Original Article

Molecular analysis of the rearranged during transfection proto-oncogene in Moroccan patients with medullary thyroid carcinoma

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ABSTRACT

Background: Germline mutations of the proto-oncogene rearranged during transfection (*RET*) are pathognomonic of hereditary medullary thyroid carcinoma (MTC). In this study, genetic analysis and familial testing of the *RET* proto-oncogene in Moroccan families with MTC were performed. **Patients and Methods:** Thirty-one index cases with MTC and 115 of their relatives were included in this study. The entire coding region of *RET* was investigated by direct sequencing of polymerase chain reaction products. Once a mutation was identified, the target exon was sequenced in available relatives. **Results:** Seven distinct germline mutations of *RET* were identified in 45.2% (14/31) of probands. The most prevalent mutations were located at codon 634 (p.C634R/Y/F) and restricted to families with multiple endocrine neoplasia type 2A (MEN2A) (50% of the 14 mutation carriers, 7/14), followed by mutation at codon 918 (p.M918T) in all MEN2B cases (21.4%, 3/14), then by mutations at codons 804 (p.V804L/M) (14.3%, 2/14); and 891 (p.S891A) (14.3%, 2/14) detected in all patients with apparently sporadic MTC. Familial screening detected *RET* mutations in 19.1% (22/115) of the studied relatives; 36.4% (8/22) were found with MEN2A symptoms, and 63.6% (14/22) were asymptomatic. About 55% (12/22) were subjected to total therapeutic or prophylactic thyroidectomy. **Conclusion:** This is the first comprehensive genetic screening showing the spectrum of mutations in *RET* in Moroccan patients with MTC, which showed a predominance of mutations at codon 634. These results further support the necessity of genetic testing in MTC patients to provide early diagnosis and adequate initial treatment of these patients. This will moreover, contribute to the definition of a national policy for the control of this cancer in Morocco.

Key words: Genetic screening, medullary thyroid carcinoma, Morocco, multiple endocrine neoplasia type 2, mutation, proto-oncogene rearranged during transfection

INTRODUCTION

Medullary thyroid carcinoma (MTC) is a malignant tumor of the calcitonin secreting parafollicular C cells of the thyroid. MTC occurs sporadically or is inherited in about 25-30% of cases as a component of multiple endocrine neoplasia type 2 (MEN2)[1,2] with variable expressivity and age-dependant penetrance. Inherited MTC syndromes (MEN2) affect approximately 1 in 30,000 individuals in the general population.[3] MEN2 occurs as one of the three distinct entities: MEN2A (MEN2, OMIM 171400), MEN2B (OMIM 162300) or familial MTC (FMTC, OMIM 155240). In all syndromes, MTC is present and can be associated with pheochromocytoma (PHEO) in the more aggressive MEN2A and MEN2B forms in about 50% of cases, and/or primary hyperparathyroidism (HPT) in up to 30% of MEN2A patients. MEN2B is also characterized by developmental abnormalities such as ganglioneuromas and marfanoid habitus.[2]

All of the MEN2 subtypes arise as a result of activating mutations in the rearranged during transfection (*RET*) proto-oncogene (OMIM 164761). *RET* is localized on 10q11.2, contains 21 exons and encodes a tyrosine kinase receptor for the glial cell line-derived neurotrophic factor family of ligands.[4] RET has a large extracellular domain

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containing a cysteine-rich region, which is thought to play a role in protein conformation and dimerization, a four cadherin-like repeat, a transmembrane domain, and an intracellular tyrosine kinase domain involved in the activation of the intracellular signalling pathways, which is crucial during cell growth and differentiation.[5] Germline mutations are described in the six exons of the *RET* gene: 10, 11, 13, 14, 15 and 16 in 92% of MEN2 and rarely in exons 5 and 8.[6-10] All the mutations reported up to now are present on public databases (http://www.arup.utah.edu/database).

Extensive reports in the literature showing strong genotype-phenotype correlations have grouped the *RET* mutations into three levels of clinical risk based on the age of onset, the aggressiveness of MTC and the presence of other endocrine tumors.[8,11] The American Thyroid Association (ATA) recently updated this classification into four risk levels A, B, C and D; A being the lowest risk level and D the highest.[3] Ninety five % of MEN2B cases exhibit mutations with the highest risk level (level D) at exon 16 (codon 918) or rarely (4%) at exon 15 (codon 883); both exons encode the cytoplasmic kinase domain(s) of the receptor. The 918 codon mutation is usually produced as a *de novo* mutation.[12] The majority of cases (80–98%) of MEM2A and FMTC have germline mutations involving the six cysteine codons 609, 611, 618, 620 (exon 10, level B), 630 and 634 (exon 11, level C) located in the extracellular domain of the protein. Mutations in cytoplasmic RET domains (level A) at codons 768, 790 and 791 (exon 13) and codons 804, 844 (exon 14) or codon 891 (exon 15) account for far fewer cases of MEN2A or FMTC.[10,13-16]

Currently, *RET* genetic testing is widely available internationally. It allows identification of asymptomatic carriers avoiding unpleasant stimulation tests (pentagastrin stimulated calcitonin test), and providing prophylactic thyroidectomy; the only effective treatment for MEN2 patients before the development of biochemical or clinical evidence of MTC.[17] Moreover, the therapeutic strategy is conditioned by the ATA risk level of the mutation carried by the patient. Preventive thyroidectomy should be done between the age of 5 and 10,[18] at the age of 5, before the age of 5 and as soon as possible in the 1st year of life, in patients with level A, B, C and D mutations, respectively.[3]

In Morocco, only two groups including ours have reported *RET* mutational analysis of MTC patients[19-21] which however, were limited to the seven relevant exons (8, 10, 11, 13–16). Herein, we present the first molecular analysis of the whole coding region of *RET* and familial genetic testing in 31 Moroccan families with MTC with the prospect of improving the diagnosis and clinical management of this cancer in our country.

PATIENTS AND METHODS

This study was approved by the Local Ethics Committee (Faculty of Medicine and Pharmacy, Rabat, Morocco) in accordance with the rules of the Helsinki declaration (www.espace.ethique.org). Consent was obtained from all patients or their parents after full explanation of the purpose and nature of all procedures.

The patients were referred to the Endocrinology Department of the Ibn Sina Hospital, Rabat, Morocco for pre and/or postoperative management of MTC. A total of 146 individuals was investigated including 31 probands and 115 relatives from different areas of Morocco. Two of the families (F01 and F06) have been previously reported.[20,21] Classification of patients as MENA, MEN2B, FMTC or sporadic MTC (sMTC), was performed according to the ATA Guideline Task Force criteria.[3] Due to the small family sizes, it was not possible to fulfill the strict international criteria for FMTC, which suggest at least four cases of MTC per family[3]. Thus, patients with no clinical evidence of PHEO, parathyroid hormone (PTH) and/or any manifestation of MEN2B were classified as apparently sporadic cases. The term "hereditary sMTC (hsMTC)" was used for those with a *RET* mutation and "sMTC" for patients negative for a *RET* mutation.

Basal serum calcitonin (bCT) levels were assessed by radioimmunoassay (IRMA, Cis-Bio International Elisa HCT; N < 10 ng/L). The pentagastrin stimulated calcitonin test is not available in Morocco. Determination of urinary metanephrine (NV: 0.2–1 µmol/24 h) and normetanephrine (NV: 0.4–2.10 µmol/24 h) was performed by high-performance liquid chromatography (CLHP) and parathormone by ummilite Diagnostics Product Corporation. MTC, PHEO and PTH were confirmed by histopathology of the surgically removed tumors.

The index patients were clinically and/or biochemically classified as sMTC, FMTC, MEN2A or MEN2B.

The clinical data of the studied patients are summarized in Tables 1 and 2.

Genetic counseling

Familial screening and genetic counseling were performed according to the ATA recommendations.[3] When symptomatic or nonsymptomatic mutation carriers were identified, clinical and biochemical management and/or thyroidectomy were suggested.

Rearranged during transfection mutational analysis

Genomic DNA was extracted from peripheral blood leukocytes using standard procedures (phenol-chloroform)

or Qiagen DNA extraction kit (Qiagen, Hilden, Germany). The exons 8, 10, 11, 13–16 and flanking intronic boundaries of *RET* were amplified as previously described[13,22] and directly sequenced. The remaining exons (1–7, 9, 12 and 17–20) were amplified using primer sets designed by primer 3.0 software (Simgene.com). Primer sequences and

Table 1: Clinical features and genetic findings of MTC probands

Patient identification	Sex	CMT				PHEO (years after [+] or before [–] MTC)	HPT	Metastase local/distant*	*RET* mutation	Age at surgery for MTC/ PHEO (years)
		Age diagnosis	CT presurgery/ postsurgery	Unilateral/ bilateral	Histology					
MEN2A										
F01-II.13	Female	23	NP/↑	Bilateral	MTC	c, b, h (+8)	c, b	Yes/Yes (10)	p.C634R	24/32
F06-II.8	Female	41	-/↑	Bilateral	MTC	c, b, h (+3)	N	No/No	p.C634Y	41/44
F16-II.7	Female	38	-/↑	Bilateral	MTC	NA	b	Yes/No	p.C634R	38/-
F21-II.3	Male	32	-/↑	Bilateral	MTC	c, b, h (−6)	N	Yes/Yes	p.C634R	39/33
F22-II.9	Male	44	NP	Bilateral	MTC	c, b, h (−1)	c, b, h	Yes (1)/No	p.C634Y	45/44
F27-II.1	Male	40	↑/↑	Bilateral	MTC	c, b, h	N	Yes/No	p.C634R	40/40
F31-II.3	Male	34	↑/NP	Bilateral	MTC	c, b, h (−1)	N	No/No	p.C634F	36/35
MEN2B										
F18-II.8	Female	31	NP	Bilateral	MTC	c, b, h (+1)	N	Yes/Yes	p.M918T	32/33
F24-II.6	Female	19	-/↑	Bilateral	MTC	N	N	Yes/Yes	p.M918T	19/-
F26-II.11	Female	16	-/↑	Bilateral	MTC	N	N	Yes/No	p.M918T	16/-
hsMTC										
F09-II.2	Female	56	-/↑	Bilateral	MTC, CCH, PTC	N	N	No/No	p.S891A	56/-
F12-II.5	Female	38	-/↑	Unilateral	MTC	N	N	No/No	p.V804L	38/-
F19-II.4	Female	55	NA	Unilateral	MTC	N	N	No/No	p.V804M	55/-
F30-II.12	Female	32	NA	Unilateral	MTC	N	N	No/No	p.S891A	33/-
MTC sporadic										
F02-II.1	Female	41	NP/↑	Unilateral	MTC	NP	NP	No/No	No	41/-
F03-II.2	Female	40	NP	NP	MTC	NP	NP	No/No	No	40/-
F04-II.4	Male	21	NP/↑	Unilateral	MTC	NP	NP	No/No	No	21/-
F05-II.1	Male	49	NP/↑	Bilateral	MTC	NP	NP	No/No	No	49/-
F07-II.3	Female	42	NP/N	Unilateral	MTC	N	N	No/No	No	50/-
F08-II.2	Male	48	NP	Unilateral	MTC	N	N	No/No	No	48/-
F10-II.5	Female	30	NP/↑	Unilateral	MTC	N	N	No/No	No	30/-
F11-II.3	Male	42	NP	NP	MTC	NP	NP	No/No	No	42/-
F13-II.2	Male	50	NP/↑	Unilateral	MTC	N	N	No/No	No	50/-
F14-II.1	Female	54	NP	Unilateral	MTC	NP	NP	No/No	No	54/-
F15-II.4	Female	33	NP/N	Unilateral	MTC	NP	NP	No/No	No	33/-
F17-II.1	Male	57	NP/↑	NP	MTC	N	N	No/No	No	57/-
F20-II.3	Female	30	NP/N	NP	MTC	N	N	No/No	No	30/-
F23-II.1	Female	49	NP	NP	MTC	N	N	No/No	No	49/-
F25-II.3	Male	58	NP	NP	MTC	NP	NP	No/No	No	58/-
F28-II.4	Female	33	NP	Unilateral	MTC	NP	NP	No/No	No	33/-
F29-II.1	Male	57	NP	Unilateral	MTC	NP	NP	Yes/No	No	57/-

*Years after the diagnosis of MTC. MEN2A: Multiple endocrine neoplasia type 2A, MEN2B: Multiple endocrine neoplasia type 2B, *RET*: Rearranged during transfection, MTC: Medullary thyroid carcinoma, hsMTC:Hereditary sporadic medullary thyroid carcinoma, CCH: C-cell hyperplasia, MEN2: Multiple endocrine neoplasia type 2, PTC: Papillary thyroid carcinoma, PHEO: pheochromocytoma, HPT: Hyperparathyroidism, bCT: Basal serum calcitonin, ↑: increased bCT, NA: Not available, NP: Not performed, c; b; h: clinical biological, histological symptoms; PHEO or HPT

Table 2: Clinical characteristics and genetic findings of the studied families with hereditary MTC

Phenotype distribution	Family *n*	*RET**	MTC	PHEO	HPT	Age range (years) (*n*)		*RET* mutation
						Individuals with clinical disease	Individuals without clinical disease	
MEN2A	F01	9/17	5	3	4	16-44 (5)	5-59 (4)	p.C634R
50% (7/14)	F06	2/9	2	1	-	31-41 (2)	-	p.C634Y
	F16	1/1	1	-	1	38 (1)	-	p.C634R
	F21	2/6	2	2	-	32-34 (2)	-	p.C634R
	F22	5/16	3	2	1	27-44 (3)	6-8 (2)	p.C634Y
	F27	1/1	1	1	-	40 (1)	-	p.C634R
	F31	1/1	1	1	-	34 (1)	-	p.C634F
MEN2B	F18	1/9	1			31 (1)	-	p.M918T
21.4% (3/14)	F24	1/2	1	-	-	19 (1)	-	p.M918T
	F26	1/2	1	-	-	16 (1)	-	p.M918T
hsMTC	F09	4/41	1	-	-	56 (1)	56-78 (3)	p.S891A
28.6% (4/14)	F12	3/5	1	-	-	38 (1)	40-46 (2)	p.V804L
	F19	1/13	1	-	-	55 (1)	-	p.V804M
	F30	4/6	1	-	-	32 (1)	6-24 (3)	p.S891A

*RET: Detected subjects with *RET* mutation/total of tested relatives. *n*: Number of individuals. MTC: Medullary thyroid cancer, PHEO: Pheochromocytoma, HPT: Hyperparathyroidism, MEN2A: Multiple endocrine neoplasia type 2A, MEN2B: Multiple endocrine neoplasia type 2B, hsMTC: hereditary sporadic medullary thyroid carcinoma

polymerase chain reaction (PCR) conditions are available on request.

Polymerase chain reaction was performed in 50 µL mixtures containing 50–100 ng of template DNA, 0.4 mM of each primer, dNTP 0.2 mM, 1X PCR buffer, 0.75–2 mM Mg^{2+}, and 0.3 U of Taq polymerase (Promega, Wisconsin, USA). The PCR reaction was run using the following conditions: Hot start at 95°C for 3 min, followed by 30–35 cycles of denaturation at 94°C for 30 s, annealing at 63–65°C for 45 s, extension at 72°C for 45 s, and a final extension cycle at 72°C for 7 min. PCR products were purified using the "PCR Clean up System Kit Purification" kit (Promega, Wisconsin, USA). DNA sequencing reactions were performed using the Big Dye Terminator V3.1 Cycle Sequencing Kit® and sequenced on a ABI PRISM 3100 Automated Sequencer (Applied Biosystems, Foster City, CA, USA). All *RET* exons were sequenced using both forward and reverse primers.

All results were confirmed using a second and independent blood sample.

RESULTS

Clinical features of patients

In this study, we screened for *RET* mutations in 31 Moroccan unrelated index cases with MTC and 115 of their relatives. Eight of them were with at least MTC; seven were diagnosed after genetic screening, and 10 were asymptomatic.

Among the 31 index cases 19 were females (61.3%), and 12 were males (38.7%). The age of diagnosis ranged from 16 to 58 years (average 39.8 years). Nine patients (9/31; 29%) underwent incomplete surgery in another institution and were then referred to our institution for postsurgical management. Thyroidectomy was performed at a mean age of 40.5 years (range 16–58). At the time of diagnosis local metastases were present in eight of the 31 index cases (25.8%) and distant metastases in three of them (3/31; 9.7%).

Based on the clinical records and information obtained during genetic counseling, and after genetic screening seven (7/31; 22.6%) families were characterized with MEN2A, three (3/31; 9.7%) with MEN2B and 21 patients (21/31; 67.7%) with apparently sMTC. Four of them (4/31, 12.9%) were reclassified as hsMTC since they carried *RET* germline mutations. The remaining 17 (17/31, 54.83%) index cases were considered so far, as sMTC cases. More than one individual was diagnosed with MTC in four families; all of them had MEN2A.

Rearranged during transfection mutational analysis

Mutational analysis of the first set of exons (exons 8, 10–11, 13–16) showed that among the 31 studied families, 14 (45.2%) carried *RET* germline mutations [Figure 1]. No mutation was detected in the remaining 17 patients clinically diagnosed with apparently sMTC. Overall, a total of seven different heterozygote missense point-mutations was identified in *RET* exons 11, 14, 15 and 16 [Table 1 and Figure 2]. Three mutations were identified at codon 634 of exon 11 (c.1900T > C, p.C634R; c.1901G > A, p.C634Y and c.1901G > T, p.C634F), two mutations at codon 804 of exon 14 (c.2410G > T, p.V804L and c. 2410G > T, p.V804M), one mutation at codon 891 within exon 15 (c.2671T > G, p.S891A), and one mutation in codon 918 within exon 16 (c.2753T > C, p.M918T) [Table 1 and Figure 2]. The amino acid at position 634 is located in the cysteine-rich region of the RET extracellular domain, 804 in TK1, 891 and 918 in TK2 tyrosine kinase subdomains of the intracellular segment of RET, respectively. No other mutations were found in the tested seven exons, except single nucleotide polymorphisms (SNPs): P.A510A (c.1529C > T), p.P622P (rs201979255), p.G691S (rs1799939), p.L769L (rs1800861), p.S836S (rs1800862) and p.S904S (rs1800863) and one intronic SNP c. 2608-24G > A (rs2472737); all but one (c.1529C > T, p.A510A) were previously reported.

The prevalence of mutations was: 22.6% (7/31) at codon p.C634 identified in seven families with MEN2A, 9.7% (3/31) at codon 918 (p.M918T) found in three families with MEN2B and 12.9% (4/31) at codon 804 or codon 891 (p.S891A) in four other families with hsMTC.

Mutations at codon 634 in multiple endocrine neoplasia type 2A

Three distinct mutations at codon 634 were found in seven probands with MEN2A (7/14, 50%) (F01-II.13, F06-II.8, F16-II.7, F21-II.3, F22-II.9, F27-II.1 and F31-II.3) and in 14 of their 44 relatives (31.8%). Four probands carried p.C634R (4/7; 57%), two had p.C634Y (2/7, 28.6%) and one p.C634F (1/7, 14.3%).

Genetic screening showed that among the 44 relatives of the 7 families, 14 (14/44, 31.8%) had the same C634 mutation as the corresponding index cases. Six of them (6/14, 43%) were children aged 5–16 years and eight (8/14, 57%) were adults aged 23–59 years. Eight (one child and seven adults) of them (8/14, 57%) were aged 16–44 years and presented with MEN2 symptoms. Unfortunately, the DNA of all the parents' probands was not available (deceased or refused genetic testing) except that of the mother F21-I.2 who was negative for the p.C634R mutation.

Among all the C634 mutation carriers with MTC (seven probands and eight relatives), PHEO was present in 66.7% (10/15), HPT in 40% (6/15), distant and/or local metastases in 10/15 (66.7%) and cutaneous lichen amyloidosis (CLA) in 26.7% (4/15) [Table 2].

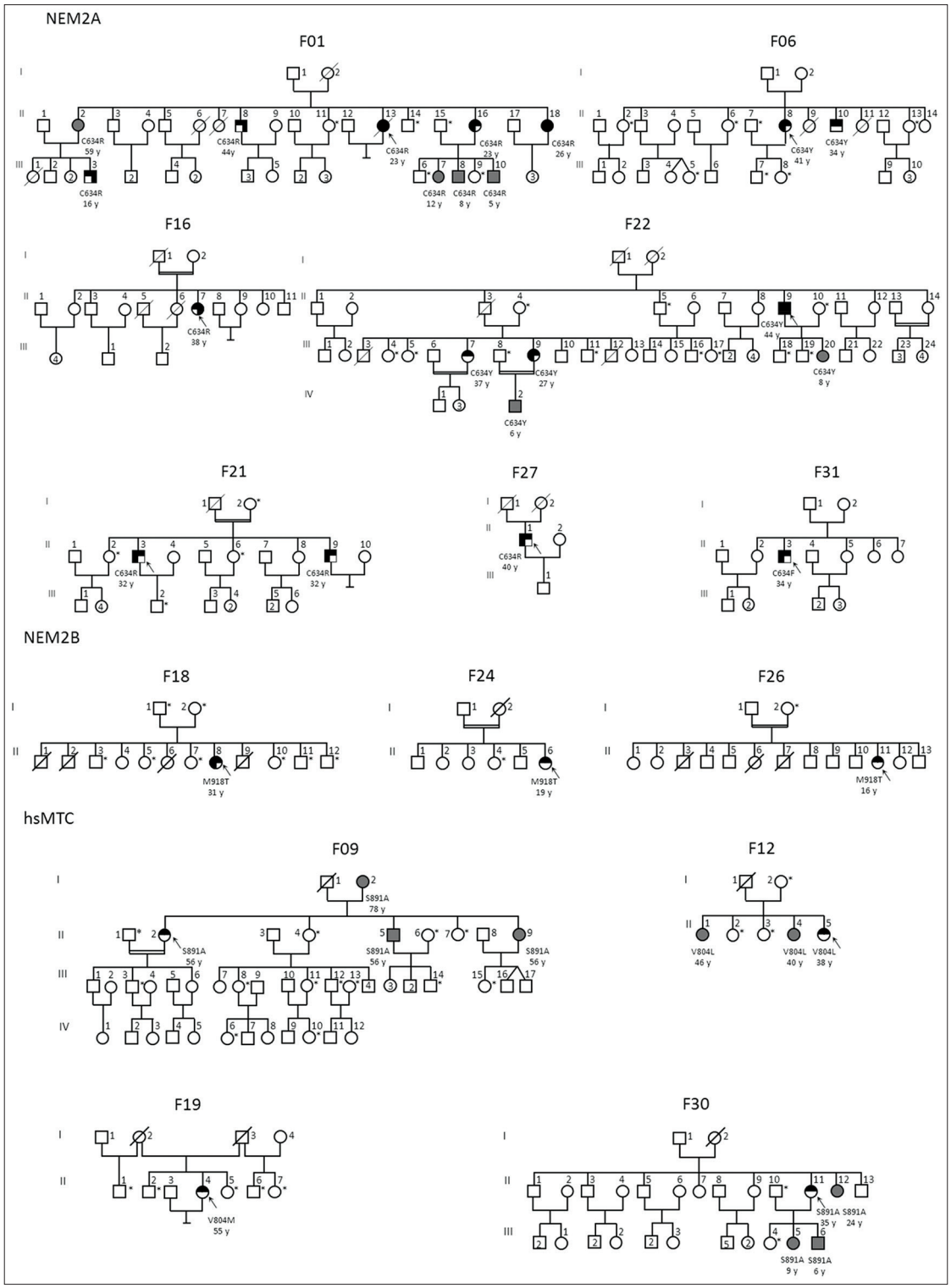

Figure 1: Pedigrees of the studied MTC families with *RET* mutations. (a) MEN2A families, (b) Hereditary sporadic MTC, (c) MEN2A families. F-n: Family number, arrows indicate the index cases and asterisks relatives negative for a *RET* mutation. All the mutations are detected in the heterozygous state and are indicated below the symbols for the available subjects. The ages at the first examination or diagnosis or of patients, are also indicated below the symbols: ◓ Patients with MTC. ◧ ◐ : Patients with PHEO. ◧ ◔ : Patients with HPT. ■ ●: Asymptomatic patients with *RET* mutation. MTC: Medullary thyroid carcinoma, RET: Rearranged during transfection, MEN2A: Multiple endocrine neoplasia type 2A, PHEO: Pheochromocytoma, HPT: Hyperparathyroidism

Of the six children, five (three from F01 and two from F22), did not present any symptoms of the disease but the bCT level was slightly increased (14–33 ng/L, NV < 10). They all underwent prophylactic thyroidectomy without lymph node resection. Histological analyses were in favor of C-cell hyperplasia in three patients (F01-III.7; F01-III.8

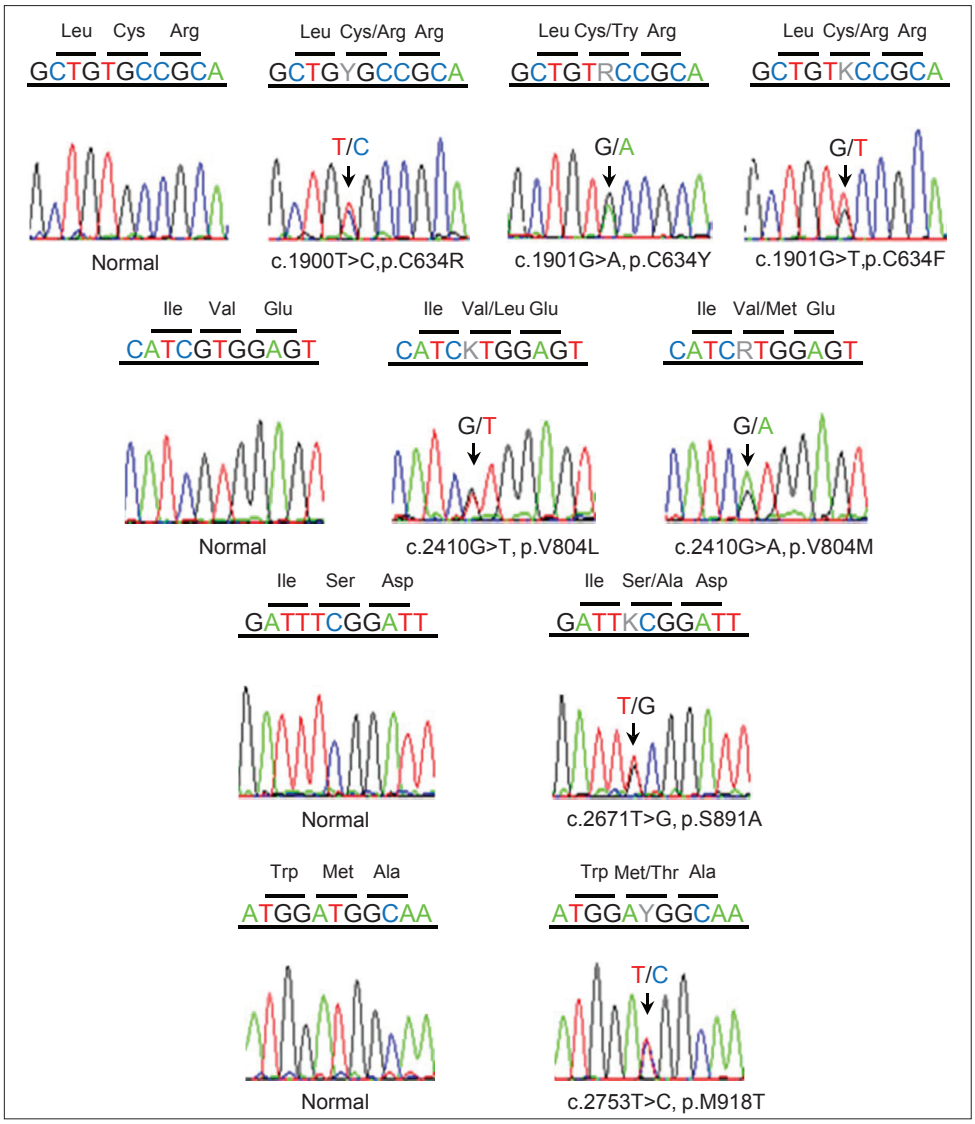

Figure 2: Detection of rearranged during transfection gene (RET) mutations in Moroccan patients with medullary thyroid carcinoma (MTC). Partial DNA sequencing chromatograms of *RET* gene. A. p.C634R/Y/F (exon 11), B. p.M918T (exon 16) and C.p.V804L/M (exon 14) and D.p.S891A (exon15) mutations detected in multiple endocrine neoplasia type 2A (MEN2A), MEN2B and apparently sporadic MTC, respectively. Control sequences are shown for purposes of comparison. The nucleotides and amino acid changes are indicated in and under each chromatogram, respectively

and F01-III.10), confirmed by immuno-histological staining in one of them but not performed for the two others. Histological data were not available for the remaining children.

Intriguingly one 59-year-old woman with a p.C364R mutation belonging to a MEN2A family (F01-II.2), did not have any clinical symptoms of the disease while her 16-year-old son (F01-III.3) presented with MTC and HPT.

Codon 918 mutation in multiple endocrine neoplasia type 2B

One mutation at codon 918 (p.M918T) was identified in three index cases (3/14, 21.4%) aged 16, 19 and 31 years (F26-II.11, F24-II.6 and F18-II.8, respectively). All presented a typical phenotype of MEN2B (e.g., mucosal neuromas, marfanoid habitus). No evidence of HPT was found in all of them and only patient F18-II.8 presented with PHEO at the time of diagnosis. Local metastases were present in the three probands (3/3, 100%) and distant metastases were present in two of them ([2/3, 66.6%], [F18-II.8, F24-II.6] [Table 1]).

Genetic testing showed that all the 11 tested relatives did not carry the mutation and were so far disease-free. In particular, in the family F18 none of the parents harbored the mutation suggesting that in this case the p.M918T mutation arises *de novo*. In the remaining families F24 and F26, only the DNA of the sister F24-II.4 and the mother F26-I.2 was analyzed [Figure 1].

Noncysteine mutations in apparently sporadic medullary thyroid carcinoma

Three distinct noncysteine mutations were found at codons 804 (p.V804L and p.V804M) and 891 in four

probands (4/14; 28.6%) aged from 33 to 54 years (F12-II.5, F09-II.2 and F19-II.4, and F30-II.11), with apparently sMTC. Interestingly, in the proband F9-01 histological analysis revealed bilateral MTC and C-cell hyperplasia, associated with papillary carcinoma.

Genetic screening showed that of the 49 tested relatives 8 (6–78 years) (from families F09, F12 and F30) carried the same mutation as their corresponding probands**,** all of them never had any symptoms suggestive of clinical evidence of MTC, and refused the management offered by the clinicians.

Among the parents' probands only the DNA of two mothers was analyzed (F09-I.2 and F12-I.2), the first harbored the same mutation (p.S891A) as her daughter (F09-II.2) and the second did not carry the mutation p.V804L.

All index cases with mutations at codons 804 or 891 did not have any biochemical evidence of PHEO, HPT or any typical characteristics of MEN2B, and were first considered as sporadic cases. Thanks to genetic testing four of them (F09-II.2, F12-II.5, F19-II.4 and F30-II.11) were reclassified as having hsMTC.

Nonrearranged during transfection mutation carriers

Among the 31 index cases, 17 patients with sMTC did not have any *RET* mutation after examination of the 7 exons that harbored hotspots. We further extended the sequence analysis to the entire *RET* coding regions. Only two additional exonic SNPs in exon 2 (GCG > GCA; p.A45A rs1800858) and exon 7 (GCA > GCG; p.A432A, rs1800860) were identified.

This group of patients with normal *RET* alleles did not show any MTC history in their first-degree relatives. The age of onset of MTC varied from 21 to 58 years (mean age 43.2 years). Of the 17 patients 10 (58.8%) had unilateral MTC, one bilateral MTC and in the 6 other patients the laterality of MTC was unspecified. These patients were considered so far, as sMTC cases.

DISCUSSION

To our knowledge and belief, this is the first comprehensive report of molecular genetic screening of the proto-oncogene *RET* in 31 independent Moroccan families with MTC. The studied patients do not overlap with any of those previously reported in neither Moroccan nor international study groups.

In Morocco, the frequency of MTC was estimated to be 2.8% (2.5% in women and 2.5% in men) according to the "Lalla Salma Foundation for Cancer Prevention and Treatment" (http://www.contrelecancer.ma/en/documents/registre-des-cancers-de-la-region-du-grand-casab-2/). So far, only two groups including ours, reported preliminary evaluation of clinical features and molecular analysis of hereditary forms of MEN2. Both analyzed only the hotspot regions of *RET*.[19-21] This is the 1st time that the entire coding exons of *RET* are explored.

Rearranged during transfection gene mutations in patients with MEN2A have been reported with variable frequency. In agreement with the majority of reports on inheritance of *RET* mutations in MEN2 family members, after genetic screening of the whole coding regions of *RET*, germline mutations were identified in about 45% (14/31) of the studied families.[23-28] In North African and Middle Eastern patients, very few groups have reported *RET* mutation frequency: 8–10%, 20–25% and 47.6%, in Algerian and Turkish,[29,30] Saudi Arabic[31] and Iranian,[23,25] and Qatari[32] reports, respectively.

Rearranged during transfection mutations with a ATA risk level D (p.M918T), C (at codon 634) or A (at codons 804 and 891) were identified in MEN2B, MEN2A and hsCMT, respectively. All have been previously described in different populations as disease-causing mutations.[7,10,22,33-35] In our cases, no mutation was identified in either exons 8, 10 and 13 or in the other exons explored.

In accordance with previous reports including Algerian,[30] Qatari,[32] Saudi Arabic,[31] Indian[23,25] and Turkish studies,[29] the most frequent mutation affected codon 634 occurring in 50% (7/14) of the mutated families studied herein,[9-11,19,23-25,36-41] followed by mutations at codon 918 (p.M918T; 4/14, 28.6%) and then by mutations at codons 804 (p.V804L/M; 2/14, 14%) and 891 (p.S891A; 2/14, 14%). However, recent studies revealed that p.V804M was the most frequent mutation in Indian and Italian FMTC,[42,43] while mutations in codon 618 were the most prevalent in Saudi Arabia MEN2A and FMTC.[31]

The differences in the mutation besides distribution frequencies might be due to the patients' ethnic origin and geographical locations, as well as the sample composition and size and explored *RET* exons.

Herein, codon 634 mutations were found in all MEN2A families; p.C634R was the most common (4/7, 57%), followed by p.C634Y (2/7, 28.6%) and p.C634F substitutions (1/7, 14.3%). These results are similar to those reported in many populations with MEN2A.[13,23,44-46] However, in Algerian, Spanish and Chinese patients p.C634Y was the most prevalent mutation in this codon.[30,39,41] In Iranian patients, Alvandi *et al*.[23] reported prevalence of p.C634R while Hedayati *et al*.[25] reported prevalence of p.C634G suggesting a difference in genetic background of those

studied populations. This mutation was also the most frequent in Qatari patients with MEN2A.[32] Codon 634 mutations have been shown to be strongly associated with the presence of PHEO, HPT, early metastases[11,24,27,46-48] and rarely with CLA.[49] In the codon 634 mutation carriers including asymptomatic children: MTC, PHEO, HPT, CLA and distant and/or local metastases were diagnosed in 71.4% (15/21); 47.6% (10/21); 28.6% (6/21); 19% (4/21) and 47.6% (10/21), respectively. This is in accordance with the previously published data.[11,24,27,46-49]

The p.C634R mutation (level risk D) is known to be associated with a severe form of MTC with intermediate risk for early development and progression of MTC (risk level C). Indeed, patients who harbor the p.C634R mutation may have evidence of MTC or C-cell hyperplasia even before the age of 5 years.[3,50] Intriguingly, a 59-year-old woman with p.C634R belonging to the MEN2A family F01 (F01-II.2), was identified with no clinical thyroid nodules or any clinical symptoms of the disease. Moreover, her 16-year-old son (F01-II.3) carrying the same mutation was detected with MEN2A. Thus, this woman could be a case with reduced penetrance or delayed onset of the disease, which might be explained by the different interactions of the mutation with different genetic background of *RET* mutation carriers as suggested by analyses of a transgenic mouse model of MTC.[51] To our knowledge and belief, this is the 1st time that a patient with a p.C634R mutation (or mutation at codon 634) is asymptomatic at an advanced age. However, we cannot exclude that this woman had histological and/or biochemical symptoms as she refused to be tested.

Interestingly, the p.S891A mutation was identified in a 56-year-old woman patient in whom MTC was associated with papillary thyroid carcinoma (PTC). Simultaneous MTC and PTC have been previously reported in 9% of MTC patients, particularly with late onset *RET* mutations such as p.S891A.[52,53] However, this has been considered as a fortuitous association.

In MEN2B, more than 95% of patients have mutations in codon 918 (exon 16) of *RET*, and more than 50% are *de novo* germline mutations.[3,54,55] In the agreement with these data, the p.M918T mutation was found in all of the three probands characterized as MEN2B (100%). Both parents of the three families were healthy and in only one family (F18) the p.M918T was shown to be a de novo mutation. In the remaining families, both parents' probands were, unfortunately, not available or deceased.

Our study confirmed that genetic screening is very useful in MTC families. Indeed, In addition to the seven asymptomatic (7/22, 31.8%) children aged 5–12 years, one symptomatic 16-year-old boy and 14 adults (14/22, 63.6%) aged 16–78 years, were identified with a risk level of A (p.V804L, p.S891A) or C germline mutations (codon 634). According to the ATA recommendations, these patients were encouraged to undergo total thyroidectomy and/or at least to have regular clinical follow-ups. It is noteworthy that 87.5% (7/8) of the symptomatic patients were unaware of their condition before the familial screening. So far, 84.7% (12/14) patients with a level C mutation have been thyroidectomized yet; 35.7% (5/14) were asymptomatic children and 42.9% (6/14) adults and one child of 16 years (1/14, 7.1%) who already presented with MEN2A symptoms at the first specialized consultation.

The remaining patients (36.4%, 8/22) were aged 6–78 years and were had risk level A mutations (codons 804 and 891), and all were asymptomatic. This is in line with a variable clinical impact and low penetrance of the disease of this group of mutations. However, as late onset of MTC and PHEO has been associated with mutations at codons 804[56] and 891,[57] one must keep in mind, that these carriers may turn out to MEN2A cases. Nevertheless, all these patients did not accept any of the clinicians' suggestions. This is probably due to the lower indolence of the disease in these families comparatively to those with risk level C or D mutations. It is worth noting that currently, all but two patients passed the recommended age of thyroidectomy.

The majority of the probands likely inherited the mutations from their deceased father or mother.

Despite accurate analysis covering the entire *RET* coding sequences, 54.8% (17/31) of index cases were negative for causative-disease germline *RET* mutations. In accordance with previous studies, all of these cases were apparently sporadic cases with unilateral MTC in 94% (16/17) and late age of diagnosis (age ranged from 40 to 58 years [mean age 48.5 years]) in 70.5% (12/17) of cases. As it was previously reported in 75% of sMTC cases,[58-60] these patients could harbor somatic *RET* mutations.[58,60] However, mutations in nonexplored regions of *RET* or other candidate genes, or loci not yet identified cannot be excluded. In this study, the distribution of the MEN2 phenotype, the frequency of hereditary forms, the *RET* mutation spectrum and frequencies were similar to those reported by some groups[9-11,19,23-25,36-41] and different from others.[31,42,43,61] The discrepancies between the different reports, may be due to differences in the patients 'ethnic origin and geographical location as well as in the composition (MEN2 types, hereditary/sporadic etc.) and the size of samples, and differential screening of *RET* exons.

Interestingly, our results showed that codon 634 mutations were exclusively associated with the MEN2A and p.M918T

with the MEN2B phenotype while codons 804 and 891 mutations to hereditary apparently sMTC. However, due to reduced size of our sample, a change in the spectrum of *RET* mutations in MEN2 patients could be expected as it was previously shown in other studies which revealed an increased frequency of "so-called" rare mutations.[43,62,63] An extended mutational analysis is obviously required to provide a precise spectrum of *RET* mutations and to establish a genotype-phenotype analysis in Moroccan MEN2 patients. Finally, this is the first comprehensive report of molecular genetic screening of *RET* giving a preliminary spectrum of mutations in Moroccan MTC patients and the importance of familial screening in MTC at-risk kindred, in particular in our country where the pentagastrin stimulated CT test is not available. In Morocco, despite efforts made by our group toward molecular diagnosis of MTC patients, this condition is still under diagnosed or diagnosed too late as in many parts of the world. This is reflected by a mean age of diagnosis of 39.8 years and relatively increased percentages of PHEO, HPT and metastases from MTC of 22.6% (7/31), 9.8% (3/31), respectively, and 39% (9/31), in the studied probands, respectively. In the absence of molecular diagnostic centers, our work will encourage the development of a national policy to fight against this cancer, because the treatment and follow-up cost are still very high, with the establishment of a national consensus adapted to our country.

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REFERENCES

1. Marsh DJ, Learoyd DL, Robinson BG. Medullary thyroid carcinoma: Recent advances and management update. Thyroid 1995;5:407-24.
2. Romei C, Pardi E, Cetani F, Elisei R. Genetic and clinical features of multiple endocrine neoplasia types 1 and 2. J Oncol 2012;2012:705036.
3. American Thyroid Association Guidelines Task Force, Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, *et al*. Medullary thyroid cancer: Management guidelines of the American Thyroid Association. Thyroid 2009;19:565-612.
4. 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 1988;3:571-8.
5. Wagner SM, Zhu S, Nicolescu AC, Mulligan LM. Molecular mechanisms of RET receptor-mediated oncogenesis in multiple endocrine neoplasia 2. Clinics (Sao Paulo) 2012;67 Suppl 1:77-84.
6. Da Silva AM, Maciel RM, Da Silva MR, Toledo SR, De Carvalho MB, Cerutti JM. A novel germ-line point mutation in RET exon 8 (Gly(533)Cys) in a large kindred with familial medullary thyroid carcinoma. J Clin Endocrinol Metab 2003;88:5438-43.
7. Krampitz GW, Norton JA. RET gene mutations (genotype and phenotype) of multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma. Cancer 2014;120:1920-31.
8. Raue F, Frank-Raue K. Genotype-phenotype correlation in multiple endocrine neoplasia type 2. Clinics (Sao Paulo) 2012;67 Suppl 1:69-75.
9. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, *et al*. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 1993;363:458-60.
10. Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, *et al*. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet 1993;2:851-6.
11. Machens A, Gimm O, Hinze R, Höppner W, Boehm BO, Dralle H. Genotype-phenotype correlations in hereditary medullary thyroid carcinoma: Oncological features and biochemical properties. J Clin Endocrinol Metab 2001;86:1104-9.
12. Brauckhoff M, Lorenz K, Ukkat J, Brauckhoff K, Gimm O, Dralle H. Medullary thyroid carcinoma. Scand J Surg 2004;93:249-60.
13. Eng C, Smith DP, Mulligan LM, Nagai MA, Healey CS, Ponder MA, *et al*. Point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours. Hum Mol Genet 1994;3:237-41.
14. Romei C, Cosci B, Renzini G, Bottici V, Molinaro E, Agate L, *et al.* RET genetic screening of sporadic medullary thyroid cancer (MTC) allows the preclinical diagnosis of unsuspected gene carriers and the identification of a relevant percentage of hidden familial MTC (FMTC). Clin Endocrinol (Oxf) 2011;74:241-7.
15. 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;92:4725-9.
16. Niccoli-Sire P, Conte-Devolx B, Groupe d'étude des Tumeurs Endocrines. RET mutations and preventive treatment of medullary thyroid cancer. Ann Endocrinol (Paris) 2005;66:168-75.
17. Jimenez C, Gagel RF. Genetic testing in endocrinology: Lessons learned from experience with multiple endocrine neoplasia type 2 (MEN2). Growth Horm IGF Res 2004;14 Suppl A: S150-7.
18. Chen H, Sippel RS, O'Dorisio MS, Vinik AI, Lloyd RV, Pacak K, *et al.* The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: Pheochromocytoma, paraganglioma, and medullary thyroid cancer. Pancreas 2010;39:775-83.
19. Ainahi A, Kebbou M, Timinouni M, Benabdeljalil N, Oufara S. Treatment evaluation, follow-up and familial screening of medullary thyroid carcinoma by highly specific calcitonin measurements. Indian J Cancer 2006;43:75-9.
20. Benazzouz B, Chraïbi A, Doghmi Y, El Bacha S, Boutayeb S, Kadiri A, *et al.* Characterization of RET proto-oncogene C634Y mutation in a Moroccan family with multiple endocrine neoplasia type 2A. Ann Endocrinol (Paris) 2006;67:21-6.
21. Benazzouz B, Hafidi A, Benkhira S, Chraibi A, Kadiri A, Hilal L. C634R mutation of the protooncongene RET and molecular diagnosis in multiple endocrine neoplasia type 2 in a large Moroccan family. Bull Cancer 2008;95:457-63.
22. Hofstra RM, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, *et al.* A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 1994;367:375-6.
23. Alvandi E, Akrami SM, Chiani M, Hedayati M, Nayer BN, Tehrani MR, *et al.* Molecular analysis of the RET proto-oncogene key exons in patients with medullary thyroid carcinoma: A comprehensive study of the Iranian population. Thyroid 2011;21:373-82.

24. Bergant D, Hocevar M, Besic N, Glavac D, Korosec B, Caserman S. Hereditary medullary thyroid cancer in Slovenia – Genotype-phenotype correlations. Wien Klin Wochenschr 2006;118:411-6.
25. Hedayati M, Zarif Yeganeh M, Sheikhol Eslami S, Rezghi Barez S, Hoghooghi Rad L, Azizi F. Predominant RET germline mutations in exons 10, 11, and 16 in iranian patients with hereditary medullary thyroid carcinoma. J Thyroid Res 2011;2011:264248.
26. Klein I, Esik O, Homolya V, Szeri F, Váradi A. Molecular genetic diagnostic program of multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma syndromes in Hungary. J Endocrinol 2001;170:661-6.
27. Egawa S, Futami H, Takasaki K, Iihara M, Okamoto T, Kanbe M, *et al.* Genotype-phenotype correlation of patients with multiple endocrine neoplasia type 2 in Japan. Jpn J Clin Oncol 1998;28:590-6.
28. Sharma BP, Saranath D. RET gene mutations and polymorphisms in medullary thyroid carcinomas in Indian patients. J Biosci 2011;36:603-11.
29. Erdogan MF, Gürsoy A, Ozgen G, Cakir M, Bayram F, Ersoy R, *et al.* Ret proto-oncogene mutations in apparently sporadic Turkish medullary thyroid carcinoma patients: Turkmen study. J Endocrinol Invest 2005;28:806-9.
30. Chikouche A, Meskine D, Boudissa D, Semrouni M, Chentli F, Ahmed Ali L, *et al.* Screening for RET mutation in patients with MTC in ALger. 31th Congres of the French endocrinology Society, Lyon 2014. Ann Endocrinol 2014;75:366.
31. Qari F. RET codon 618 mutations in Saudi families with multiple endocrine neoplasia Type 2A and familial medullary thyroid carcinoma. Ann Saudi Med 2013;33:155-8.
32. Zirie M, Mohammed I, El-Emadi M, Haider A. Multiple endocrine neoplasia type iia: Report of a family with a study of three generations in Qatar. Endocr Pract 2001;7:19-27.
33. Hansford JR, Mulligan LM. Multiple endocrine neoplasia type 2 and RET: From neoplasia to neurogenesis. J Med Genet 2000;37:817-27.
34. Cosci B, Vivaldi A, Romei C, Gemignani F, Landi S, Ciampi R, *et al.* In silico and *in vitro* analysis of rare germline allelic variants of RET oncogene associated with medullary thyroid cancer. Endocr Relat Cancer 2011;18:603-12.
35. Mulligan LM, Eng C, Attié T, Lyonnet S, Marsh DJ, Hyland VJ, *et al.* Diverse phenotypes associated with exon 10 mutations of the RET proto-oncogene. Hum Mol Genet 1994;3:2163-7.
36. Frank-Raue K, Höppner W, Frilling A, Kotzerke J, Dralle H, Haase R, *et al.* Mutations of the ret protooncogene in German multiple endocrine neoplasia families: Relation between genotype and phenotype. German Medullary Thyroid Carcinoma Study Group. J Clin Endocrinol Metab 1996;81:1780-3.
37. Puñales 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;88:2644-9.
38. Qi XP, Chen XL, Ma JM, Du ZF, Fei J, Yang CP, *et al.* RET proto-oncogene genetic screening of families with multiple endocrine neoplasia type 2 optimizes diagnostic and clinical management in China. Thyroid 2012;22:1257-65.
39. Sánchez B, Robledo M, Biarnes J, Sáez ME, Volpini V, Benítez J, *et al.* High prevalence of the C634Y mutation in the RET proto-oncogene in MEN 2A families in Spain. J Med Genet 1999;36:68-70.
40. Mulligan LM, Eng C, Healey CS, Clayton D, Kwok JB, Gardner E, *et al.* Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. Nat Genet 1994;6:70-4.
41. Zhou Y, Zhao Y, Cui B, Gu L, Zhu S, Li J, *et al.* RET proto-oncogene mutations are restricted to codons 634 and 918 in mainland Chinese families with MEN2A and MEN2B. Clin Endocrinol (Oxf) 2007;67:570-6.
42. Pinna G, Orgiana G, Riola A, Ghiani M, Lai ML, Carcassi C, *et al.* RET proto-oncogene in Sardinia: V804M is the most frequent mutation and may be associated with FMTC/MEN-2A phenotype. Thyroid 2007;17:101-4.
43. Romei C, Mariotti S, Fugazzola L, Taccaliti A, Pacini F, Opocher G, *et al.* Multiple endocrine neoplasia type 2 syndromes (MEN 2): Results from the ItaMEN network analysis on the prevalence of different genotypes and phenotypes. Eur J Endocrinol 2010;163:301-8.
44. Machens A, Dralle H. Familial prevalence and age of RET germline mutations: Implications for screening. Clin Endocrinol (Oxf) 2008;69:81-7.
45. Schuffenecker I, Virally-Monod M, Brohet R, Goldgar D, Conte-Devolx B, Leclerc L, *et al.* 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'etude des tumeurs à calcitonine. J Clin Endocrinol Metab 1998;83:487-91.
46. 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;276:1575-9.
47. Raue F, Frank-Raue K. Genotype-phenotype relationship in multiple endocrine neoplasia type 2. Implications for clinical management. Hormones (Athens) 2009;8:23-8.
48. Marsh DJ, Mulligan LM, Eng C. RET proto-oncogene mutations in multiple endocrine neoplasia type 2 and medullary thyroid carcinoma. Horm Res 1997;47:168-78.
49. 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 (Oxf) 2003;59:156-61.
50. Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, *et al.* Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab 2001;86:5658-71.
51. Cranston AN, Ponder BA. Modulation of medullary thyroid carcinoma penetrance suggests the presence of modifier genes in a RET transgenic mouse model. Cancer Res 2003;63:4777-80.
52. Machens A, Dralle H. Simultaneous medullary and papillary thyroid carcinomas in carriers of the V804M RET germline mutation-a spurious association? Surgery 2010;147:895-6.
53. Biscolla RP, Ugolini C, Sculli M, Bottici V, Castagna MG, Romei C, *et al.* Medullary and papillary tumors are frequently associated in the same thyroid gland without evidence of reciprocal influence in their biologic behavior. Thyroid 2004;14:946-52.
54. Carlson KM, Dou S, Chi D, Scavarda N, Toshima K, Jackson CE, *et al.* Single missense mutation in the tyrosine kinase catalytic domain of the RET protooncogene is associated with multiple endocrine neoplasia type 2B. Proc Natl Acad Sci U S A 1994;91:1579-83.
55. Schuffenecker I, Ginet N, Goldgar D, Eng C, Chambe B, Boneu A, *et al.* Prevalence and parental origin of de novo RET mutations in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma. The calcitonin tumors study group. Am J Hum Genet 1997;60:233-7.
56. Mukherjee S, Zakalik D. RET codon 804 mutations in multiple endocrine neoplasia 2: Genotype-phenotype correlations and implications in clinical management. Clin Genet 2011;79:1-16.
57. Jimenez C, Habra MA, Huang SC, El-Naggar A, Shapiro SE, Evans DB, *et al.* Pheochromocytoma and medullary thyroid carcinoma: A new genotype-phenotype correlation of the RET protooncogene 891 germline mutation. J Clin Endocrinol Metab 2004;89:4142-5.
58. Elisei R, Cosci B, Romei C, Bottici V, Renzini G, Molinaro E, *et al.* Prognostic significance of somatic RET oncogene mutations in

sporadic medullary thyroid cancer: A 10-year follow-up study. J Clin Endocrinol Metab 2008;93:682-7.
59. Romei C, Elisei R, Pinchera A, Ceccherini I, Molinaro E, Mancusi F, *et al.* Somatic mutations of the ret protooncogene in sporadic medullary thyroid carcinoma are not restricted to exon 16 and are associated with tumor recurrence. J Clin Endocrinol Metab 1996;81:1619-22.
60. Eng C, Thomas GA, Neuberg DS, Mulligan LM, Healey CS, Houghton C, *et al.* Mutation of the RET proto-oncogene is correlated with RET immunostaining in subpopulations of cells in sporadic medullary thyroid carcinoma. J Clin Endocrinol Metab 1998;83:4310-3.
61. Mulligan LM, Marsh DJ, Robinson BG, Schuffenecker I, Zedenius J, Lips CJ, *et al*. Genotype-phenotype correlation in multiple endocrine neoplasia type 2: Report of the International RET Mutation Consortium. J Intern Med 1995;238:343-6.
62. Frank-Raue K, Rondot S, Schulze E, Raue F. Change in the spectrum of RET mutations diagnosed between 1994 and 2006. Clin Lab 2007;53:273-82.
63. Niccoli-Sire P, Murat A, Rohmer V, Franc S, Chabrier G, Baldet L, *et al*. Familial medullary thyroid carcinoma with noncysteine ret mutations: Phenotype-genotype relationship in a large series of patients. J Clin Endocrinol Metab 2001;86:3746-53.

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