REVIEW

Multiple endocrine neoplasia: the Chilean experience

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Multiple endocrine neoplasia (MEN) types 1 and 2 are genetic diseases that are inherited as autosomal traits. The major clinical manifestations of multiple endocrine neoplasia type 1 include the so-called “3 P’s”: parathyroid, pituitary, and pancreatic tumors, including gastroenteronduroendocrine tumors. Genetic testing can be performed on patients and the potential carriers of the menin gene mutation, but the genotype-phenotype correlation in multiple endocrine neoplasia type 1 is less straightforward than multiple endocrine neoplasia type 2. Most likely, the main advantage of genetic testing in MEN1 is to exclude from further studies those who are negative for the genetic mutation if they belong to a family with a known history of MEN1. In Chile, we started with rearranged during transfection proto-oncogene genetic testing (MEN2) 15 years ago. We carried out a prophylactic total thyroidectomy to prevent medullary thyroid carcinoma in a three-year-old girl who presented with microscopic medullary thyroid carcinoma. More than 90% of the individuals who tested positive using a genetic test achieved a biochemical cure compared with only 27% of patients who receive a clinical diagnosis. Mutations are mainly located in exon 11; the most common is C634W, rather than C634R. Hypertensive crisis was the cause of death in three patients, and extensive distant metastases occurred in nine (including two patients with multiple endocrine neoplasia type 2B) of 14 patients. Earlier recognition of medullary thyroid carcinoma and the other features of the disease, especially pheochromocytoma, will improve the survival rate of patients with multiple endocrine neoplasia.

KEYWORDS: RET; Menin; Mutations; MEN1; MEN2.

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MUTIPLE ENDOCRINE NEOPLASIA TYPE 1

Multiple endocrine neoplasia type 1 (MEN1; MIM 131100) is an autosomal dominant disease that predisposes individuals to develop parathyroid tumors (100% penetrance by age 50), pancreatic islet cells tumors (30–75%), and pituitary adenoma (10–60%) (1). Some patients may also develop adrenal cortical tumors, carcinoid tumors, facial angiofibromas, collagenomas and lipomas. They can also develop a variable combination of more than 20 endocrine and nonendocrine tumors (Table 1) (2,3).

A diagnosis of MEN1 should be considered when two of the three principal MEN1 tumors affecting the parathyroids, pancreatic islets, or anterior pituitary are present. Familial MEN1 is diagnosed when at least one other first-degree relative has one of the three main MEN1 tumors (4).

MEN1 is a rare disease (the prevalence has been estimated at 2–3/100,000) caused by a mutation in the MEN1 gene (menin, MIM 613733) located on chromosome 11q13 and is characterized by a high penetrance (94% by the fifth decade), high mortality, and no clear genotype-phenotype correlation compared with MEN2. De novo mutations have been described in up to 10% of cases (5–9).

The MEN1 gene consists of 10 exons that encode a 610-amino acid protein, Menin, a nuclear protein that is ubiquitously expressed. Menin is a tumor suppressor protein and also plays a role in maintaining DNA stability and gene regulation. Approximately 1133 germline mutations in the MEN1 gene have been reported (9). The model for tumorigenesis in familial MEN1, according to Knudson’s “two hits” theory, is that a mutated copy of MEN1 is inherited at the germline level from an affected parent (first hit), whereas the wild-type copy, which is inherited from the healthy parent, is lost at the somatic level (second hit), thereby resulting in a tumor. This mechanism is supported by the observation that heterozygosity is lost, or allelic loss occurs, when tumor DNA is compared with constitutive DNA (2).

Genetic studies should be offered to patients who meet the clinical criteria for MEN1 (sporadic or familial), their asymptomatic relatives, and patients who do not meet MEN1 criteria but have suspicious/atypical features that are suggestive of MEN1. Genetic testing has enabled the confirmation of clinical diagnosis, identification of asymptomatic carriers who require screening for tumor detection and early treatment and identification of family members who do not have the MEN1 mutation and, therefore, do not require further clinical examinations (10).
Parathyroid tumors are the first manifestation of MEN1 in more than 87% of patients; however, other tumors, like pancreatic or pituitary adenoma, have also been reported as the first manifestation (7).

Physical examination for dermal tumors should start in childhood and be carried out periodically. Annual biochemical screening is recommended after five years of age, and tumor imaging should be performed less frequently (Table 2) (4).

We started MEN1 genetic testing in 2001, initially with one family, and then helped other researchers in Chile. We gathered 22 patients from four families and three sporadic cases, who were the youngest and the oldest patients included (two patients). Genetic testing was performed on two families (30 individuals), which identified 13 members with the IVS9 +1G mutation and three members with the IVS6 +1G mutation. Partial data from one family was published in 2004 (11).

Table 3 shows the prevalence of each manifestation and the age at diagnosis. Regarding hyperparathyroidism (HPT), all three of the unaffected individuals were younger than 24 years and the diagnosis was confirmed by genetic testing. They were followed according to the criteria shown in Table 2. Of the 22 other HPT individuals, the mean age at onset was 39 years (19–86 years) and two patients were also diagnosed with concomitant parathyroid carcinoma (at ages of 31 and 75 years).

With regard to the other manifestations of MEN, their prevalence does not differ significantly from other published studies, with the exception of carcinoid tumors, which our data indicate are more frequent even though all of these cases occurred in the same family (7). One patient had papillary thyroid microcarcinoma, a finding that could be incidental. Regarding cutaneous manifestations, we observed collagenoma, lipomas, and angiofibromas, but not all patients were meticulously examined; therefore, definitive conclusions cannot be reached.

## MULTIPLE ENDOCRINE NEOPLASIA TYPE 2

MEN type 2 (MEN2) is an autosomal dominant syndrome that affects approximately one in 30,000 individuals (4,12–14). The major components of the syndrome are medullary thyroid carcinoma (MTC), pheochromocytoma (PHEO) and HPT. Germline mutations in the proto-oncogene RET (MIM 164761), located on chromosome 10q11.2, are responsible for the three subtypes: MEN2A (80%; MIM 171400), MEN2B (5%; MIM 162300) and familial MTC (FMTC, 15%; MIM 155240). MTC commonly presents alongside the three subtypes, and

### Table 1 - MEN1-related tumors (penetrance at age 40).

| Organ/tumor                          | Features                  | %     |
|--------------------------------------|---------------------------|-------|
| Parathyroid hyperplasia/adenoma      | Multiglandular disease    | >90   |
| Gastroenteropancreatic tract         | Gastrinoma                | 40    |
|                                      | Insulinoma                | 10    |
|                                      | Others (VIPoma, PPoma, SSoma, glucagonoma) | 2     |
|                                      | Nonfunctioning            | 20    |
| Pituitary                            | Prolactinoma              | 20    |
|                                      | Acromegaly                | 5     |
|                                      | GH/PRL                    | 5     |
|                                      | Nonfunctioning adenomas   | 17    |
| Foregut carcinoids                   | Thymic                    | 2     |
|                                      | Bronchial                 | 2     |
|                                      | Gastric enterochromaffin tumor | 10   |
| Adrenal gland                        | Nonfunctioning            | 20–40 |
|                                      | Pheochromocytoma          | <1    |
|                                      | Cortisol, aldosterone     | Rare  |
| Skin tumors                          | Facial angiofibromas      | 85    |
|                                      | Collagenomas              | 70    |
|                                      | Lipomas                   | 30    |
| Central nervous system               | Meningiomas               | 5–8   |
|                                      | Ependymomas               | 1     |
|                                      | Leyomiomas                | 10    |

VIP: vasoactive intestinal peptide. PP: pancreatic polypeptide. SS: somatostatin. GH: growth hormone. PRL: prolactin.

### Table 2 - Clinical surveillance for subjects known to have MEN1 Adapted from (2,4).

| Tumor                              | Biochemical tests       | Age to begin (yr) | Frequency |
|------------------------------------|-------------------------|-------------------|-----------|
| Hyperparathyroidism                | Serum calcium           | 5                 | Yearly    |
|                                    | PTH                     | 8                 | Yearly    |
| Insulinoma                         | Glycemia, fasting insulin, proinsulin | 5     | Yearly    |
| Anterior pituitary                 | PRL, IGF-1              | 5                 | Yearly    |
| Gastrinoma                         | Fasting and/or stimulated gastrin | 20   | Yearly    |
|                                    |                         |                   |           |
| Tumor                              | Imaging tests           | Age to begin (yr) | Frequency |
|------------------------------------|                        |                   |           |
| Enteropancreatic and carcinoid tumor | Chest and Abdominal CT or MRI | 20         | Yearly to every 3–5 yr |
| Anterior pituitary                 | Head MRI                | 5                 | Yearly to every 3–5 yr |
| Other enteropancreatic             | Somatostatin Receptor Scintigraphy (SRS) | Yet to be defined | Yet to be defined |
|                                    | Octreotide scan         |                   |           |

CT: computed tomography. MRI: magnetic resonance imaging.
the risk of developing MTC is nearly 100%. Patients with MEN2A/2B have a 30–50% risk of developing PHEO, especially those who harbor mutations in codon 634, codon A883F, and M918T (12–15). Diverse features differentiate both subtypes: on one hand, only patients with MEN2A are at risk of developing HPT and cutaneous lichen amyloidosis (CLA) (10–30% and 12%, respectively) and only patients with MEN2B have marfanoid habitus, mucosal neuromas on the tongue, lips and subconjunctival areas and diffuse ganglioneuromas of the gastrointestinal tract (14). In FMTC, affected patients suffer exclusively from MTC; to prove that a particular member of a family has FMTC, it is necessary to demonstrate the absence of PHEO and HPT in two or more generations within a family or provide evidence of an identified RET mutation in families with FMTC (American Thyroid Association) (13). Rarely, families with both MEN2 and Hirschsprung’s disease have been found to have MEN2-specific codons 609, 611, 618, and 620 while also presenting with the MEN2A or FMTC subtypes (15,16). MTC, when presenting along with MEN2B, develops earlier and has a more aggressive course than MTC in the other two subtypes. During the first year of life, the classic features of MEN2B may be lacking, so physicians must recognize the more subtle features of this condition, such as the inability to cry and constipation (17). Straightforward genotype-phenotype correlations in MEN2 have also been described, and, as expected, the aggressiveness of MTC is influenced by the specific RET mutation. For instance, MEN2 has been confirmed using thyroidectomy specimens collected from MEN2A patients as young as 15 months and in a 9-month-old child with MEN2B (M918T); their conditions progressed to N1 (TNM staging AJCC 2002) at 5 and 2.7 years old, respectively (12,18–20). Therefore, identification of the specific RET mutation that is present in at-risk patients allows for effective clinical screening and optimal clinical and surgical management. In 2009, after extensive review of the literature, the American Thyroid Association (ATA) replaced the consensus guidelines for diagnosis that were released in 2001 and developed a categorization system based on new evidence in order to offer age-based recommendations for performing prophylactic thyroidectomy, predict phenotypes and to establish who should be screened for PHEO and HPT. Table 4 shows the adapted ATA guidelines and other guidelines (12,13).

We started RET genetic testing in 1997 and the most important findings have been published (16,21,22). Sequencing of the RET proto-oncogene was carried out in 60 sporadic MTC patients and 15 families with the MEN2 phenotype, totaling almost 200 individuals. In one family, genetic testing is pending but their phenotype is MEN2A. From the apparently sporadic patients, we found three new MEN2 (probably FMTC) individuals (5%), one with codon 618, one with codon V804M, and another with codon S891A. This percentage is within the range of occurrence of apparently sporadic hereditary cases (4–7%) (23,24). Table 5 shows the main clinical features of 19 families. MEN2A accounted for 73% of all cases, while FMTC and MEN2B accounted for 21% and 6% of cases, respectively. The female-male ratio was 1.27:1. For patients who received genetic testing (61 out of 63), the most common mutation was located in exon 11 (62%), followed by exon 10 (25%), exon 16 (6%), exon 14 (3.5%), and exon 15 (3.5%). In our population, the C634W mutation was the most common (28%), followed by mutations on exon 10 at codon C620R (21%) and the C634R

Table 3 - Prevalence of manifestations and age of diagnosis of MEN1 Chilean families.

| Manifestation                      | % (affected/total) | Median age at diagnosis, years (range) | N   |
|------------------------------------|--------------------|----------------------------------------|-----|
| Hyperparathyroidism                | 88 (22/25)         | 39 (19–86)                             | 22  |
| Pituitary tumor                    | 28 (7/25)          | 40 (18–86)                             |     |
| Gastroenteropancreatic tumor       | 32 (8/25)*         | 35 (19–60)                             |     |
| Foregut carcinoids                 | 16 (4/25)†         | 52 (23–67)                             |     |
| Adrenal gland                      | 24 (6/25)          | 47 (23–86)                             |     |

*Two patients presented with gastrinoma and pancreatic neuroendocrine tumor concomitantly.
†One patient had lung and duodenal carcinoids.

Table 4 - Recommendations for screening procedures and time of prophylactic thyroidectomy. Adapted from (12,13).

| ATA Level | D                     | C                     | B                     | A                     |
|-----------|-----------------------|-----------------------|-----------------------|-----------------------|
| RET Mutation codon | 918, 883 | 634 | 609, 611, 618, 620, 630 | 768, 790, 804, 891 |
| Age of prophylactic thyroidectomy | ASAP or within the first year of life | <5 years | Consider surgery before age 5. May delay surgery beyond age of 5 years if stringent criteria are met* | May delay surgery beyond age 1 5 years if stringent criteria are met* |
| Age of screening for PHEO | Start at 8 years, then annually | Start at 8 years, then annually | Start at 20 years, then annually | Start at 20 years, then annually |
| Age of screening for PHP | Does not apply | Start at 8 years, then annually | Start at 20 years, then annually | Start at 20 years, then periodically |

ASAP: as soon as possible.
*Normal annual basal/stimulated serum calcitonin level, normal annual neck ultrasound, less aggressive MTC family history and family preference.
mutation (18%). These frequencies differ from the largest published series, where C634R is the reported as the most common mutation (13,15). We identified 56 patients with MTC, with a median age of 28.5 years (range: 1–73 years), and seven patients with CCH, with a median age of 7 years (range: 4–28 years). Interestingly, a V804M carrier who was operated on at 28 years of age showed only CCH, demonstrating that this mutation may have low penetrance in some cases (12,14).

The median preoperatory serum calcitonin level was 11 pg/ml (range: 2.9–27 pg/ml) in patients with CCH and 149 pg/ml (range: 13–5549 pg/ml) in patients with MTC. There was a high correlation between the preoperatory serum calcitonin level and tumor size and stage (0.79 and 0.69 respectively, \(p < 0.005\)).

Table 6 shows clinical outcomes and compares biochemical cure, persistent disease, and death with the method of diagnosis (e.g., genetic testing or clinical diagnosis):

| Method of Diagnosis | Outcome | Genetic Testing | Clinical | Total |
|---------------------|---------|----------------|----------|-------|
| Cured               | 28      | 8              | 36       |
| %                   | 90.3    | 27.6           | 60       |
| Persistent          | 2       | 8              | 10       |
| %                   | 6.5     | 28             | 18.7     |
| Dead                | 1       | 13             | 14       |
| %                   | 3.2     | 44.8           | 23       |
| Total               | 31      | 29             | 60       |
| %                   | 100     | 100            | 100      |

Fisher’s exact test = 0.000.

Table 5 - Clinical characteristics and RET mutations in families with MEN2.

| Family number | Individuals analyzed/ mutated | MTC/CCH | ATA risk | Age of proband at diagnosis (yr) of any tumor | Phenotype | Codon/protein substitution | PHEO (number) | HPT (number) |
|---------------|-------------------------------|---------|----------|-----------------------------------------------|-----------|----------------------------|---------------|--------------|
| 1             | 26/11                         | 8/3     | C        | 42                                            | MEN2A/CLA | C634W                      | 3             | 0            |
| 2             | 7/5                           | 5/0     | C        | 28                                            | MEN2A     | C634W                      | 2             | 0            |
| 3             | 3/1                           | 1/0     | C        | 43                                            | MEN2A/CLA | C634W                      | 1             | 0            |
| 4             | 4/1                           | 1/0     | C        | 23                                            | MEN2A     | C634R                      | 1             | 1            |
| 5             | 6/5                           | 5/0     | C        | 38                                            | MEN2A     | C634R                      | 1             | 1            |
| 6             | 7/4                           | 4/0     | C        | 33                                            | MEN2A     | C634R                      | 1             | 0            |
| 7             | 3/1                           | 1/0     | C        | 26                                            | MEN2A     | C634R                      | 1             | 0            |
| 8             | 6/4                           | 4/0     | C        | 35                                            | MEN2A     | C634G                      | 2             | 0            |
| 9             | 9/5                           | 5/0     | C        | 29                                            | MEN2A     | C634F                      | 2             | 0            |
| 10            | 9/2                           | 2/0     | C        | 37                                            | FMTMC     | C634Y                      | 0             | 0            |
| 11            | 2/2                           | 2/0     | B        | 40                                            | FMTMC     | C618R                      | 0             | 0            |
| 12            | 17/6                          | 5/1     | B        | 50                                            | FMTMC     | C620R                      | 0             | 0            |
| 13            | 11/7                          | 5/2     | B        | 18                                            | MEN2A     | C620R                      | 2             | 0            |
| 14            | 6/2                           | 1/1     | A        | 40                                            | FMTMC     | V804M                      | 0             | 0            |
| 15            | 3/2                           | 2/0     | A        | 47                                            | FMTMC     | S891A                      | 0             | 0            |
| 16            | 2/2                           | 2/0     | D        | 32                                            | MEN2B     | M918T                      | 1             | 0            |
| 17            | 3/1                           | 1/0     | D        | 14                                            | MEN2B     | M918T                      | 0             | 0            |
| 18            | 3/1                           | 1/0     | D        | 4                                             | MEN2B     | M918T                      | 0             | 0            |
| 19            | 2/2                           | 2/0     | pending  | 16                                            | MEN2A     | pending                    | 1             | 0            |
| total         | 129/63                        | 56/7    |          |                                               |           |                            | 17            | 2            |

In summary, the various types of MEN are inherited autosomal dominant syndromes. Molecular biology techniques have made it possible to identify the genes responsible for causing MEN1 and 2. The identification of the carriers of mutant MEN1 and 2 genes who are at risk of developing these syndromes requires frequent biochemical screening for the development of endocrine tumors. The identification of those who are negative will save resources and reassure both the patient and the family. Carriers of the genes responsible for MEN2 should be advised to have thyroidectomy in childhood, according with the ATA risk level of the patient. Some cases of sporadic MTC are determined to actually be MEN2 following RET proto-oncogene testing; therefore, the routine application of this test is recommended in all cases of apparent sporadic MTC.

**AUTHOR CONTRIBUTIONS**

Wohllk N and Diaz R were responsible for the data collection, statistical analysis, supervision of the study, and writing of the manuscript. Wohllk N was also responsible for the genetic testing results and communications with patients and their parents.
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