Research Article

Multiple Endocrine Neoplasia Type 2A: Genetics and Prophylaxis

Karima Sifi*[1,2], Nourredine Abadi[1,2], Khalida Boudaoud[3], Nassim Nouri[3], Karima Benmohamed[3], Sabah Hanachi[1,2], Karima Benmebarek[1,2], EL Kacem Lezzar[3], Chérifa Benlatreche[1,2]

[1]Laboratoire de Biologie et Génétique Moléculaire, Faculté de Médecine, Université Constantine 3, Algérie.
[2]Laboratoire de Biochimie, CHU Dr-Benbadis, Rue Benseghir-Abdelouahab, Constantine, Algérie.
[3]Service d'endocrinologie, CHU Dr-Benbadis, Rue Benseghir-Abdelouahab, Constantine, Algérie.
***Corresponding author's E-mail:** sifikarima@yahoo.fr

Received: 28-12-2016; **Revised:** 27-02-2017; **Accepted:** 12-03-2017.

ABSTRACT

MEN 2, which includes MEN2A, MEN 2B, and FMTC is caused by autosomal dominant gain-of-function mutations of RET proto-oncogene. MEN2A is the most frequent form. This syndrome is characterized by a medullary thyroid carcinoma (MTC) and/or pheochromocytoma (PHEO) and/or multiple tumors of the parathyroid glands. Early prophylactic total thyroidectomy before the development of MTC is currently the only curative treatment. The aims of this study were to characterize point mutations in RET proto-oncogene in the family index case, to Identify and assess the effectiveness of the management of the index case relatives who have presented the RET proto-oncogene mutation and to search the existence of a possible correlation between the phenotype and genotype among the members of this MEN 2A family. Our work focused on a family of NEM 2A with an index case with MTC and pheochromocytoma, and 13 1st degree relatives. Exons 8, 9, 10, 11, 13, 14, 15 and 16 of the *RET proto-onco*gene were analysed by PCR/sequencing. We characterized the exon 11 RET proto-oncogene germline mutation C634Y in the index case MEN 2A and in 7 of his 13 1st degree relatives. The haplotype G691S / S904S of RET in the homozygous state was found in the relatives who presented the mutation suggesting that this haplotype have a modifying effect on the age of onset of MTC in MEN2A. In our patients, the C634Y mutation was significantly associated with the presence of pheochromocytoma, which is consistent with the literature data. The C634Y is characterized by a particularly agressiveness, thyroidectomy is then recommended before the age 5 years. All patients described in this report have undergone thyroidectomy (6 cases), removal of PHEO (only 4 cases).

Keywords: MEN2A, RET proto-oncogene, mutation C634Y, prophylactic thyroidectomy, Pheochromocytoma ablation.

INTRODUCTION

Multiple endocrine neoplasia type 2 (MEN 2) is an inherited multiglandular disorder[1], with an estimated prevalence of 2.5 per 100,000 in the general population in Europe. MEN2 syndrome includes three clinically different inherited syndromes.

-MEN2A associating medullary thyroid carcinoma (MTC) and/or pheochromocytoma (PHEO) and/or multiple tumors of the parathyroid glands.

-MEN2B characterized by MTC, pheochromocytoma, Marfan type dysmorphia and ganglioneuromatosis submucosa and digestive.[2,3]

-Familial medullary thyroid carcinoma (FMTC), affected family members develop MTC only.

These three phenotypic variants have as a constant the presence of an MTC with high penetrance. Affected individuals develop early primary C-cells hyperplasia (CCH) which progresses to invasive MTC. However, the C cells hyperplasia is a primary neoplastic lesion.

Serum calcitonin (CT) is an MTC recognized biomarker.[4,5] Its base elevation associated with its increase after injection of pentagastrin (Pg test) is pathognomonic of MTC.

These three subtypes of MEN 2 differ in their incidence, age of onset, MTC aggressiveness, genetics, association with other diseases, and prognosis.[6-9]

MEN 2A, is the most common form (60% of MEN 2).[10,11] It is characterized by MTC, pheochromocytoma, and hyperparathyroidism[12] in one patient, or the presence of two or more tumors in multiple members of the same family.[9]

The MEN 2A MTC (frequency over 90%) is not always the first manifestation of the disease. It then associates a PHEO in 20% to 50% of cases. These are usually bilateral sometimes with a dissociation time.[13-15] It can be completely asymptomatic (60% of cases) and constitute by their presence a real threat of death by a fatal cardiogenic shock.[16,17] Their research is absolutely indispensable in the presence of MTC because their treatment must precede that of the MTC. Primary hyperparathyroidism (HPT1) is in 15-30% of cases practically always asymptomatic.[18,19]

In some cases, MEN 2A can be associated with paraneoplastic syndromes such a rash pruritic sitting in the upper back, lichen amyloid or "notalgia" incriminating a keratin deposit.[20-22]

A skin biopsy at their level reveals amyloid deposits at the dermal-epidermal junction[23-26] or a thickening of the corneal nerve fibers, previously considered characteristic

MEN 2B, but can be observed in some cases of MEN2A. A motor diarrhea, flush and an Hirschsprung syndrome can also be seen. About 15% of MTC are associated with ectopic ACTH production and Cushing's syndrome.[27]

The three MEN 2 variants are linked to activating RET proto-oncogene germline mutations and are inherited in an autosomal dominant pattern.

RET proto-oncogene is located on the long arm of chromosome 10 at 10q11.2, and spans about 60kb. Its coding region is divided into 21 exons[28-30] and encodes a tyrosine kinase transmembrane receptor, expressed in several developing tissues and mainly in those derived from the neural crest.

RET appears to transduce growth and differentiation signals in several developing tissues.[31]

RET proto-oncogene germline mutations have been reported in 97% of MEN 2A cases and are crucial for the onset and the progression of MTC.

These mutations are mostly localized in exon 11 at codon 634, in exon 10 at codons 609-611-618-620 and other mutations located in exons 8, 10, 11 and 13-15 have been also observed.

Actually, especially after the introduction of *RET* genetic screening in the work-up of all patients with MTC, there is a change in the spectrum of detecting *RET* mutations and an increase of diagnosed cases from 10% to 39% of the families with mutations in exon 13-15, so called "rare mutations".[32,33]

Characterization of RET proto-oncogene germline mutations, has made possible genetic testing for MEN 2. The efficiency of this screening justifies it to be advocates for children and adults or a preventative treatment would be possible.

The aims of this study were to characterize point mutations in RET proto-oncogene in an index case and his relatives, to Identify and assess the effectiveness of the management of the index case relatives who have presented the RET proto-oncogene mutation and to search the existence of a possible correlation between the phenotype and genotype among the members of this MEN 2A family.

PATIENTS AND METHODS

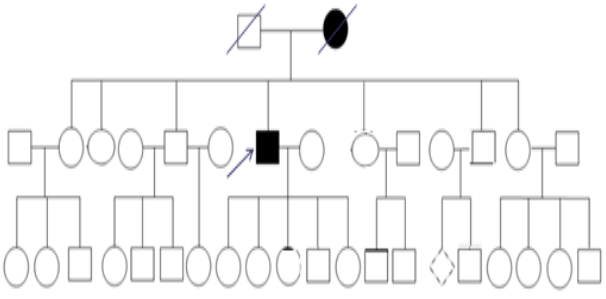

Figure 1: The Index Case Family Pedigree

A 33-year-old man, the fourth in a family of seven brothers and sisters, married with five children alive and well has consulted for a permanent hypertension.

The interrogation and clinical examination revealed: The presence of a thyroid nodule, diarrhea and notalgia. In his family history, there is the notion of the death of his mother at a young age in a total thyroidectomy on nodular goiter and hypertension (Fig.1.). A number of investigations were performed for our patients as a blood calcitonin, which reverted to 106 pg/ml, urinary metanephrines 6.30 µmol/24 h. Serum calcium, PTH and CEA were normal. The MIBG scintigraphy revealed bilateral adrenal tumor and cervical ultrasound showed a thyroid nodule.

Basal serum calcitonin (bCT), PTH and CEA levels were assessed by immune - chemiluminescent/magnetic particle (ICMA)(Abbott Diagnostics assay), Determination of urinary metanephrine was performed by high-performance liquid chromatography (CLHP) and serum calcium by arsenaso III color. Bilateral PHEO ablation followed by total thyroidectomy was done and MTC and PHEO were confirmed by histopathology of the surgically removed tumors.[34]

The relatively young age of the discovery of the PHEO and the MTC suggested that it is about a potential MEN2A syndrome and thus the analysis of RET proto-oncogene exons: 8.9.10.11.13.14.15 and 16 has been performed.

After extracting DNA from peripheral blood leukocytes using the standard salting out method[35], the search for mutations in the RET proto-oncogene was carried out by PCR/sequencing as previously reported.[36]

DNA sequencing reactions were performed using the Big Dye Terminator V3.1 Cycle Sequencing Kit and sequenced on an ABI PRISM 3130 Automated Sequencer (Applied Biosystems, Foster City, CA, USA). Al *RET* exons were sequenced using both forward and reverse primers.

RESULTS

We have identified, in the index case, the RET proto-oncogene, exon 11 germline mutation C634Y a transition G > A at nucleotide level c2096 resulting in TGC conversion to CGC, substituting cysteine to arginine, in the heterozygous state (Fig.2.) and the G691S polymorphisms (exon11) (Fig.3.) S904S (exon15) in the heterozygous state.

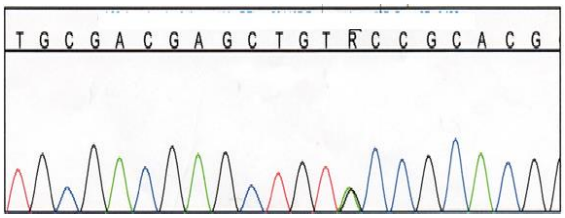

Figure 2: Partial DNA sequence for exon 11 of RET proto-oncogene, showing c. 2096G > A (C634Y) heterozygous variant found in the proband

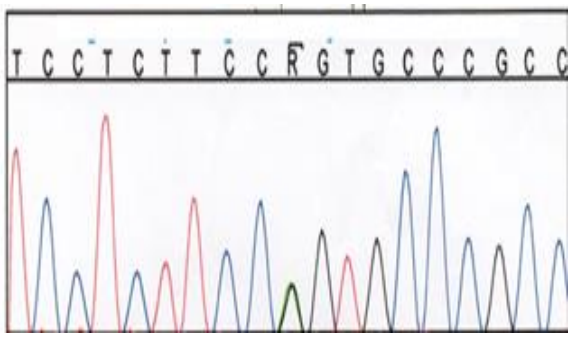

Figure 3: Partial DNA sequence for exon 11 of RET proto-oncogene, showing **G691S** heterozygous variant found in the proband

Then the screening of the C634Y mutation of RET has been offered to all the relatives (Fig.4.) recommendation 7 of the revised ATA Guidelines.[37]

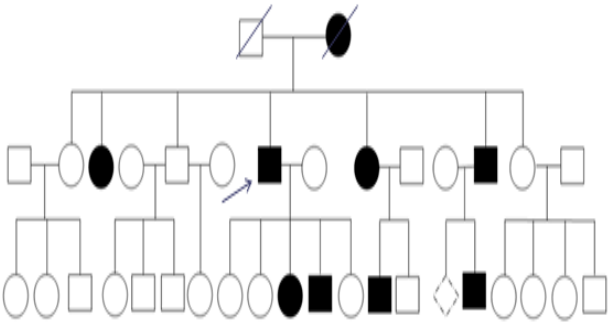

Figure 4: The index case family pedigree after the genetic screening of the C634Y mutation

Table 1: Frequency of Mutation C634Y in Relatives

Number of Relatives	Diagnosis	Mutation	Frequency
7 relatives	NEM2A	C634Y	58.33 %
5 relatives	Unharmed	-	48.33 %

In relatives, Exon 11 RET proto-oncogene analysis identified the C634Y exon 11 mutation in the heterozygous state with a frequency of 58.33% (Table 1).

The screening of the other exons in search of polymorphisms showed that the latter were identical to those of the index case but on the homozygous state (Fig.5) (Fig.6).

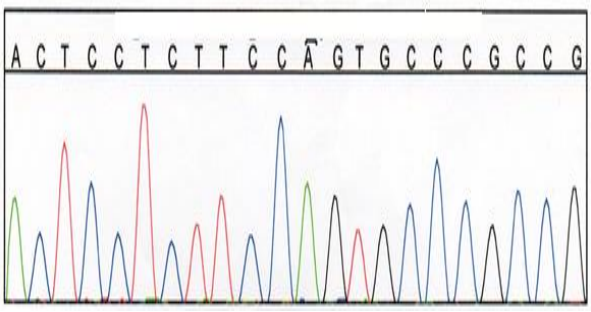

Figure 5: Partial DNA sequence for exon 15 of RET proto-oncogene, showing G691S homozygous variant found in the relatives

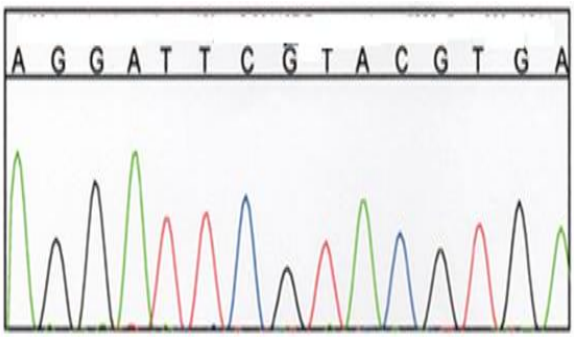

Figure 6: Partial DNA sequence for exon 15 of RET proto-oncogene, showing S904S homozygous variant found in the proband (TCC/TCG)

The average age of the discovery of the mutation in relatives was 18.57 ± 14.87 years (Tabl.2) with extremes of 4 and 40 years.

RET mutated relatives were submitted to clinical and biochemical examination to ascertain thyroid, parathyroid, and adrenal gland involvement.

Screening for PHEO and hyperparathyroidism was performed both at diagnosis and annually during the follow-up for a possible prophylactic taking load.

Sixty-six point sixty-six percent (66.66%) of the MEN 2A index case relatives showed a high rate of calcitonin before surgery. And 50% of them have seen the persistence of high rates following surgery (Tableau.2).

The measurement of urinary catecholamine incomes positive in 42% of the relatives who made the determination.

Al the relatives were recommended for a preventive total thyroidectomy and pheocytochroma ablation (Table 2).

Table 2: Biological, Genetic and Clinical Features of the 7 Relatives Patients

Index Case Relatives	Age at Diagnosis by Years 18.57 ± 14.87	Mutation	CT (pg/ml) Before Surgery	CT after Thyroidectomy Surgery	Urinary Meta Nephrine	Intervention
Nephew (III16)	4 years	C634Y	11	<2	0.37	Thyroidectomized
Son (III-11)	4 years	C634Y	756	760/766/478/423	0.30	Thyroidectomized
Daughter (III-10)	10 years	C634Y	5	< 2	O.25	Thyroidectomized
Nephew (III-13)	11 years	C634Y	11	< 2	0.20	Thyroidectomized
Younger brother (II-12)	27 years	C634Y	86	106/429	4.50	Thyroidectomized+ pheochromocytoma ablation
Sister (III-3)	40 years	C634Y	558.82 /403.8	116/ 76.6 /154	11.03	Thyroidectomized+ pheochromocytoma ablation
Sister (II-9)	34 years	C634Y	4,87	< 2	3.22	Thyroidectomized+ pheochromocytoma ablation

Serum (pg/ml) calcium, parathyroid hormone, Serum CEA were Normal

CT (pg/ml) : Normal value 8.4 pg / ml in men and 5 pg/ml in women

Urinary metanephrine : Normal value = 0,20-1 µmol/24 h

DISCUSSION

Genetic analysis of the RET proto-oncogene has become indispensable in the management of MEN2 syndrome. It allows early surgical indications in genetically predisposed. During this work, we have identified a MEN 2A family from Constantine and characterized the RET proto-oncogene, exon 11 germline mutation C634Y in the heterozygous state. Among the germline mutations of cysteine codon, mutations at codon 634 position are the most highly transforming, more common and occur in ~85% of NEM 2A families, also found in FMTC (30%).[32,38]

The C634Y represents approximately 26% of all mutations at this codon while the C634R mutation (Cys>Arg) represents more than 50%.[39]

The C634Y mutation was first described in Morocco in MEN 2A.[40] Sánchez and al[41], In a study, showed that the most frequent RET mutation in MEN 2A Spanish families was the C634Y[41] also in Japanese and Chinese.[42,43]

In MEN 2A populations, the C634Y incidence is high relatively to C634R. The RET proto-oncogene mutation analyses in French hereditary MEN2A revealed that the most frequent mutations in this population were the C634R and C634Y.[44,45]

This mutation is also present in Caucasians and Americans in which the C634R mutation is the most common.[37]

Exon 11 codon 634 mutation causes ligand-independent dimerization of receptor molecules, enhanced phosphorylation of intracellular substrates and cell transformation.[46]

The mutation of a cysteine residue leaves the unpaired partner cysteine free to form intermolecular disulfide bonds, there by generating covalent receptor dimers that display the constitutive activity.[47]

Among the relatives, 7 (58.33%) C634Y carriers have been identified and these patients were examined for the MTC, the PHEO and HPT for possible taking prophylactic load. And 5 of the relatives (48.33%) will not be followed.

The G691S/S904S RET haplotype was present in the homozygous state in relatives. Several studies suggested that this haplotype appears to influence the age of onset of MTC in patients with the MEN 2A syndrome and patients homozygous for these polymorphisms were, on average, diagnosed with MTC 10 years earlier than patients sharing this haplotype in the heterozygous or wild state.[48-51]

The G691S polymorphism is considered as a functional polymorphism. The change of the aminoacid G → S (glycine to serine) in the RET protein sequence creates a new phosphorylation site, which affects the downstreamsignaling.[48,49] It could also lead to a change in the secondary structure of RET, affecting the flexibility and accessibility of protein solvents.[48,52] The S904S SNP does not lead to an aminoacid change but, appears to be related to the SNP G691S due to a founding effect.

Fifty percent (50%) of the relatives have seen the persistence of high rates of Calcitonin (CT) following surgery. As a part of the MTC, the preoperative CT assay must allow to propose an adequate surgery immediately. Moreover the results of the surgical recovery are assessed on the postoperative CT dosage.[53,54]

The measurement of urinary méthoxyamines income was positive in 42% of the relatives. Indeed PHEO diagnosis is in first-line a biological diagnosis based on the rise of urinary or plasma méthoxyamines.[53]

For mutations at codon 634, the risk of developing unilateral or bilateral pheochromocytoma is high. It appears in 57% of patients, and 15–30% of codon 634 mutations carriers will develop hyperpathyroidism.[6,55]

Patients with C634YRET mutation should be screened periodically from 8 years of age for hyperparathyroidism[8] by measuring serum calcium and parathyroid hormone.

In MEN2, it has shown that specific RET mutations may provide the phenotypic expression of the disease, the MTC aggressiveness, and can provide information on MTC prognosis, could guide prophylactic thyroidectomy and the screening of PHEO and HPT.[32,56]

Phenotypic analysis of the MEN 2A family has shown that: In one case the MTC has been preceded by 4 years the occurrence of PHEO. In 3 cases, the discovery of the MTC and PHEO was simultaneous and in 2 cases the MTC was the only endocrine neoplasia present both at diagnosis and during follow-up. A paraneoplastic clinical and biological cushing was observed in the elder sister. Notalgia and diarrhea in the index case and the remaining relative was asymptomatic.

Several studies have shown a strong correlation between the codon 634 mutation and risk of PHEO and/or HPT in affected MEN 2A families.[44,57,58] We also observed this association MTC and PHEO in the members of this family.

These PHEO are almost synchronous or metachronous to the MTC. They are the first symptom in 13–27% of individuals with pheochromocytomas and MEN 2A.[59-61]

Their penetrance increases with age, being 25% by age 30 years, 52% by age 50 years, and 88% by age 77 years[62], almost always benign and are usually bilateral, and confined to the adrenal gland. The tumors are usually associated with diffuse nodular adrenal medullary hyperplasia.[63]

Children with MEN2A and RET codon 634 mutations (ATA-H category) often develop MTC during the first years of life; therefore, annual physical examination, cervical US, and measurement of serum CT levels should begin at 3 years of age.[56,64]

Because, related and unrelated individuals, with the same germline RET mutations, develop MTC and PHEO at different ages, many authors have suggested that other genetic or epigenetic events can trigger the tumorigenesis including the presence of SNP in RET.[65]

Diarrhea, represents the most frequent systemic manifestation, occurs in affected persons with a plasma calcitonin concentration of more than 10 ng/mL and implies a poorprognosis.[66,67]

Notalgia was observed in the MEN 2A case index. Indeed the notalgia in MEN 2A has been observed in some families.[20]

This lichenoid skin lesion is located over the upper portion of the back and may appear before the onset of MTC[68] and is associated in 36% of cases to the mutation at RET 634 codon.[15,69]

The paraneoplastic Cushing syndrome was observed in 15% of MEN 2A cases. It is associated with ectopic ACTH production.[27,70,71]

According to the revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma and especially the recommendation that says that the current level C category should be changed to a new category, ''high risk'' (H) that includes patients with the RET codon C634 mutations and the RET codon A883F mutation.[36]

The C634Y (ATA-H category) is then characterized by a particular aggressiveness. Thyroidectomy is then recommended before the age 5 years (Recommendation 35 ATA/GTE.[6,36,72,73]

Recommendations for the timing of prophylactic thyroidectomy and the extent of surgery are based upon a model that utilizes these genotype-phenotype.[6,74]

All patients described in this report have undergone thyroidectomy (7 cases), and PHEO ablation only in 4 cases.

The relatives that having been operated on the basis of a germline RET mutation, C-cell hyperplasia, a pre-malignant lesion, has been found in one case, microscopic MTC in 2 cases, MTC in 3 cases.

Three patients had lymph node metastases. These results are identical to those found by different studies.[55,75,76]

In the literature, for mutations located in exon 10 or 11, mainly corresponding to the NEM2A 634 mutation, the prevalence of cancer at the age of five years, is from 50 to 60%, but the possibility of the development of a micro cancer as early as at the age of 15 months has been reported[46,77,78,79].

Similarly lymph node involvement is considered rare before the age of ten years[80]. In children, the cure at ten years is in 100% after MTC surgery in the absence of lymph node involvement, it drops to 84%, in case of lymph node involvement.[81]

Conversely, in patients with a MEN2 A phenotype, monitoring should be prolonged, indefinite, yearly to detect the occurrence of pheochromocytoma. An annual dosage of calcium, supplemented if hypercalcemia by a parathyroid hormone assay will complement the assessment carried out in search of pheochromocytoma.

The screening for PHEO should begin by age 11 years for children in the ATA-H as saying the ATA Recommendation 37[82], while the European Thyroid Association recommends the beginning of the biochemical screening for pheochromocytoma and hyperparathyroidism at 8 years of age (Recommendation 7).[34]

For the timing of prophylactic thyroidectomy and the extent of surgery are based upon a model that utilizes these genotype-phenotype.[83]

The age of thyroidectomy in patients with the C634Y mutation was not respected because the related screening has been achieved, when the MEN 2A case index was identified, but also for psychological and cultural reasons. It is therefore evident that, although these interventions were scheduled to be prophylactic, over 80% of them have been therapeutic.

In fact, recommendations for thyroidectomy are respected in practice less than 15% of the cases for fear of complications of early surgery.[84]

CONCLUSION

This work contributed to the development of genetic testing for MEN 2 in Algeria.

This molecular screening has become an essential tool. It allows, very early, to distinguish individuals at risk of carrying the mutation those that are not carriers and which can be excluded from monitoring for the rest of their life. The surgery is an alternative treatment (prophylactic and/or curative).

Through this work, we hope to contribute to the formation of a multidisciplinary team whose nucleus already exists for the development of genetic testing for MEN 2 and the establishment of a national consensus adapted to our country, for the MEN2 prophylactic thyroidectomy.

This will encourage the development of a national policy against this cancer whose care is very expensive.

REFERENCES

1. Schlumberger M, Pacini F. Thyroid tumors, Paris Edition Nucleon, Chapter 18, 2006, 313-340.
2. Scheuba C, Kaserer K, Moritz A, Drosten R, Vierhapper H, Bieglmayer C, Haas O A and Niederle B. Sporadic hypercalcitoninemia: Clinical and therapeutic consequences, Endocr Relat Cancer, 16, 2009, 243–253.
3. Kommino P. RET proto-oncogene and thyroid cancer, Endocr Pathol, 8(3), Autumn, 1997, 235-239.
4. Deftos LJ, Lee MR, Potts Jr JT. A radioimmunoassay for thyrocalcitonin, Proceedings of the National Academy of Sciences of the United States of America, 60, 1968, 293-299.
5. Neher R, Riniker B, Rittel W, Zuber H. Human calcitonin, Structure of calcitonin M and D, He/v Chim Acta, 51(8), 1968, 1900-l905.
6. Brandi M L, Gagel R F, Angeli A, Bilezikian J P, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, Gheri R G, Libroia A, Lips C J M, Lombardi G, Mannelli M, Pacini F, Ponder B A J, Raue F, Skogseid B,

Tamburrano G, Thakker R V, Thompson N W, Tomassetti P, Tonelli F, Wells S A, Marx S J. Guidelines for diagnosis and therapy of MEN Type 1 and Type 2. J Clin Endocrinol, 86, 2001, 5658–71.

7. RaueF, Frank-RaueK. Multiple Endocrine Neoplasia Type 2: 2007 Update: Horm Res, 68(suppl 5), 2007, 101–104.

8. Kloos R T, Eng C, Evans D B, Francis G L, Gagel R F, Gharib H, Moley JF, Pacini F, Ringel MD, Schlumberger M, Wells S A, Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid, 19, 2009, 565–612.

9. Kouvaraki MA, Shapiro SE, Perrier ND, Cote GJ, Gagel RF, Hoff AO, Sherman SI, Lee JE Evans DB. RET proto-oncogene: a review and update of genotype-phenotype correlations in hereditary medullary thyroid cancer and associated endocrine tumors, Thyroid, 15, 2005, 531-544.

10. Frank-Raue K, Rondot S, Raue F. Molecular genetics and phenomics of RET mutations: Impact on prognosis of MTC Molecular and Cellular Endocrinology, 322, 2010, 2–7.

11. Frank-Raue K, Rondot S, Schulze E, Raue F. Change in the spectrum of RET mutations diagnosed between 1994 and 2006, Clin Lab, 53, 2007, 273–282.

12. Chong GC, Beahrs OH, Sizemore GW, Woolner LH. Medullary carcinoma of the thyroid gland Cancer, 35, 1975, 695–704.

13. Lenders JWM, Eisenhofer G, Mannelli M, Pacak K. Phaechromocytoma, Lancet, 366, 2005, 665-675.

14. Machens A, Brauckoff M, Holzhausen H, Thanh PN, Lehnert H, Dralle H. Codon-specific development of pheochromocytoma in multiple endocrine neoplasia type 2, J Clin Endocrinol Metab, 90(7), 2005, 3999-4003.

15. Verga U, Fugazzola L, Cambiaghi S, Pritelli C, Alessi E, Cortelazzi D, Gangi E, Beck-Peccoz P. Frequent association between MEN2A and cutaneous lichen amyloidosis, Clin Endocrinol (Oxf), 59, 2003, 156–61.

16. Kim J, Reutrakul S, Davis DB, Kaplan EL, Refetoff S. Multiple endocrine neoplasia 2A syndrome presenting as peripartum cardiomyopathy due to catecholamine excess, Eur J Endocrinol, 151, 2004, 771–777.

17. Yun Jung Lee, Suk Chon, Sang-Ho Lee, Tae-Won Lee, Chun-Gyoolhm, Tae June Noh, Seungjoon Oh, Jeong-Taek Woo, Sung-Woon Kim, Jin-Woo Kim, and Young Seol Kim. A family Presenting with Catastrophic Features due to Pheochromocytoma Associated with Multiple Endocrine Neoplasia 2A, Endocrinol metab, 25(2), 2010 Jun, 135-141.

18. Gimm O. Multiple endocrine neoplasia type 2: clinical aspects, Frontiers of Hormone Research, 28, 2001, 103–130. Multiple endocrine neoplasms Alberto Falchetti MD, PhD. Best Practice & Research Clinical Rheumatology, Vol 22, 2008, 149–163.

19. Alexander L, Shifrin MD, FACS Cristina, Xenachis MD, Angela Fay, Neptune and Matawan NJ. One hundred and seven family members with the rearranged during transfection V804M proto-oncogene mutation presenting with simultaneous medullary and papillary thyroid carcinomas, rare primary hyperparathyroidism, and no pheochromocytomas: Is this a new syndrome-MEN 2C? Surgery, Volume 146, Number 6, 2009, 998-1005.

20. Gagel RF, Levy ML, Donovan DT, Alford BR, Wheeler T, Tschen JA. Multiple endocrine neoplasia type 2a associated with cutaneous lichen amyloidosis, Ann Intern Med, 111, 1989, 802–806.

21. Nunziata V, Giannattasio R, Di Giovanni G, D'Armiento MR, Mancini M. Hereditary localized pruritus in affected members of a kindred with multiple Endocrine neoplasia type 2A Sipple`s syndrome. Clinendocrinol Oxf, 30, 1989, 57-63.

22. Donovan DT, Levy ML, Furst EJ, Alford BR, Wheeler T, Tschen JA, Gagel RF. Familial cutaneous lichen amyloidosis in association with multiple endocrine neoplasia type 2A : a new variant, Henry Ford Hosp Med J, 37, 1989, 147–50.

23. Hassan M. Heshmati, MD, Hossein Gharib, MD, Jon A. Van Heerden, MD, Rochester, Minnesota, Glen W. Sizemore, MD, Maywood, Illinois. Advances and Controversies in the Diagnosis and Management of Medullary Thyroid Carcinoma, The American Journal of Medicine T, Volume 103, 1997, 60–69.

24. Nelson Wohllk, Heiko Schweizer, Zoran Erlic, Kurt Werner Schmid, Path d, Martin K Walz, Friedhelm Raue, Hartmut PH Neumann. Multiple endocrine neoplasia type Best Practice & Research Clinical Endocrinology & Metabolism, 24, 2010, 371–387.

25. Alberto Falchetti. Multiple endocrine neoplasms, Best Practice & Research Clinical Rheumatology, Vol 22, No 1, 2008, 149–163.

26. Modigliani E. Le cancer médullaire de la thyroïde, Rev Med Interne, 20, 1999, 490-503.

27. Rubinstein WS. Endocrine Cancer Predisposition Syndromes, Hereditary Paraganglioma, Multiple Endocrine Neoplasia Type 1, Multiple Endocrine Neoplasia Type 2 and Hereditary Thyroid Cancer, Hematol Oncol Clin, N 24, 2010, 907–937.

28. Mulligan LM, Eng C, Attie T, Lyonnet S, Marsh DJ, Hyland VJ, Robinson BG, Frilling A, Verellen-Dumoulin C, Safar A, Venter DJ, Munnich A, Ponder BAJ. Diverse phenotypes associated with exon 10 mutations of the RET proto-oncogene, Hum Mol Genet, 3, 1994, 2163-7.

29. Lairmore TC, Dou S, Howe JR, Chi D, Carlson K, VeileR, S K Mishra, S A Wells Jr, and H Donis-Keller. A 1.5-megabase yeast artificial chromosome contig from human chromosome 10q11.2 connecting three genetic loci (RET, D10S94, and D10S102) closely linked to the MEN2A locus, Proc Natl Acad Sci USA, 90, 1993, 492-6.

30. Takahashi M, Buma Y, Iwamoto T, Inaguma Y, Ikeda H, Hiai H. Cloning and expression of the ret proto-oncogene encoding a tyrosine kinase with two potential transmembrane domains, Oncogene, 3, 1988, 571-8.

31. Hubner RA, Houlston RS. Molecular advances in medullary thyroid cancer diagnosis, Clin Chim Acta, 370, 2006, 2-8.

32. Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, Van Amstel HK, Lips CJ, Nishisho I, Takai SI, Marsh DJ, Robinson BG, Frank-Raue K, Raue F, Xue F, Noll WW, Romei C, Pacini F, Fink M, Niederle B, Zedenius J, Nordenskjöld M, Komminoth P, Hendy GN, Mulligan LM, Gharib H, Thibodeau S N, Lacroix A, Frilling A, Ponder B A J, Mulligan L M. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis, JAMA, 276, 1996, 1575-1579.

33. Raue F, Frank-Raue K. Genotype-phenotype relationship in multiple endocrine neoplasia type 2. Implications for clinical management, *Hormones,* 8(1), 2009, 23-28.

34. Elisei M, Alevizaki B, Conte-Devolx K, Frank-Raue V, Leite G.R, Williams F. European Thyroid Association Guidelines for Genetic Testing and Its Clinical Consequences in Medullary Thyroid Cancer Eur Thyroid J, 1, 2012, 216–231.

35. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells, *Nucleic Acids Research,* 16(3), 1988 Feb 11, 1215.

36. Elisei R, Cosci B, Romei C, Bottici V, Sculli M, Lari R, Barale R, Pacini F, PincheraA. RET exon 11 (G691S) polymorphism is significantly more frequent in sporadic medullary thyroid carcinoma than in the general population, J Clin Endocrinol Metab, 89, 2004, 3579–3584.

37. Samuel A, Wells Jr, Sylvia L, Asa, Henning Dralle, Rossella Elisei, Douglas B Evans, Robert F Gagel, Nancy Lee, Andreas Machens, Jeffrey F Moley, Furio Pacini, Friedhelm Raue, Karin Frank-Raue, Bruce Robinson, M Sara Rosenthal, Massimo Santoro, Martin Schlumberger, Manisha Shah, and Steven G. Waguespack, Revised American Thyroid Association Guidelines for the Management of

Medullary Thyroid Carcinoma The American Thyroid Association Guidelines Task Force on Medullary Thyroid Carcinoma, Thyroid Volume, 25, Number 6, 2015.

38. T Gill JR, Reyes-Mugica M, Lyengar S, Kidd KK, Touloukian RJ, Smith C, Keller MS, Genel M. Early presentation of metastatic medullary carcinoma in multiple endocrine neoplasia type IIA: implications for therapy, J Pediatr, 129, 1996, 459–63.

39. Mulligan LM, Eng C, Healey CS, Clayton D, Kwok JB, Gardner E, Ponder MA, Frilling A, Jackson CE, Lehnert H, Neumann H P H, Thibodeau S N and Ponder B A J. Specific mutations of the RET proto-oncogene are related to disease phenotype in Men 2A and FMTC, Nature Genetics, 6, 1993, 70-4.

40. Benazzouz A, Chraïbi Y, Doghmi S, El Bacha S, Boutayeb A, Kadiri L, Hilal. Caractérisation de la mutation C634Y du proto-oncogène RET dans une famille Marocaine de néoplasie endocrinienne multiple de type 2AB, Ann Endocrinol, 67, Vol 1, 2006, 21-26.

41. Sánchez B, Robledo M, Biarnes J, Sáez ME, Volpini V, Benítez J, Navarro E, Ruiz A, Antiñolo G and Borrego S. High prevalence of the C634Y mutation in the RET proto-oncogene in MEN 2A families in Spain, J Med Genet, 36, 1999, 68–70.

42. YuraShiharama, Ogura K, Takami H, Ito K, Tohsen T, Miyauchi A, Nakamura Y. Mutational analysis of the RET proto-oncogene In 71 japanase patients with medullary thyroid cancercarcinoma, J Hu Genet, 43, 1998, 101-106.

43. Yulin Zhou, Yongju Zhao, Bin Cui, LiqunGu, Shaoxin Zhu, Jianjun Li, Jinbo Liu, Ming Yin, Tieyun Zhao, Zhiqiang Yin, Chaoli Yu, Changyou Chen, Liming Wang, Buyun Xiao, Jie Hong, Yifei Zhang, Zhengyi Tang, Shu Wang, Xiaoying Li and Guang Ning. RET proto-oncogene mutations are restricted to codons 634 and 918 in mainland Chinese families with MEN2A and *Clinical Endocrinology,* 67, 2007, 570–576.

44. Schuffenecker M, Virally-Monod R, Brohet, Goldgar D, Conte-Devolx B, Leclerc L, Chabre O, Boneu A, Caron J, Houdent C, Modigliani E, Rohmer V, Schlumberger M, Eng C, Guillausseau PJ, Lenoir GM." Risk and penetrance of primary hyperparathyroidism in multiple endocrine neoplasia type 2A families with mutations at codon 634 of the RET proto-oncogene, Groupe d'étude des tumeurs à calcitonine" *Journal of Clinical Endocrinology & Metabolism*, Vol 83, No. 2, 1998, 487–491.

45. Berard J. L, Kraimps F, Savagner, Murat A, Renaudin K, Nicolli-Sire P, Bertrand G, MoisanJ-P, Bezieau S." Germline sequence variants S836S and L769L in the RE arranged during transfection (RET) proto-oncogene are not associated with predisposition to sporadic medullary carcinoma in the French population," *Clinical Genetics*, vol. 65, No. 2, 2004, 150–152.

46. Machens A, Niccoli-Sire P, Hoegel J, Frank-Raue K, Van Vroonhoven J.J, Roeher HD and al, Wahl RA, Lamesch P, Raue F, Conte-Devolx B, Dralle H, European Multiple Endocrine Neoplasia (EUROMEN) Study Group, Earlymalignant progression of hereditarymedullarythyroid cancer, N Engl J Med, 349, 2003, 1517-1525.

47. Asai N, Iwashita T, Matsuyama M, Takahashi M. Mechanism of activation of the ret proto-oncogene by multiple endocrine neoplasia 2A mutations, *Mol Cell Biol,* 15, 1995, 1613–1619.

48. Robledo M, Gil L, Pollan M, Cebrian A, Ruiz S, Azanedo M, Benitez J, Menarguez J, Rojas JM. Polymorphisms G691S/S904S of RET as genetic modifiers of MEN 2A, Cancer Res, 63, 2003, 1814–1817.

49. Cardot-Bauters C, Leteurtre E, Leclerc L, Vantyghem M-C, Do cao C, Wemeau J-L, d'Herbomez M, Carnaille B, Barbu V, Pinson S and P Pigny, Groupe d'Etude des Tumeurs Endocrines (GTE). Does theRET variant G691S influence the features of sporadic medullary thyroid carcinoma? Clinical Endocrinology, 69, 2008, 506–510.

50. Shifrin A L, Ogilvie J B, Stang MT, Musial Fay A, Yen-Hong K, Matulewicz T, Xenachis C Z and Vernick J J. Single nucleotide polymorphisms act as modifiers and correlate with the development of medullary and simultaneous medullary/the RET 804M proto-oncogene mutation, Surgery, 148, 2010, 1274-81.

51. Siqueira D R, RomittiM, da Rocha A P, Ceolin L, Meotti C, Estivalet A, Punales M, Maia A L. The RET polymorphic alleleS836S is associated with early metastatic disease in patients with hereditary or sporadic medullary thyroid carcinoma, *Endocr Relat. Cancer,* 17, 2010, 953-963.

52. Colombo C, Minna E, Rizzetti MG, RomeoP, Lecis D, Persani L, Mondellini P, Pierotti M A, Greco A, Fugazzola L. The modifier role of RET-G691S polymorphism in hereditary medullary thyroid carcinoma: functional characterization and expression/penetrance studies, Orphanet Journal of Rare Diseases, 10, 2015, 25.

53. Proye C. Cancers médullaires de la thyroïde hyperthyrocalcitoninémie persistante après chirurgie, réinterventions-résultats, Annales de chirurgie, Volume, 128, Juin 2003, 289-292.

54. Proye C, Marmousez T, Modigliani E. Stratégie diagnostique et conduite à tenir dans les hypercalcitoninémies résiduelles après traitement chirurgical du cancer médullaire de la thyroïde, *Rev Fr Endocrinol Clin,* 34, 1993, 549-562. 6 55

55. Modigliani E, Cohen R, Campos JM, Conte-Devolx B, Maes B, Boneu A, Schlumberger M, Bigorgne JC, Dumontier P, Leclerc L, Corcuff B, Guilhem I. Prognostic factors for survival and for biochemical cure in medullary thyroid carcinoma: results in 899 patients. The GETC Study Group. Groupe d'étude des tumeurs à calcitonine, Clin. Endocrinol, 48, 1998, 265–273.

56. YipL, Cote GJ, Shapiro SE, Ayers GD, Herzog CE, Sellin RV, Sherman SI, Gagel RF, Lee JE, Evans DB. Multiple Endocrine Neoplasia Type 2: Evaluation of the Genotype-Phenotype Relationship, Arch Surg, 138, 2003, 409-16.

57. Berndt I, Berndt I, Reuter M, Saller B, Frank-Raue K, Groth P, Grussendorf M, Raue F, Ritter, M M, Hoppner, W. A new hot spot for mutations in the ret proto-oncogene causing familial medullary thyroid carcinoma and multiple endocrine neoplasia type 2A, J Clin Endocrinol Metab, 83, 1998, 770–774.

58. Hansford J R, Lois M Mulligan LM. Review article Multiple endocrine neoplasia type 2 and *RET*: from neoplasia to neurogenesis, *J Med Genet,* 37, 2000, 817-827.

59. Inabnet WB, Caragliano P, Pertsemlidis D. Pheochromocytoma. Inherited associations, bilaterality, and cortex preservation, Surgery, 128, 2000, 1007–1011.

60. Frank-Raue K, Rybicki LA, Erlic Z, Schweizer H, Winter A, Milos I, Toledo SP, Toledo RA, Tavares MR, Alevizaki M, Mian C, Siggelkow H, Hüfner M, Wohllk N, Opocher G, Dvořáková S, Bendlova B, Czetwertynska M, Skasko E, Barontini M, Sanso G, Vorländer C, Maia AL, Patocs A, Links TP, de Groot JW, Kerstens MN, Valk GD, Miehle K, Musholt TJ, Biarnes J, Damjanovic S, Muresan M, Wüster C, Fassnacht M, Peczkowska M, Fauth C, Golcher H, Walter MA, Pichl J, Raue F, Eng C, Neumann HP. International RET Exon 10 Consortium Risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germline RET mutations located in exon 10, Hum Mutat, 32, 2011, 51–58.

61. Pomares FJ, Canas R, Rodriguez JM, Hernandez AM, Parrilla P, Tebar FJ. Differences between sporadic and multiple endocrine neoplasia type 2A phaeochromocytoma, Clin Endocrinol (Oxf), 48, 1998, 195–200.

62. Imai T, Uchino S, Okamoto T, Suzuki S, Kosugi S, Kikumori T, Sakurai A. High penetrance of pheochromocytoma in multiple endocrine neoplasia 2 caused by germ line RET codon 634, Eur J Endocrinol, 168(5), Apr 152013, 683-7.

63. Lairmore TC, Ball DW, Baylin SB, Wells SA Jr. Management of pheochromocytomas in patients with multiple endocrine neoplasia type 2 syndromes, Ann Surg, 217, 1993, 595–601.

64. Punales M K, Graf H, and Gross J L, Maia A L. RET codon 634 mutations in multiple endocrine neoplasia type 2: variable clinical features and clinical outcome, J Clin Endocrinol Metab, 88, 2003, 2644–2649.

65. De Groot J W B, Links T P, Plukker J TM, Lips C J M, and Robert M. W. Hofstra RMW. *RET* as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumors, Endocrine Reviews, 27(5), 2006, 535–560.

66. DeLellis R, Lloyd RV, Heitz PU, Eng C. Pathology and genetics: tumours of the endocrine organs. World Health Organization classification of tumours Oxford, UK, Oxford University Press, 2004.

67. Ponder BA, Ponder MA, Coffey R, Pembrey ME, Gagel RF, Telenius-Berg M, Semple P, Easton DF. Risk estimation and screening in families of patients with medullary thyroidcarcinoma, Lancet, 1, 1988, 397–401.

68. Ceccherini I, Romei C, Barone V, Pacini F, Martino E, Loviselli A, Pinchera A, Romeo G. Identification of the Cys634 –Tyr mutation of the RET proto-oncogene in a pedigree with multiple endocrine neoplasia type 2A and localized cutaneous lichen amyloidosis, J Endocrinol Invest, 17, 1994, 201–204.

69. Santoro M, Carlomagno F, and Melillo R. RET A multi-faceted gene in human cancer, Endocrinol Metab, 27(3), 2012, 173-179.

70. Wójtowicz M, Rękas-Wójcik A, Lipa A, Prystupa A, Dzida G. Paraneoplastic syndromes in daily clinical practice, Journal of Pre-Clinical and Clinical Research, Vol 8, No 2, 2014, 71-75.

71. Ares J, Díaz-Naya L, Martín-Nieto A, Joaquín Pertierra J. MEN 2A with double mutation in RET gene: Ectopic ACTH production in medullary thyroid carcinoma, Letters to the Editor Med Clin (Barc), 146(9), 2016, 418–421.

72. Niccoli – Sire P. Cancer médullaire de la thyroïde et néoplasies endocriniennes multiples de type 2, Livret de recommandations pour la prise en charge diagnostique et thérapeutique élaboré par le conseil scientifique du GTE, Edition Janvier 2006.

73. You YN, Lakhani V and Wells SA. New directions in the treatment of thyroid cancer. *Journal of the American College of Surgeons*, 205, 2007, S45–S48.

74. Frank-Raue K, Buhr H, Dralle H. Long-term outcome in 46 gene carriers of hereditary medullary thyroid carcinoma after prophylactic thyroidectomy: impact individual *RET* genotype, Eur J Endocrinol, 155, 2006, 229-236.

75. Gagel RF, Cote GJ, Martins Bugalho MJ, Boyd AE 3rd, Cummings T, Goepfert H, Evans DB, Cangir A, Khorana S, Schultz PN. Clinical use of molecular neoplasia type 2A, J Intern Med, 238(4), 1995 Oct, 333-41.

76. Learoyd DL, Marsh DJ, Richardson AL, Twigg SM, Delbridge L, Robinson BG. Genetic testing for familial cancer Consequences of RET proto-oncogene mutation analysis in multiple endocrine neoplasia, type 2, Arch Surg, 132(9), 1997 Sep, 1022-5.

77. Ukkat J, Lorentz K, Hinze R, Thomusch O, Dralle H. Importance of early screening and prophylactic thyroidectomy in asymptomatic non index RET germline carriers, World J. Surg, 25, 2001, 713-717.

78. Niccoli-Sire P, Murat A, Baudin E, Henry JF, Proye C, Bigorne JC, B standig B, Modigliani E, Morange S, Schlumberger M, Conte-Devolx B. Early prophylactic thyroidectomy in MEN 2/FMTC gene carriers: results in 71 thyroidectomized patients, The French Calcitonin Tumors Study Group (GZTC), Eur. J. Endocrinol, 141, 1999, 468-474.

79. Machens A, Holzhausen H J, Thanh PN, Dralle H. Malignant progression from C cell hyperplasia to medullary thyroid carcinoma in 167 carriers of RET germline mutations, Surgery, 134, 2003, 425-431.

80. Skinner M A, Moley J A, Dikkey W G, Owzar K, Debenedetti M K, Wells S A Jr. Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A, N. Engl. J. Med, 353, 2005, 1105-1113.

81. Raval M V, Sturgeon C, Bentrem D J, Elaraj D M, Stewart A K, Winchester D J, Ko CY, Reynolds M. Influence of lymp mode metastases on survival in pediatric medullary thyroid cancer, J. Pediatr. Surg, 45, 2010, 1947-1954.

82. Wells Jr SA, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, Lee N, Machens A, Moley JF8, Pacini F9, Raue F10, Frank-Raue K10, Robinson B11, Rosenthal MS12, Santoro M13, Schlumberger M14, Shah M15, Waguespack SG6. Revised American Thyroid Association guidelines for the management of medullary thyroidcarcinoma, Thyroid, 25, 2015, 567–610.

83. Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, Gheri RG, Libroia A, Lips CJ, Lombardi G, Mannelli M, Pacini F, Ponder BA, Raue F, Skogseid B, Tamburrano G, Thakker RV, Thompson NW, Tomassetti P, Tonelli F, Wells SA Jr, Marx SJ. Guidelines for diagnosis and therapy of MEN Type 1 and Type 2, J Clin Endocrinol Metab, 86, 5658-5671, 17.

84. Piolat C, Dyon J F, Sturm N, Pinson S, BostM, Jouk P S, Jouk PS, Plantaz D, Chabre O. Very early prophylactic thyroid surgery for implants with a mutation of the RET proto-oncogene at codon 634: evaluation of the implementation of interval guidelines for MEN type 2 in a single center, Clin.Endocrinol (Oxf), 65, 2006, 118-124.

Source of Support: Nil, **Conflict of Interest:** None.