). Again, considering the analysis of prevalence, the asymptomatic MEN1 mutation carrier (case 12) was excluded. Thus, considering full screening (hormonal and radiologic), pituitary adenoma was evident in 52% (12/23) of cases: 58.3% (7/12) had prolactinomas only, 33.3% (4/12) had non-functioning pituitary adenoma (NFPT) and one presented a double adenoma (prolactinoma and luteinizing hormone (LH)-secreting tumor: case 18, Table 1).

There was no case with Cushing disease or acromegaly. Microadenoma was identified in 83.3% (10/12) and macroadenoma in 16.7% (2/12) of cases. The mean age at the time of diagnosis of PIT was 32.25 ± 15.89 years (range, 14–55), and it was found before ages 20 and 25 years in 33 and 50% of cases respectively. The frequency of prolactinoma was 29.6% (8/27; case 12, the asymptomatic MEN1 carrier, was not included) and the frequency of NFPT was 17.4% (4/23). The mean age at the time of diagnosis of prolactinoma and NFPT was 29.5 ± 15.71 years (range, 16–55) and 38.25 ± 16.1 years (range, 16–54) respectively. Mean prolactin serum levels were 106.7 ± 76.9 ng/ml, ranging from 30 to 251 ng/ml (normal values for males, 2–10 ng/ml; for females, 2–15 ng/ml).

PETs. PETs were found in 68.2% (15/22) of the cases that were submitted to full radiologic exams, including endoscopic ultrasound (Table 1). The frequency of non-functioning pancreatic endocrine tumor (NFPT) was 80% (12/15), the frequency of gastrinoma was 73.3% (11/15), and the frequency of insulinoma was 13.3% (2/15). Co-occurrence of functioning and non-functioning tumors was evident in 60% (9/15) of cases: seven gastrinoma/NFPT, one gastrinoma/insulinoma/NFPT, and one insulinoma/NFPT. However, six other cases presented only one tumor type: three (20%) gastrinomas and three (20%) NFPT. The three cases with NFPT only were younger than 40 years of age at the time of diagnosis. As with functioning PET, 25% of the cases (3/12) were diagnosed before 30 years of age (one insulinoma and two gastrinomas). Gastrinoma was diagnosed after 30 years of age in 81.8% of cases. The mean age at the time of diagnosis of PET was 39.93 ± 14.08 years (range, 16–61). The prevalence of NFPT, gastrinoma, and insulinoma was 54.5% (12/22), 40.7% (11/27) and 7.4% (2/27) respectively. The mean age at the time of diagnosis of gastrinoma and NFPT was 43.09 ± 13.26 years (range, 22–61) and 39.36 ± 14.17 years (range, 16–61) respectively.

Other tumors

AIFs were documented in 45.5% of cases (10/22). The mean age at diagnosis was 49 ± 9.14 years (range, 33–61), and all were older than 30 years of age (Table 1).
Table 3 Phenotype of 20 hyperparathyroidism (HPT)/multiple endocrine neoplasia type 1 (MEN1) cases.

A: Clinical findings

<table>
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<th>Cases</th>
<th>Age (year old)/sex</th>
<th>Ionized calcium (mg/dl)</th>
<th>Calcium (mg/dl)</th>
<th>Serum phosphorus (mg/dl)</th>
<th>PTH (pg/ml)</th>
<th>Age at the time of HPT diagnosis (years)</th>
<th>Presence/history of renal calculi at the diagnosis of HPT</th>
<th>Age at the first episode of renal calculi (years)</th>
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<td>Mean values ± s.e.m.</td>
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<td>11 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>118 ± 28.6</td>
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<td>Reference values</td>
<td>4.6–5.3</td>
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<td>2.7–4.5</td>
<td>11–62</td>
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<td>NA</td>
<td>NA</td>
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</tbody>
</table>

B: Densitometric data

<table>
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<tr>
<th>Cases</th>
<th>Age at the time of HPT diagnosis (years)/sex</th>
<th>1/3 proximal distal radius (1/3 R)</th>
<th>Femoral neck (FN)</th>
<th>Lumbar spine (L1–L4)</th>
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<tr>
<td></td>
<td></td>
<td>T</td>
<td>Z</td>
<td>g/cm²</td>
</tr>
<tr>
<td>5</td>
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<td>6</td>
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<tr>
<td>20</td>
<td></td>
<td>−4</td>
<td>−2.9</td>
<td>0.452</td>
</tr>
</tbody>
</table>
All cases were asymptomatic with normal values of adrenal hormones and were diagnosed by radiological exams and, in 30%, the tumors were bilateral.

BC tumors were found in 2/23 cases (8.7%) and diagnosed at 57 and 59 years of age (Table 1: cases 25 and 27). Two patients had GC tumors at 32 and 41 years of age (8.7%; Table 1: cases 5 and 13).

Clinical versus genetic diagnosis

In the 30 MEN1 mutation carriers, 17 were clinically diagnosed before genotyping and 13 were recognized after the mutation analysis. The mean age at the diagnosis of the 17 cases (46.7 ± 12.34) was higher than in the 13 cases diagnosed by genetic screening (27 ± 14; P = 0.001). Only 30.7% of the genetically screened cases were asymptomatic (P < 0.05). The prevalence of classic MEN1-related tumors within the 17 clinically diagnosed cases was: HPT (100%), PET (78.6%), PIT (50%), AT (64.3%), BC (11.8%), and GC (11.8%). Tumor prevalences in ten genetically identified cases (Table 1) were: HPT (80%), PET (50%), PIT (55%), and AT (12.5%); no carcinoid tumor was documented in these cases. Case 12 (asymptomatic MEN1 carrier) and cases 29 and 30 (MEN1 carriers deceased before clinical evaluation) were excluded from this analysis.

Deceased cases

The 81 deceased family members were dispersed among the first to seventh generation of the genealogy (Fig. 3), and 26 of them were MEN1-related cases (Table 1: cases 26–50; including the founder couple). The mean age of death in this group was 57.65 ± 15.73 years (range, 26–84). A clinical history strongly suggestive of MEN1 was identified in 69% (18/26) of cases, and death certificates revealed MEN1-related mortality in 53.8% (14/26). The most frequent causes of death were pulmonary and enteropancreatic tumors, renal insufficiency, and complicated gastro-duodenal ulcers.

Prevalence of classic MEN1-related tumors

We analyzed the prevalence of classic MEN1-related tumors observed in our family (F6) and in very large families, F1–F5 (Table 4: Fig. 6). Also, data from a subset of nine large informative MEN1-S represented by 343 MEN1 families with 873 affected cases were analyzed (Supplemental Table 1) (12, 13, 15, 20, 28, 49, 50–52).

Very large MEN1 families and MEN1-S

The prevalence of HPT, PIT, and PET observed in 360 MEN1-affected cases in the subset F1–F6 were 93, 25,
and 49% respectively (Table 4). The frequencies of the same MEN1-related tumors in the nine informative MEN1-S were 95, 40, and 57% respectively (Table 4 and Supplemental Table 1).

No difference in HPT frequency was noticed between F1–F6 and MEN1-S (P > 0.253); however, the prevalence of PIT (P < 0.0001) and PET (P = 0.021) in F1–F6 was lower than in MEN1-S. If MEN1 prolactinoma variant (F5) was excluded from this analysis, considering its exceedingly low prevalence of PET, the frequencies of PET in these five very large families and MEN1-S became similar (P = 0.10). However, the difference in prevalence of PIT still remained (P < 0.001; Table 4). Considering tumor subtypes, NFPT was more prevalent in families 1–6 than in MEN1-S (28% vs 8%; P = 0.037; Table 4).

All very large MEN1 families presented at least one of the major MEN1-related tumors with prevalence divergent from that of MEN1-S (Table 5; Fig. 6).

### Very large MEN1 families

Families 1–6 presented a similar prevalence of HPT (P > 0.18–0.90; Table 4; Fig. 6). Our family (F6) presented the highest combined prevalence of PIT/PET (52%, 68%). Three families had low expression of one of the classic tumors: F1–F2 (PIT: 16%, 18%) and F5 (PET, 10%). Another two families, F3 and F4, presented dominance of one of these tumors (PET: 72 and 74%; Table 4; Fig. 6).

**PETs** Families F1, F3, F4, and F6 presented a homogeneous tumor pattern characterized by a high prevalence of PET (65–74%) when compared with F2 and F5 (P < 0.05). The MEN1_Burin (F5) had the lowest prevalence of PET (10 versus 49–74%; P < 0.0001), whereas the F2 family presented an intermediate prevalence of PET, which was lower than F3 (P = 0.012) and F4 (P = 0.038; Table 4 and Supplemental Table 3A, which can be viewed online at http://www.eje-online.org/supplemental/; Fig. 6).

Families with a higher prevalence of PET (F1, F3, F4, and F6; 65–74%) also had the highest prevalence of NFPT (20–69%) (Fig. 6). Families more recently reported, such as F3 and F4 and ours, had higher frequencies of NFPT (39–69%) than families that were reported earlier, such as F1, F2, and F5 (3.2–20.3%; P < 0.0001; Table 4). Family 4 had the highest frequency of NFPT among the six MEN1 families (Fig. 6). In our family, NFPT was the most prevalent pancreatic tumor (54.5%) and the second most frequent tumoral subtype after HPT (Table 4; Fig. 6).

F1–F3 and F6 presented similar prevalence of gastrinoma (36–49%; P > 0.33). Gastrinoma was more uncommon in the Finnish family, F4 (3%), and in the MEN1 prolactinoma variant, F5 (4.3%; and...
We observed a heterogeneous pattern of combined prevalence of the pancreatic tumors in F1–F6. Our family (F6) and MEN1 Burin had the highest (41 and 54.5% respectively) and the lowest (4.3 and less than 6% respectively) combined frequency of gastrinoma and NFPT. Family 4 had the highest and the lowest prevalence of NFPT (69%) and gastrinoma (3%) respectively. In contrast, the American family (F2) had the lowest and the highest frequency of NFPT (3.2%) and gastrinoma (49%) respectively. F1 and F3 had intermediate prevalence of these pancreatic tumors (Table 4; Fig. 6).

PITs Our family presented the highest prevalence of PIT among the six very large MEN1 families, but this value was significant only relative to F1–F3 (52 vs 16–26%; P < 0.036; Table 4; Supplemental Table 3A; Fig. 6). Most very large families presented similar prevalence of prolactinoma (16–32%; Table 4 and Supplemental Table 3C; Fig. 6). However, prolactinoma was clearly more frequent in the MEN1 Burin (F5) than in the Tasman 1 family (F1; p < 0.019; Supplemental Table 3C). Families F5–F6 had the highest combined prevalence of prolactinoma (F5–F6: 29.6–32% versus F1–F4: 14.5–21%; P < 0.011). Furthermore, our family presented the highest prevalence of NFPiT (F6: 17.4% versus F1 and F3–F5: <2–8%; P < 0.025). No cases of acromegaly were reported in our family or in F1, F3, or F4. This tumor was suspected in one case from F2, and there was no informative data from F5.

Our family (F6) presented the highest (29.6 and 17.4%) and F2 the lowest (16 and 0%) combined

---

**Table 4**

<table>
<thead>
<tr>
<th>Very large MEN1 families</th>
<th>Country</th>
<th>MEN1 carrier</th>
<th>affected members</th>
<th>HPT</th>
<th>PIT</th>
<th>PRL</th>
<th>NFPiT</th>
<th>ACRO</th>
<th>PET</th>
<th>G</th>
<th>NFPT</th>
<th>I</th>
<th>AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 (Burgess, 1996)</td>
<td>Australia</td>
<td>165/124</td>
<td>92 18</td>
<td>16 14</td>
<td>4.5</td>
<td>65 9 1.6 16</td>
<td>49 37</td>
<td>1.6</td>
<td>68 9</td>
<td>- 0</td>
<td>28 32</td>
<td>4 27</td>
<td>NA</td>
</tr>
<tr>
<td>F2 (Marx, 1986)</td>
<td>USA</td>
<td>74/63</td>
<td>95 16</td>
<td>16 16</td>
<td>4.5</td>
<td>65 9 1.6 16</td>
<td>49 37</td>
<td>1.6</td>
<td>68 9</td>
<td>- 0</td>
<td>28 32</td>
<td>4 27</td>
<td>NA</td>
</tr>
<tr>
<td>F3 (Vierimaa, 2007)</td>
<td>Finland</td>
<td>55/39</td>
<td>97 16</td>
<td>16 16</td>
<td>4.5</td>
<td>65 9 1.6 16</td>
<td>49 37</td>
<td>1.6</td>
<td>68 9</td>
<td>- 0</td>
<td>28 32</td>
<td>4 27</td>
<td>NA</td>
</tr>
<tr>
<td>F4 (Vierimaa, 2007)</td>
<td>Finland</td>
<td>35/29</td>
<td>97 16</td>
<td>16 16</td>
<td>4.5</td>
<td>65 9 1.6 16</td>
<td>49 37</td>
<td>1.6</td>
<td>68 9</td>
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<td>28 32</td>
<td>4 27</td>
<td>NA</td>
</tr>
<tr>
<td>F5 (Green, 1999)</td>
<td>Canada</td>
<td>95/78</td>
<td>93 16</td>
<td>16 16</td>
<td>4.5</td>
<td>65 9 1.6 16</td>
<td>49 37</td>
<td>1.6</td>
<td>68 9</td>
<td>- 0</td>
<td>28 32</td>
<td>4 27</td>
<td>NA</td>
</tr>
<tr>
<td>F6 (Lourenc¸o-Jr, 2007)</td>
<td>Brazil</td>
<td>50/27</td>
<td>93 16</td>
<td>16 16</td>
<td>4.5</td>
<td>65 9 1.6 16</td>
<td>49 37</td>
<td>1.6</td>
<td>68 9</td>
<td>- 0</td>
<td>28 32</td>
<td>4 27</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Figure 6** Prevalences of tumoral subtypes in the six very large MEN1 families (F1–F6)* and in nine large MEN1 series (MEN1-S). *The six MEN1 families with more than 25 affected cases: F1, Tasman 1; F2, American family (Pennsylvania); F3 and F4, Finnish families (Northern); F5, MEN1 Burin; and F6, Brazil Southeast. HPT, hyperparathyroidism; PIT, pituitary adenoma; PRL, prolactinoma; NFPiT, non-functioning pituitary tumor; PET, enteropancreatic endocrine tumor; G, gastrinoma; and NFPT, non-functioning pancreatic tumor. Percentages of MEN1-S correspond to mean frequencies obtained from a group of nine large MEN1 series with a predominance of several small families.
prevalence of prolactinoma and NFPiT respectively, whereas F1, F3, and F4 exhibited similar combined frequencies of these tumors (prolactinoma: 14.5–21%; NFPiT: 4.5–8%). Additionally, F5 presented a dominance of prolactinoma (32%) and very low frequencies of other PITs (less than 2%; Table 4; Fig. 6).

**Age-related penetrance of main MEN1 endocrine tumors**

The age-related penetrance of the main MEN1-related endocrine tumors was examined in this MEN1 family (F6). Considering the age at the time of HPT diagnosis, we found that penetrance varied by age: 7.1% at 20 years, 28.6% at 30 years, 38.3% at 40 years, 64.3% at 50 years, and 89.3% at 60 years. When the age at the time of nefrolithiasis onset was considered, the penetrance of HPT was 21.4% at 20 years, 57.1% at 30 years, and 60.7% at 40 years. The age-related penetrances of the other main MEN1-related endocrine tumors, considering the age at the time of diagnosis, are shown in Fig. 7.

**Discussion**

We documented the clinical and genetic features of the fifth largest MEN1 family reported so far. This family included 50 MEN1 cases that either harbored the founding germline MEN1 mutation, 308delC, or were obligatory mutation carriers (Figs 1–3; Table 1). Despite the paucity of very large MEN1 families identified in the literature (1.1%), the ones that have been documented have contributed to a better characterization of the MEN1 phenotype (12, 13, 14, 20, 21, 24–27, 36, 37). For example, the description of prolactinoma-variant MEN1Burin was reported in one of the largest MEN1 families (12, 14). This variant was further suggested in other small MEN1 families (38, 39) and confirmed in two large USA families, reinforcing the existence of this clinical variant (24).

**Prevalence of classic MEN1-related tumors**

We found an excessive number of index cases (39%) in the nine informative MEN1-S (Supplemental Table 1), which may not reflect tumor frequency in their respective MEN1-affected relatives. Meanwhile, the nine MEN1-S and several other less informative MEN1-S are usually considered to represent the classic MEN1-related tumor pattern (typical MEN1) and are frequently used as references for the MEN1 phenotype (24). On the contrary, tumor frequencies in all of the very large F1–F6 MEN1 families diverged from those of MEN1-S in at least one major MEN1-related tumor (Table 5; Fig. 6).

**Table 5** Comparisons of classic multiple endocrine neoplasia type 1 (MEN1)-related tumors between the six very large (with more than 25 affected cases) MEN1 families (F1–F6) and the combined nine MEN1 series (MEN1-S).

<table>
<thead>
<tr>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>MEN1 tumors</th>
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<tr>
<td>0.164</td>
<td>0.909</td>
<td>0.485</td>
<td>0.206</td>
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<td>0.186</td>
<td>0.226</td>
<td>0.032a</td>
<td>0.099</td>
<td>&lt;0.0001b</td>
<td>0.297</td>
<td>PET</td>
</tr>
<tr>
<td>0.525</td>
<td>0.044b</td>
<td>0.491</td>
<td>0.009b</td>
<td>&lt;0.0001b</td>
<td>0.455</td>
<td>G</td>
</tr>
<tr>
<td>0.022b</td>
<td>0.167</td>
<td>&lt;0.0001b</td>
<td>&lt;0.0001b</td>
<td>–</td>
<td>&lt;0.0001b</td>
<td>NFPT</td>
</tr>
<tr>
<td>0.048b</td>
<td>0.329</td>
<td>0.464</td>
<td>0.720</td>
<td>0.257</td>
<td>0.631</td>
<td>PRL</td>
</tr>
<tr>
<td>0.857</td>
<td>–</td>
<td>0.361</td>
<td>0.470</td>
<td>0.246</td>
<td>0.049a</td>
<td>NFPiT</td>
</tr>
</tbody>
</table>

HPT, hyperparathyroidism; PET, enteropancreatic endocrine tumor; G, gastrinoma; NFPT, non-functioning pancreatic tumor; PIT, pituitary adenoma; PRL, prolactinoma; NFPiT, non-functioning pituitary adenoma.

aHigher prevalence than MEN1-S.
bLower prevalence than MEN1-S.

**Figure 7** Age-related penetrance of main endocrine tumors in the large Brazilian MEN1 family (F6). HPT, hyperparathyroidism; PIT, pituitary adenoma; PRL, prolactinoma; PET, enteropancreatic endocrine tumor; G, gastrinoma; NFPT, non-functioning pancreatic tumor; NFPiT, non-functioning pituitary tumor and AT, adrenocortical tumor. Penetrance was estimated based on age at the time of MEN1 tumor diagnosis; HPT penetrance was also estimated based on the age of nefrolithiasis (calculi) onset. We may note that PIT, PRL, PET and NFPiT curves were highly superimposed.
In addition, the prevalence of classic MEN1-related tumors within F1–F6 revealed marked phenotypic variability, as shown in Table 4. Families F1–F3 presented similar prevalence of functioning classic MEN1-related tumors: HPT (92–97%); prolactinoma (14.5–18%); and gastrinoma (36–49%), along with very low frequencies of acromegaly (0–1.6%) and insulinoma (0–8.7%). On the contrary, the prevalence of non-functioning pancreatic tumors (NFPTs) diverged markedly in these families (3.2–39%). This might be due to the variable use of somatostatin receptor scintigraphy and possibly endoscopic US, as in F3 (26). Moreover, early studies reported up to 25% of gastrinoma/MEN1 occurring in the pancreas, in contrast to recent papers showing that pancreatic gastrinomas are exceptionally rare in MEN1 (40). Conversely, frequent duodenal gastrinomas have been described in association with NFPT. Thus, NFPT reported in early studies could have been erroneously considered to be gastrinoma (41). Further, F1 and F2 were clinically diagnosed before genotyping, when clinical screening for non-functioning tumors was still inefficient (34). F4 presented similar prevalence of classic tumors to F3, except for very low prevalence of gastrinoma and excessively high prevalence of NFPT. This suggests that F4 followed an atypical MEN1 pattern and may represent a potentially new clinical variant with low expression of gastrinoma. F5 has been characterized previously as a clinical variant of MEN1 with a dominance of prolactinoma and paucity of gastrinoma. Our family (F6) and MEN1Burin had an equivalent prevalence of prolactinoma, which was higher than that of F1–F4 (F5–F6: 29.6–32% versus F1–F4: 14.5–21%; P = 0.011) but did not differ from MEN1-S.

Additionally, F6 had the highest prevalence of NFPT when compared with the nine MEN1-S (F6: 17.4% vs 5%; P = 0.049) and F1 and F3–F5 (17.4% versus F1 and F3–F5: <2–8%; P = 0.025). All of our NFPT cases were asymptomatic and had pituitary microadenoma, indicating that the extensive screening performed in MEN1 mutation carriers may have contributed to its early diagnosis. In accordance, families that were clinically diagnosed before genetic analysis (F1, F2, and F5) presented lower prevalence of NFPT (less than 4.5%) than those recognized after genetic screening (7–17.4%).

Our family also presented a high prevalence of NFPT among the very large families (54.5%), and F1–F6 had higher frequency of NFPT than MEN1-S (28 vs 8%; P = 0.037). However, recent reports focusing on PET in MEN1 emphasized the finding of higher prevalence of NFPT than described previously, reaching similar or greater frequency than gastrinoma (32, 40). These new PET frequencies may be in part related to the identification of positive mutation carriers; the routine use of endoscopic US applied for early diagnosis of NFPT (40, 42), as well as the biochemical screening with serum pancreatic polypeptide (16). Accordingly, the more recently reported families (F3, F4, and F6) exhibited markedly higher prevalence of NFPT than the very large MEN1 families (F1, F2, and F5) described earlier (P < 0.0001).

Patients with gastrinoma/MEN1 frequently have concomitant NFPTs (32, 40). Accordingly, our family had 60% of cases of PET with concomitant gastrinoma/NFPT, reinforcing cumulative data of more recently described MEN1 cases (32, 40, 41). However, no data were available on this specific topic in F1–F5. Furthermore, the highest combined prevalence of pancreatic and PTTs was found in F6 (68%/52%).

In summary, our family (F6) presented the highest prevalence of non-functioning MEN1-related tumors (NFPT and NFPiT) compared with the other five very large MEN1 families (Fig. 6) and MEN1-S. This may indicate either higher tumor penetrance in F6 or better patient adherence to clinical screening.

Burgess (1996) did not report any cases of acromegaly or sub-clinical GH hypersecretion in the Tasman 1 family (F1) (27). In contrast, early reports mentioned a high percentage of acromegaly in MEN1 (20%) accounting for 27–37% of all PIT/MEN1 (27). Concordantly, no cases of acromegaly or GH hypersecretion were diagnosed in our family or in the Finnish MEN1 families (F3–F4), and this tumor was not confirmed or clearly reported in the other very large families (F2, F5). Recently, an even lower prevalence of acromegaly (3–7.4%; 10–17% of PIT) has been reported in MEN1 (43). In addition, the nine MEN1-S reviewed here presented only 5% of cases with acromegaly (8, 10, 16, 17, 19). These data suggest that the prevalence of acromegaly in MEN1 may have been generally underestimated, and the absence of acromegaly in the Tasman 1 family seems to be more the rule than the exception.

The age-related penetrance of MEN1-related tumors is an important factor that may interfere with its prevalence (24). The absence of informative data regarding the age of each patient at the time of diagnosis in F1–F5 partially limited the comparison of prevalence of MEN1 tumors. In most of the MEN1-S and in F1–F5, the mean age at diagnosis was ~40 years (Supplemental Table 1); however, most insulinomas occurred before 40 years of age (17). There was no difference in the prevalence of PTTs before or after 40 years (19). Gastrinoma/MEN1 has high penetrance and is frequently reported after 30–40 years, but an active search for this tumor in younger cases is recommended. Except for F4 and F5, having exceptionally low prevalence of gastrinoma, the frequency of this tumor was similar in F1, F3, F6, and MEN1-S (Table 5; Fig. 6).

**HPT and BMD**

Most cases of primary HPT are represented by its sporadic form (greater than 90%). The pattern of BMD in this condition is well established and is characterized by

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preferential bone loss in the compact bone (one-third of DR), intermediate loss in mixed bone (FN), and relative protection of the trabecular bone (lumbar spine) (44, 45). In familial forms of HPT, such as MEN1, BMD has rarely been reported. Burgess addressed this topic by studying T- and Z-scores in two bone sites, the lumbar spine and the FN, in a group of 20 uncontrolled and nine controlled HPT/MEN1 cases from Tasman 1 family (31).

We compared data from 20 cases of uncontrolled HPT reported by Burgess (BMD analyzed before surgery or in cases with less than 4 years of surgery) with our 20 uncontrolled HPT/MEN1 cases. It was observed that in both families, bone demineralization of the lumbar spine (F1, 70%; F6, 60%) and FN (F1, 90%; F6, 65%) were very frequent. In our cases, osteoporosis was predominant in the lumbar spine (L1–L4, 40%/FN, 20%), and osteopenia was more prevalent at the FN (45%; L1–L4, 20%). In contrast, osteoporosis was predominant in the FN (50%; L1–L4, 30%), while osteopenia was equivalent in both sites (40%) in Burgess’ cases.

A relative protection of trabecular bone (vertebral spine) has been observed in sporadic HPT when PTH values are two to three times higher than the upper limit of normal values. Thus, vertebral osteopenia occurs in about 15% of cases with sporadic HPT (46). Burgess’ and our cases also presented similar PTH values. Despite this, the anabolic effect of these serum levels of PTH on trabecular bone was not supported by data from HPT/MEN1 patients reported by Burgess and in our present cases (Fig. 4) (31).

We also originally documented the preferential demineralization of cortical bone (proximal one-third of the DR) in our cases with HPT/MEN1 (T < −1.0 in 90%; Fig. 4). In addition, this bone site was the most compromised one (Fig. 5). Our data are similar to that observed in the sporadic form of HPT in which the cortical bone (compact) is classically described as being more prone to the catabolic effect of elevated PTH values (44–46).

Most of our cases (85%; 17/20), without any previous surgical treatment of HPT and presenting a long-term clinical history of renal calculi, were examples of the natural history of bone disease secondary to MEN1 syndrome. The bone mineral loss pattern observed in this specific MEN1 family should be supported by further BMD studies in other MEN1 families.

The BMD analysis of our patients with HPT/MEN1 revealed a precocious, severe, and frequent bone demineralization of the three main bone sites (Table 3B; Figs 4 and 5). This specific pattern of bone demineralization at the three bone sites in HPT/MEN1, emphasizing the absence of protection of trabecular bone could be, in part, due to hypogonadism secondary to the combined occurrence of prolactinoma/MEN1 in some cases; early onset of HPT interfering with the normal peak of bone mass; environmental factors, such as vitamin D deficiency; direct action of MEN1 deficiency in the development of bone tissue; or associated factors and others (31, 47). Environmental factors such as vitamin D deficiency could be excluded in our cases.

**Founding mutation**

Recurrent MEN1 germline mutations are frequent (33–49%) in MEN1 families, making up 8–25% of all mutations (7–9, 28, 48). Most of them occur in the MEN1 gene regions susceptible to slippage (C–G-rich area). Thus, the 308delC frameshift mutation occurred in a C–G-rich area of the MEN1 gene, in the 299–386 region that we recently suggested as a possible hotspot for MEN1 mutations (33).

Founding mutations in MEN1 are relatively rare (7–9). Only 13 founder effects have been reported so far in this disease (Supplemental Table 4, which can be viewed online at http://www.eje-online.org/supplemental/). The description of the full MEN1 phenotype was available in six of these cases, coinciding with the very large families analyzed here (Table 4) (14, 28–30). The founder effect documented in the present MEN1 family is the first reported in Latin America.

We identified 37 reported Italian MEN1 families whose genetic analysis was conducted, and MEN1 germline mutations were found in only 24 families (52–54). None of them harbored the 308delC mutation, suggesting the possible occurrence of a de novo mutation in one of the ancestors who migrated from Italy to Brazil.

In conclusion, we described a high frequency of functioning and non-functioning MEN1-related tumors in a very large Brazilian MEN1 family. We also described, for the first time, the occurrence of striking bone demineralization in the cortical bone of this MEN1 family.

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