

REVIEW

Multiple endocrine neoplasia: the Chilean experience

René E. Diaz,^{I,II} Nelson Wohlk^{II}

^IFellow in Endocrinology, Universidad de Chile, Santiago de Chile. ^{II}Department of Endocrinology, Hospital del Salvador, Universidad de Chile, Santiago de Chile.

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 gastroenteroneuroendocrine 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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E-mail: nwohlk@gmail.com

Tel.: 562 378 0517

MULTIPLE 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).

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No potential conflict of interest was reported

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

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

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

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>A mutation and three members with the IVS6 + 1G>A 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 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) Octreotide scan	Yet to be defined	Yet to be defined

CT: computed tomography. MRI: magnetic resonance imaging.

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)	3
			Acromegaly
			Prolactinoma
			GH/PRL
			Nonfunctioning adenomas
Gastroenteropancreatic tumor	32 (8/25)*	35 (19–60)	5
			Gastrinoma
			Nonfunctioning enterochromaffin
Foregut carcinoids	16 (4/25)†	52 (23–67)	3
			Bronchial
			Duodenal
Adrenal gland	24 (6/25)	47 (23–86)	4
			Nonfunctioning
			Bilateral Hyperplasia
			2

*Two patients presented with gastrinoma and pancreatic neuroendocrine tumor concomitantly.

†One patient had lung and duodenal carcinoids.

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

ATA Level	D	C	B	A
<i>RET</i> 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 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 periodically
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.

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

Family number	Individuals analyzed/ mutated	Age of proband at diagnosis (yr) of any tumor		Phenotype	Codon/protein substitution	PHEO (number)	HPT (number)	
		MTC/CCH	ATA risk					
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	FMTC	C634Y	0	0
11	2/2	2/0	B	40	FMTC	C618R	0	0
12	17/6	5/1	B	50	FMTC	C620R	0	0
13	11/7	5/2	B	18	MEN2A	C620R	2	0
14	6/2	1/1	A	40	FMTC	V804M	0	0
15	3/2	2/0	A	47	FMTC	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

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 preoperative 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 preoperative 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): 14 patients died in total, and of these three died from hypertensive crisis, two from surgical complications (one was diagnosed by genetic testing) and nine from distant metastases (including two out of the four patients with MEN2B). Forty-nine patients are still alive: 36 are biochemically cured, 10 have persistent disease, two have not been operated on yet and one has unknown condition that is affecting his postoperative calcitonin level. As expected,

Table 6 - Outcome according to method of diagnosis and serum calcitonin level.

Outcome	Method of Diagnosis		Total
	Genetic Testing	Clinical	
Cured	28	8	36
%	90.3	27.6	60
Persistent	2	8	10
%	6.5	28	16.7
Dead	1	13	14
%	3.2	44.8	23
Total	31	29	60
%	100	100	100

Fisher's exact test = 0.000.

patients who were prophylactically operated on were more likely to be biochemically cured and demonstrated less persistent disease states than those who were operated on after clinical diagnosis. Regarding the other main features, PHEO was present in 27% of patients, which presented bilaterally in most patients with PHEO (70%), and the patients who developed PHEO had a median age of 34 years (range: 26–47 years). HPT was very uncommon (3%) and only seen in two patients with the C634R mutation, as has been reported in other studies (13,14,25). CLA was seen in three patients with the C634W mutation, which is in agreement with other reports in the literature (12,13,26). We did not find any cases of Hirschsprung's disease, a rarely associated condition that has been described in patients with mutations in exon 10 (16,27,28). We found four patients with the MEN2B phenotype; *de novo* mutation was present in two of these patients and, retrospectively, both displayed the precocious symptoms of the inability to cry and constipation during the first year of life.

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

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

REFERENCES

1. Wermer P. Genetic aspects of adenomatosis of endocrine glands. *Am J Med.* 1954;16(3):363-71, [http://dx.doi.org/10.1016/0002-9343\(54\)90353-8](http://dx.doi.org/10.1016/0002-9343(54)90353-8).
2. Falchetti A, Marini F, Luzi E, Giusti F, Cavalli L, Cavalli T, et al. Multiple endocrine neoplasia type one (MEN1): not only inherited endocrine tumors. *Genet Med.* 2009;11(12):825-35, <http://dx.doi.org/10.1097/GIM.0b013e3181be5c97>.
3. Wohlk N, Becker P, Véliz J, Pineda G. Multiple endocrine neoplasia: a clinical model for applying molecular genetic techniques. *Rev Med Chil.* 2000;128(7):791-800.
4. Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, et al. Guidelines for diagnosis and therapy of MEN type one and type 2. *J Clin Endocrinol Metab.* 2001;86(12):5658-71, <http://dx.doi.org/10.1210/jc.86.12.5658>.
5. Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, et al. Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science.* 1997;276(5311):404-7, <http://dx.doi.org/10.1126/science.276.5311.404>.
6. 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.* 1997;6(7):1177-83, <http://dx.doi.org/10.1093/hmg/6.7.1177>.
7. Trump D, Farren B, Wooding C, Pang JT, Besser GM, Buchanan KD, et al. Clinical studies of multiple endocrine neoplasia type 1 (MEN1). *QJM.* 1996;89(9):653-69.
8. Dean PG, van Heerden JA, Farley DR, Thompson GB, Grant CS, Harmsen WS, et al. Are patients with multiple endocrine neoplasia type I prone to premature death? *World J Surg.* 2000;24(11):1437-41, <http://dx.doi.org/10.1007/s002680010237>.
9. Thakker RV. Multiple endocrine neoplasia type 1 (MEN1). *Best Pract Res Clin Endocrinol Metab.* 2010;24(3):355-70, <http://dx.doi.org/10.1016/j.beem.2010.07.003>.
10. Newey PJ, Thakker RV. Role of multiple endocrine neoplasia type 1 (MEN1) mutational analysis in clinical practice. *Endocr Pract.* 2011;31(1):1-24.
11. Carrasco CA, González AA, Carvajal CA, Campusano C, Oestreicher E, Arteaga E, et al. Novel intronic mutation of MEN1 gene causing familial isolated primary hyperparathyroidism. *J Clin Endocrinol Metab.* 2004;89(8):4124-9, <http://dx.doi.org/10.1210/jc.2003-032101>.
12. Wohlk N, Schweizer H, Erlic Z, Schmid KW, Walz MK, Raue F, et al. Multiple endocrine neoplasia type 2. *Best Pract Res Clin Endocrinol Metab.* 2010;24(3):371-87, <http://dx.doi.org/10.1016/j.beem.2010.02.001>.
13. Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid.* 2009;19(6):565-612, <http://dx.doi.org/10.1089/thy.2008.0403>.
14. Kouvaraki MA, Shapiro SE, Perrier ND, Cote GJ, Gagel RF, Hoff AO, et al. RET proto-oncogene: a review and update of genotype-phenotype correlations in hereditary medullary thyroid cancer and associated endocrine tumors. *Thyroid.* 2005;15(6):531-44, <http://dx.doi.org/10.1089/thy.2005.15.531>.
15. Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. *JAMA.* 1996;276(19):1575-9.
16. Frank-Raue K, Rybicki LA, Erlic Z, Schweizer H, Winter A, Milos I, et al. Risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germline RET mutations located in exon 10. *Hum Mutat.* 2011;32(1):51-8, <http://dx.doi.org/10.1002/humu.21385>.
17. Brauckhoff M, Machens A, Hess S, Lorenz K, Gimm O, Brauckhoff K, et al. Premonitory symptoms preceding metastatic medullary thyroid cancer in MEN 2B: an exploratory analysis. *Surgery.* 2008;144(6):1044-50, <http://dx.doi.org/10.1016/j.surg.2008.08.028>.
18. Machens A, Niccoli-Sire P, Hoegel J, Frank-Raue K, van Vroonhoven TJ, Roehrer HD, et al. Early malignant progression of hereditary medullary thyroid cancer. *N Engl J Med.* 2003;349(16):1517-25, <http://dx.doi.org/10.1056/NEJMoa012915>.
19. Kaufman FR, Roe TF, Isaacs H Jr, Weitzman JJ. Metastatic medullary thyroid carcinoma in young children with mucosal neuroma syndrome. *Pediatrics.* 1982;70(2):263-7.
20. Wohlk N, Cote GJ, Evans DB, Goepfert H, Ordóñez NG, Gagel RF. Application of genetic screening information to the management of medullary thyroid carcinoma and multiple endocrine neoplasia type 2. *Endocrinol Metab Clin North Am.* 1996;25(1):1-25, [http://dx.doi.org/10.1016/S0889-8529\(05\)70310-8](http://dx.doi.org/10.1016/S0889-8529(05)70310-8).
21. Wohlk N, Becker P, Youton R, Cote GJ, Gagel RF. [Germline mutations of the ret proto-oncogene in Chilean patients with hereditary and sporadic medullary thyroid carcinoma]. *Rev Med Chil.* 2001;129(7):713-8.
22. Milos IN, Frank-Raue K, Wohlk N, Maia AL, Pusiol E, Patocs A, et al. Age-related neoplastic risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germ line RET Cys634Trp (TGC>TGG) mutation. *Endocr Relat Cancer.* 2008;15(4):1035-41, <http://dx.doi.org/10.1677/ERC-08-0105>.
23. Wohlk N, Cote GJ, Bugalho MM, Ordóñez N, Evans DB, Goepfert H, et al. Relevance of RET proto-oncogene mutations in sporadic medullary thyroid carcinoma. *J Clin Endocrinol Metab.* 1996;81(10):3740-5, <http://dx.doi.org/10.1210/jc.81.10.3740>.
24. Elisei R, Romei C, Cosci B, Agate L, Bottici V, Molinaro E, et al. RET genetic screening in patients with medullary thyroid cancer and their relatives: experience with 807 individuals at one center. *J Clin Endocrinol Metab.* 2007;92(12):4725-9, <http://dx.doi.org/10.1210/jc.2007-1005>.
25. Punales MK, Graf H, Gross JL, Maia AL. RET codon 634 mutations in multiple endocrine neoplasia type 2: variable clinical features and clinical outcome. *J Clin Endocrinol Metab.* 2003;88(6):2644-9, <http://dx.doi.org/10.1210/jc.2002-021422>.
26. Verga U, Fugazzola L, Cambiaghi S, Pritelli C, Alessi E, Cortelazzi D, et al. Frequent association between MEN 2A and cutaneous lichen amyloidosis. *Clin Endocrinol.* 2003;59(2):156-61, <http://dx.doi.org/10.1046/j.1365-2265.2003.01782.x>.
27. Eng C. Seminars in medicine of the Beth Israel Hospital, Boston. The RET proto-oncogene in multiple endocrine neoplasia type two and Hirschsprung's disease. *N Engl J Med.* 1996;335(13):943-51, <http://dx.doi.org/10.1056/NEJM199609263351307>.
28. Romeo G, Ceccherini I, Celli J, Priolo M, Betsos N, Bonardi G, et al. Association of multiple endocrine neoplasia type 2 and Hirschsprung disease. *J Intern Med.* 1998;243(19):515-20, <http://dx.doi.org/10.1046/j.1365-2796.1998.00332.x>.