Research Article

**RET** Proto-Oncogene Mutational Analysis in 45 Iranian Patients Affected with Medullary Thyroid Carcinoma: Report of a New Variant

Elia Damavandi †,1,2 Fatemeh Vand-Rajabpour,3 Maliheh Javadi-Arjmand †,3 Mohammad-Reza Mohjeri Tehrani,4 Bagher Larijani †,4 Majid Kabuli †,3 and Mohsen Ghadami †,4,5

1Specialized Medical Genetic Center (SMGC) of ACECR, Tehran, Iran
2Department of Photo Healing and Regeneration, Medical Laser Research Center, Yara Institute, ACECR, Tehran, Iran
3Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
4Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
5Cardiac Primary Research Center, Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran

Correspondence should be addressed to Mohsen Ghadami; mghadami@tums.ac.ir

Received 16 May 2021; Revised 10 October 2021; Accepted 17 October 2021; Published 3 November 2021

Academic Editor: Anjali Mishra

Copyright © 2021 Elia Damavandi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Background.** The aim of this study was to identify germline mutation of the RET (rearranged during transfection) gene in patients with medullary thyroid carcinoma (MTC) and their first-degree relatives to find presymptomatic carriers for possible prophylactic thyroidectomy. **Methods/Patients.** We examined all six hot spot exons (exons 10, 11, 13, and 14–16) of the RET gene by PCR and bidirectional Sanger sequencing in 45 Iranian patients with MTC (either sporadic or familial form) from 7 unrelated kindred and 38 apparently sporadic cases. First-degree relatives of RET positive cases were also genotyped for index mutation. Moreover, presymptomatic carriers were referred to the endocrinologist for further clinical management and prophylactic thyroidectomy if needed. **Results.** Overall, the genetic status of all of the participants was determined by RET mutation screening, including 61 affected individuals, 22 presymptomatic carriers, and 29 genetically healthy subjects. In 37.5% (17 of 45) of the MTC referral index patients, 8 distinct RET germline mutations were found, including p.C634R (35.3%), p.M918T (17.6%), p.C634Y (11.8%), p.C634F (5.9%), p.C611Y (5.9%), p.C618R (5.9%), p.C630R (5.9%), p.L790F (5.9%), and one uncertain variant p.V648I (5.9%). Also, we found a novel variant p.H648R in one of our apparently sporadic patients. **Conclusion.** RET mutation detection is a promising/golden screening test and provides an accurate presymptomatic diagnostic test for at-risk carriers (the siblings and offspring of the patients) to consider prophylactic thyroidectomy. Thus, according to the ATA recommendations, the screening of the RET proto-oncogene is indicated for patients with MTC.

1. Introduction

Calcitonin-secreting parafollicular C cells of the thyroid gland are the origin of 5–10% of thyroid cancers, so called medullary thyroid carcinoma (MTC). MTC has a worse prognosis than the most common form of thyroid cancer, papillary thyroid carcinoma (PTC), which accounts for 60–80% of thyroid carcinomas and originates from follicular cells [1]. MTC has two forms of sporadic (isolated) and hereditary that compromise about 75% and 25% of the cases, respectively. Hereditary forms are transmitted as autosomal dominant pattern of inheritance and are seen either as isolated familial MTC (FMTC) with a prevalence of 10% or as a syndromic form of cancer, also known as multiple endocrine neoplasia type 2 (MEN2A and MEN2B, which have a prevalence of 85% and 5%, respectively) [2, 3].
familial medullary thyroid carcinoma, the only lesion present is MTC, but MEN2A is characterized by MTC and pheochromocytoma with or without parathyroid hyperplasia or adenoma [1–3].

PTC and MTC have clear clinical and pathological differences, but RET proto-oncogene contributes somehow to the carcinogenesis of both types. Activation of the RET gene by rearrangement (inversion or translocation) appears to be only seen in patients suffering from PTC [4] or thyroid adenoma [5], and germline missense mutations of the RET gene have been shown to be the cause of the hereditary form of MTC (MEN2) [6]. Interestingly, somatic mutations in RET have been found in 23–70% of sporadic MTCs and 10–15% of sporadic pheochromocytoma patients, as well [7, 8]. Gain of function mutations in exons 10, 11, 13, 14, and 15 of the RET gene have mostly been reported in MEN2A (e.g., codons 609, 611, 618, 620, and 634) but about 95% of the patients with MEN2B have a mutation in exon 16 (codon 918). Therefore, to obtain a reliable screening genetic test for MTC patients, exons 10, 11, 13, 14, 15, and 16 should be considered as the hot spots for RET gene mutations.

Identification of the germline mutation of the RET gene in a patient with MTC provides an accurate presymptomatic diagnostic test for the siblings and offspring of the patients. Here, we report the results of the mutational analysis of the RET proto-oncogene in 45 Iranian patients with MTC (either sporadic or familial form) from 7 unrelated kindreds and 38 apparently sporadic cases.

2. Subjects and Methods

2.1. Patients and Specimen Collection. Seven unrelated families and 38 apparently sporadic cases with medullary thyroid carcinoma referred to Endocrinology and Metabolism Research Institute of Tehran University of Medical Sciences participated in this study. First, 45 index cases whose MTC was diagnosed by an endocrinologist and confirmed histopathologically and underwent thyroidectomy based on the ATA guideline were genetically consulted. Then, each index case was evaluated for mutations in six hot spot exons of the RET gene, and segregation analysis was done for their at-risk relatives if a causative mutation was found [3]. Overall, 112 participants were examined for RET mutations. All procedures were in accordance with the ethical standards of Tehran University of Medical Sciences and the Helsinki Declaration of 1975 revised in 1983. Written informed consent was obtained from all participants or their parents for their participation and also their permission for the publication of the results.

2.2. Molecular Analysis. Genomic DNA was extracted from EDTA peripheral venous blood using the standard salting-out/proteinase K method. Primers were designed to amplify all six exons, 10, 11, 13, 14, 15, and 16, and their exon-intron boundaries using the NCBI Primer-design tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and Gene Runner software (version 6.0.28). Fifty nanograms of the extracted DNA were used as a PCR template. PCR conditions are available upon request. The PCR products were run on a 2% agarose gel, and subsequently, all six exons were bidirectionally sequenced using the ABI3130 automated sequencer and analyzed with the Chromas version 2 (http://chromas.software.informer.com/2.0/). The sequence data were compared with the RET sequence data (Ref Seq NM_020975) from the NCBI BLAST human database (http://www.ncbi.nlm.nih.gov/BLAST/) and Ensembl human database (GRCh37).

2.3. In Silico Analysis. A comprehensive in silico analysis was performed to evaluate the pathogenic potential of the detected variants by checking in Ensembl.org, dbSNP (http://www.ncbi.nlm.nih.gov/snp), 1000 Genomes databases (http://browser.1000genome.org), ClinVar, HGMD® Professional, MEN2 database (http://www.arup.utah.edu/database/MEN2/MEN2_display.php), and recently published articles. Additionally, Polyphen 2, SIFT, Mutation Taster, and InterVar (http://wintervar.wglab.org/) were investigated to predict the possible pathogenic effect of novel variants.

3. Results

3.1. Clinical and Molecular Findings. Of 45 referred MTC patients, 7 cases had FMTC or MEN syndrome, with more than one affected members. Thirty-eight patients seemed to be apparently sporadic MTC. All six RET exons (10, 11, 13, 14, 15, and 16 exons) were sequenced in 45 index patients. Eight RET known distinct mutations, one unclassified variant, and one novel variant were detected (Table 1).

The family IR-F4 was an extended kindred with two affected individuals in the second generation (I2 and II2), two affected (III3 and III2), three carriers in the third generation (III5–7), and two carriers in the fourth generation (IV2 and IV9) (Figure 1(a)). The 44-year-old proband III3 and other affected individuals of the pedigree had a known heterozygous mutation (c.257G>T) in exon 13 (p.L790F). According to the ATA risk classification, p.L790F mutation is stratified as ATA-MOD (A level), indicating a moderate risk of aggressive MTC with ∼10% incidence of PHEO. Carriers of this mutation are advised to undergo prophylactic thyroidectomy only if it occurs as FMTC, or they have a high level of calcitonin. Therefore, these carriers were referred to the endocrinologist for more clinical follow-up.

The proband of the family IR-F15 (I2) also had a known mutation (c.1900T>C) in exon 11 (p.C634R). Her mother (I2) died because of MTC and was not available but two other family members did not have any RET mutations in the six tested exons (Figure 1(b)).

The family IR-F18 was another family with three affected daughters (II5, II6, and II7), one affected son (II4), three affected grandchildren (III3, III4, and II8), and one carrier granddaughter (II8) (Figure 1(c)). A known heterozygous mutation in exon 11 (c.1900T>C (p.C634R)) was found in all affected individuals (II5, II6, II7, III3, III4, and III8) and one carrier granddaughter (II8). Interestingly, individual II6 had two genetically normal triplets. According to the
Table 1: RET mutations and number of affected subjects, carriers, and healthy individuals.

| Family ID | Type   | Mutation (polymorphism) | Affected number (percent) | Affected individuals (diagnosed primarily with symptoms) | Presymptomatic carriers (diagnosed primarily with genetic testing) | ATA risk based on 2015 ATA risk guideline (ATA risk based on 2009 ATA risk guideline) |
|-----------|--------|-------------------------|---------------------------|----------------------------------------------------------|---------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| IR-F4     | MEN2A  | L790F (Polymorphism: L769L, S836S) | 4 (11.4%)                 | II-2, II-3: Confirmed histopathologically and underwent thyroidectomy Ctn level (before surgery): high Ctn level (after surgery): decreased to normal level III-3: Ctn level (before surgery): high Pathologic staging: T1 N0 MX Ctn level (after surgery): decreased to normal level III-10: Ctn level (before surgery): high Pathologic staging: T1 NX MX Ctn level (after surgery): decreased to normal level I-2: Died Ctn level (before surgery): high Ctn level (after surgery): decreased to normal level | III-5: Ctn level: high Thyroidectomy done III-6: Ctn level: high Thyroidectomy done | MOD (A) |
| IR-F15    | MTC    | C634R                   | 2 (5.7%)                  | II-2: Ctn level: 320 pg/mL Ctn level (after surgery): decreased to normal level II-2, II-4, II-6, II-7, III-3, III-4 and III-8: Confirmed histopathologically, Thyroidectomy done Ctn level (before surgery): high Ctn level (after surgery): decreased to normal level | III-6: Ctn level: high Thyroidectomy done | H (C) |
| IR-F18    | MEN2A  | C634R (Polymorphism: G691S, S904S) | 8 (22.8%)                 | II-2: Thyroidectomy, parathyroidectomy and adrenalectomy done. Ctn level (before surgery): high Ctn level (after surgery): 1.67 pg/mL | III-4: Ctn level: >1000 pg/mL Prophylactic thyroidectomy done. III-6: Ctn level: 62 pg/mL, Prophylactic thyroidectomy done. IV-1: Ctn level: high, Prophylactic thyroidectomy done. IV-2: Ctn level: Normal Prophylactic thyroidectomy recommended IV-4: Ctn level: high, Prophylactic thyroidectomy done. IV-9: Ctn level: high, Prophylactic thyroidectomy done. | H (C) |
| IR-F25    | MEN2A  | C630R (Polymorphism: G691S, L769L, S904S) | 3 (8.6%)                  | III-1: Thyroidectomy done. Ctn level (before surgery): high Ctn level (after surgery): 11.7 pg/mL | III-2: Thyroidectomy, parathyroid and adrenalectomy done. Ctn level (before surgery): >1000 pg/mL Ctn level (after surgery): NA | MOD (B) |
| Family ID | Type       | Mutation (polymorphism) | Affected number (percent) | Affected individuals (diagnosed primarily with symptoms) | Presymptomatic carriers (diagnosed primarily with genetic testing) | ATA risk based on 2015 ATA risk guideline | ATA risk based on 2009 ATA risk guideline |
|-----------|------------|-------------------------|---------------------------|----------------------------------------------------------|---------------------------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| IR-F31    | MEN2A      | C634R                  | 4 (11.4%)                 | I-1: Thyroidectomy and adrenalectomy done. (Ctn level: NA) | II-2: Thyroidectomy, adrenalectomy done. (Ctn level: NA)       | II-1: Thyroidectomy done. (Ctn level: NA) |                                          |
| IR-F33    | MEN2A      | C618R                  | 2 (5.7%)                  | II-5: Thyroidectomy and adrenalectomy done. Ctn level (before surgery): 200 pg/mL Ctn level (after surgery): 20 pg/mL | II-2: Thyroidectomy and adrenalectomy done. Ctn level (before surgery): >1000 pg/mL Ctn level (after surgery): 300 pg/mL | II-1: Thyroidectomy done. (Ctn level: decreased to normal level) II-2: Thyroidectomy done. (Ctn level: decreased to normal level) | MOD (B)                                       |
| IR-F36    | MEN2A      | C634R                  | 2 (5.7%)                  | II-2: Thyroidectomy, adrenalectomy done. III-1: Died | III-2, III-4: Prophylactic thyroidectomy recommended. |                                           |                                          |

_Apparently Sporadic Cases_

| Family ID | Type       | Mutation (polymorphism) | Affected number (percent) | Affected individuals (diagnosed primarily with symptoms) | Presymptomatic carriers (diagnosed primarily with genetic testing) | ATA risk based on 2015 ATA risk guideline | ATA risk based on 2009 ATA risk guideline |
|-----------|------------|-------------------------|---------------------------|----------------------------------------------------------|---------------------------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| IR-F11    | Metastatic MTC | M918T                | 1 (2.8%)                  | Thyroidectomy done. Relapse with high Ctn level: 2000 pg/mL Metastatic MTC reported in histopathology report and sonography of neck Confirmed histopathologically and underwent thyroidectomy | Ctn level (before surgery): 8600 pg/mL | HST (D)                                       |                                           |
| IR-F14    | MTC        | C611Y (Polymorphism: G691S, L769L) | 1 (2.8%)                 | Ctn level (before surgery): high Ctn level (after surgery): decreased to normal level Thyroidectomy and unilateral adrenalectomy done. | III-2: Prophylactic thyroidectomy recommended. | MOD (B)                                       |                                           |
| IR-F17    | MEN2A      | C634R                  | 1 (2.8%)                  | Ctn level (before surgery): 352 pg/mL Relapse with high Ctn level. Thyroidectomy done years ago. | III-1: Ctn level: 20 pg/mL Thyroidectomy recommended |                                           |                                           |
| IR-F19    | MTC        | V648I (uncertain)      | 1 (2.8%)                  | Relapse with Ctn level of 31 pg/mL. | — | — |                                           |
ATA risk classification, she was advised to undergo prophylactic thyroidectomy before 5 years of age.

The family IR-F25 was an extended kindred consisting of a healthy father (II3) and an affected mother (II4) with one affected son (III1), one affected daughter (III2), two carrier daughters (III4 and III6), one phenotypically healthy son (III3), one genetically healthy daughter (III5), three carrier grandchildren (IV1, IV4, and IV9), one carrier granddaughter (IV2), and three genetically healthy grandchildren (IV3, IV7, and IV8) (Figure 1(d)). The 63-year-old proband (II4) was diagnosed with MEN2A (MTC, pheochromocytoma (PHEO), and hyperparathyroidism) and underwent thyroidectomy, parathyroidectomy, and adrenalectomy. Sequencing of six \( RET \) exons of the proband and her affected children revealed a heterozygous mutation (c.1888T>C) in exon 11 (p.C630R) classified as a pathogenic variant. All carrier relatives of c.1888T>C mutation with elevated calcitonin levels, III1, III4, IV1, IV4, and IV9, underwent prophylactic thyroidectomy at the time of diagnosis (age: 36, 24, 13, 12, and 5 years old, respectively). The 19-year-old carrier granddaughter (IV2), who had a normal calcitonin level, did not undergo prophylactic thyroidectomy. However, according to the ATA risk level, p.C630R \( RET \) mutation is stratified as ATA-MOD (level B) with a moderate risk of aggressive MTC, and the patients are recommended to receive prophylactic thyroidectomy before the age of 5 years old.

The family IR-F31 was another kindred comprising an affected father (I1), a healthy mother (I2), two affected daughters (II2 and II4), one affected son (II5), one genetically healthy son (II7), and two carrier grandsons (III3 and III4) (Figure 1(e)). MEN2A was diagnosed in the 45-year-old grandfather as the proband with clinical manifestations of MTC and pheochromocytoma. He underwent thyroidectomy. His affected children also had MEN2A, including MTC and pheochromocytoma. We identified a heterozygous known mutation in exon 11 (c.1900T>C, p.C634R) in this family which has been classified as a pathogenic variant with a high ATA risk (ATA-H, level C). Prophylactic thyroidectomy is recommended for this mutation before 5 years of age because of the high risk of aggressive MTC. To conduct a follow-up study, two carrier grandsons (III3 and III4) were referred to the endocrinologist and underwent prophylactic thyroidectomy at age 5.

The family IR-F37 was another kindred comprising an affected father (I1), a healthy mother (I2), two affected daughters (II2 and II4), one affected son (II5), one genetically healthy son (II7), and two carrier grandsons (III3 and III4) (Figure 1(e)). MEN2A was diagnosed in the 45-year-old grandfather as the proband with clinical manifestations of MTC and pheochromocytoma. He underwent thyroidectomy. His affected children also had MEN2A, including MTC and pheochromocytoma. We identified a heterozygous known mutation in exon 11 (c.1900T>C, p.C634R) in this family which has been classified as a pathogenic variant with a high ATA risk (ATA-H, level C). Prophylactic thyroidectomy is recommended for this mutation before 5 years of age because of the high risk of aggressive MTC. To conduct a follow-up study, two carrier grandsons (III3 and III4) were referred to the endocrinologist and underwent prophylactic thyroidectomy at age 5.

The family IR-F39 had a known \( RET \) mutation (c.1852T>C) in exon 10 (p.C618R) (Figure 1(f)). The proband (II5) was first referred for \( RET \) genetic testing because of a high calcitonin level (above 1,500), and histopathological results showed MTC. Initially, a heterozygous known mutation (c.1852T>C) was seen in exon 10 (p.C618R) in the proband (II5) at age 27, and subsequently, this variant was found in his affected father I1 and three carrier siblings (II1, II2, and II3). According to the ATA risk

### Table 1: Continued.

| Family ID | Type | Mutation (polymorphism) | Affected number (percent) | Affected individuals (diagnosed primarily with symptoms) | Presymptomatic carriers (diagnosed primarily with genetic testing) | ATA risk based on 2015 ATA risk guideline (ATA risk based on 2009 ATA risk guideline) |
|-----------|------|-------------------------|---------------------------|----------------------------------------------------------|-----------------------------------------------------------------|-------------------------------------------------------------------------------------|
| IR-F22    | MTC  | C634F (Polymorphism: G691S, L769L, S836S, S904S + A>T IVS16)     | 1 (2.8%)                  | Ctn level (before surgery): 553 pg/mL Ctn level (after surgery): 1.2 pg/mL Metastatic MTC to lymph nodes and both lungs confirmed histopathologically Total thyroidectomy and LND done. Ctn level: >2000 pg/mL | —                                                                                   | H (C)                                                                             |
| IR-F23    | MEN2B (metastatic MTC + ambiguous genitalia) | M918T                  | 1 (2.8%)                  | Thyroidectomy and bilateral adrenalectomy done. Ctn level: NA | IV-1: Ctn level: Normal Prophylactic thyroidectomy recommended | —                                                                                   |
| IR-F29    | MEN2A | C634R                  | 1 (2.8%)                  | NA                                                        | —                                                                                   | H (C)                                                                             |
| IR-F37    | MTC  | M918T                  | 1 (2.8%)                  | NA                                                        | —                                                                                   | HST (D)                                                                           |
| IR-F38    | MTC  | C634Y, H658R (novel)   | 1 (2.8%)                  | NA                                                        | —                                                                                   | H (C)                                                                             |
| IR-F39    | MTC  | (Polymorphism: G691S)   | 1 (2.8%)                  | NA                                                        | —                                                                                   | H (C)                                                                             |
classification, p.C618R mutation is stratified as ATA-MOD (level B), and carriers are recommended to undergo prophylactic thyroidectomy before 5 years of age. Follow-up visits showed that the calcitonin level was above the normal range (300 pg/ml) in II5 even after prophylactic thyroidectomy.

Thirty-eight index patients who seemed to be apparently sporadic MTC were tested for RET mutation at six mentioned exons. The results showed that 10 out of 38 patients had germline RET mutations. In addition, in four families, we found four carriers of a causative mutation in pre-symptomatic children who had affected parents, including family IR-F14 with c.1832G > A mutation in exon 10 (p.C611Y), families IR-F17 and IR-F29 with c.1900T > C mutation in exon 11 (p.C634R), and family IR-F22 with c.1901G > T mutation in exon 11 (p.C634F).

In the family IR-F17, a 32-year-old affected mother (II8) with a p.C634R mutation had a 4-year-old daughter with elevated calcitonin who was found to have the same mutation as observed in her mother. The proband in the family IR-F29 (III6) had an asymptomatic 10-year-old boy who carried the same mutation (p.C634R). Moreover, a 15-year-old carrier of p.C611Y in the family IR-F14 with a moderate ATA (ATA-MOD, Level B) and a 12-year-old carrier of p.C634F in the family IR-F22 were found. All of the carriers in these four families were referred to the endocrinologist for further clinical follow-up and prophylactic thyroidectomy. Furthermore, three index patients in families IR-F11, IR-F23, and IR-F37 had a known RET germline mutation (c.2735T > C) in exon 16 (p.M918T), which is a MEN2B specific mutation with the highest ATA risk (ATA-HST, level D). However, no available persons in their families had this mutation. The apparent sporadic patient IR-F39 had a known RET mutation (c.1901G > A) in exon 11 (p.C634Y). Likewise, another apparently sporadic patient (IR-F38) had a RET germline mutation (p.C634Y) and a novel uncertain variant (c.1973A > G) causing p.H658R, both in exon 11. RET novel variant p.H658R was predicted to be of uncertain significance by InterVar, benign by polyphen2, disease causing by mutation taster, and tolerated with SIFT score 0.5 by SIFT web server (Table 2).

4. Discussion

We examined all six exons (10, 11, 13, 14, 15, and 16) by PCR and direct Sanger sequencing. First-degree relatives of RET positive cases were also genotyped and underwent
Table 2: Variants according to in silico analysis.

| Variant   | InterVar          | PolyPhen2                    | Mutation taster                      | SIFT       | MEN2 database | Report     |
|-----------|-------------------|------------------------------|--------------------------------------|------------|---------------|------------|
| 1 p.H658R | Uncertain         | Benign                       | Disease causing (NMD, amino acid    | Tolerated  | Novel         |            |
|           | significance      | with 0.006 score:            | sequence change, protein features    | (SIFT score |               |            |
|           |                   | Sensitivity: 0.97             | might be affected, and splice site   | 0.5        |               |            |
|           |                   | Specificity: 0.75             | changes)                             |            |               |            |
| 2 p.V648I | Likely benign     | Benign                       | Disease causing (NMD, amino acid    | Tolerated  | Uncertain     | [9]        |
|           |                   | with 0.006 score:            | sequence change, protein features    | (SIFT score|               | (2002)     |
|           |                   | Sensitivity: 0.97             | might be affected, and splice site   | 0.43       |               |            |
|           |                   | Specificity: 0.75             | changes)                             |            |               |            |

prophylactic thyroidectomy if they had the causative mutation. According to the results (Figure 2), 37.5% (17 of 45) of the MTC referral index patients were found to have eight distinct RET germline mutations, including p.C634R (35.3%), p.M918T (17.6%), p.C634Y (11.8%), p.C634F (5.9%), p.C611Y (5.9%), p.C618R (5.9%), p.C630R (5.9%), p.L790F (5.9%), and an uncertain variant p.V648I (5.9%). In addition, we found one new variant in an apparently sporadic patient. The distribution of the detected RET mutations was as follows: 64.7% in exon 11, 17.6% in exon 16, 11.8% in exon 10, 5.9% in exon 13, and no mutation in exons 14 and 15 was found. Overall, the genetic status of all participants (61 affected individuals, 22 presymptomatic carriers, and 29 genetically healthy) was determined by RET mutation screening, regarding all six exons of RET (10, 11, 13, 14, 15, and 16). Moreover, 22 genetically presymptomatic carriers were referred to the endocrinologist for further clinical management.

Medullary thyroid cancer (MTC) is responsible for about 10% of thyroid cancers, but its prognosis is worse than the most common thyroid cancer, papillary thyroid cancer (PTC). However, 25% of MTCs are hereditary, known as MEN2 syndromes, including MEN2A and MEN2B. Germline missense mutations in hot spot exons of the RET gene (exons 10, 11, 13–16) cause 90% of all MEN2 syndromes. Biochemical tests used for screening at-risk cases of MEN2 have false positive/negative results. By contrast, RET mutation detection is a promising/golden screening test. Accurate identification of genotype-phenotype correlation in MEN2 syndromes is essential for presymptomatic detection of carriers and clinical management of the affected subjects. In addition, RET genetic testing can contribute to distinguishing between three types of MEN2 syndromes that have some manifestation similarities.

According to the results, 37.5% (17 out of 45) of the MTC index patients had nine distinct RET germline mutations, including p.C634R (35.3% in 6 patients), p.M918T (17.6% in 3 patients), p.C634Y (11.8% in 2 patients), p.C634F (5.9% in 1 patient), p.C611Y (5.9% in 1 patient), p.C618R (5.9% in 1 patient), p.C630R (5.9% in 1 patient), and p.L790F (5.9% in 1 patient). The distribution of the detected mutations in hot spot exons of RET gene was as follows: 64.7% (11/17) in exon 11, 17.6% (3/17) in exon 16, 11.8% (2/17) in exon 10, 5.9% (1/17) in exon 13, and no mutations in exons 14 and 15 were found. We were able to determine the clinical condition of all 112 participants by RET screening test, including 61 affected individuals, 22 presymptomatic carriers, and 29 genetically healthy subjects. Presymptomatic carriers were referred to the endocrinologist for further clinical evaluation and possible prophylactic thyroidectomy.

In this study, 7 Iranian kindred with hereditary MTC had four distinct RET germline mutations (4 families had p.C634R mutation, and three other families had p.C630R, p.L790F, and p.C618R mutations). Three families (IR-F18, IR-F31, and IR-F36) with p.C634R mutation had FMTC or other MEN2A types phenotypes (MTC, PHEO, and PHPT). Based on the ATA guideline, p.C634R mutation is seen in MEN2A syndromes. Our findings support the role of p.C634R mutation in MEN2A manifestation. The ATA guideline also indicates that p.C634R mutation is seen in FMTC (as a type of MEN2A). Substitution mutations at codon 634 in exon 11, especially cytosine to arginine, are predominately seen in Iranian MEN families [10–14]. It has been stated that mutations at codon 634 are the most frequent mutation in Caucasians [15]. This mutation comprised 53% of all detected mutations in this study. The present study showed that mutations in exon 11, especially at codon 634, are commonly observed in the Iranian population, which is consistent with other studies [10–14].

Interestingly, follow-up studies in the IR-F25 family showed that a 19-year-old carrier, IV-2, did not manifest any MEN2A phenotypes, unlike other carriers. The carrier IV-2 might have some other modifier genes that give her an opportunity to be a healthy carrier with a normal calcitonin level until 19 years of age. In addition, a review of the literature showed that p.L790F mutation is reported for the second time in Iranian families with FMTC [16]. Similarly, it is the second report of p.C618R mutation in Iranian MTC families [13]. Nevertheless, p.C618R mutation is the most common mutation in MEN2A in Saudi Arabian families [17].

The present study showed five distinct RET mutations in ten out of thirty-eight (26.3%) apparently sporadic Iranian MTC cases, including two p.C634R, two p.C634Y, one p.C634F, three p.M918T, and one p.C611Y mutations. Additionally, one RET uncertain variant (p.V648I) was detected in one apparently sporadic MTC Iranian patient for the first time. Moreover, it is the first report of an apparently sporadic MTC Iranian patient who had a p.C634R mutation and a novel H658R uncertain variant. Further molecular studies are needed to identify the clinical effect of these two variants.
On the other hand, in this study, p.M918T mutation was another common mutation in sporadic Iranian MTC cases, which is inconsistent with findings in European populations [11, 16]. To the best of our knowledge, p.M918T mutation has been reported in two cases and has not been detected in Iranian Azari and other populations [10–14]. Different genetic backgrounds could be the reason for the mentioned conflicting results, which requires more investigations. On the other hand, it seems that some modifier polymorphisms that were found in some carriers of RET mutation in our study might contribute to MTC manifestations [18]. Additional genetic evidence is required to clarify the additive effect of modifier polymorphisms.

In conclusion, evaluation of RET mutations in hot spot exons resulted in the genetic diagnosis of 37.5% of the Iranian MTC patients. The genotype of all participants in this study was precisely determined, and 22 presymptomatic carriers were referred for further clinical management and prophylactic thyroidectomy. Thus, RET mutation screening could serve as an essential detection method for any MTC patient to find presymptomatic RET carriers.

**Data Availability**

The data used to support the findings of this study are included within the article.

**Disclosure**

This manuscript has been presented in “preprint” with the following link: https://www.researchsquare.com/article/rs-227836/v1

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

E. D. designed and F. V-R-P carried out the experiment. F. V-R-P and M. J-A wrote the manuscript with support from M. G. and E. D. M-R. M-T and B. L. diagnosed and introduced the patients. M. G. and M. K. helped supervise the project. All authors discussed the results and contributed to the final manuscript.

**References**

[1] D. J. Ledbetter and P. R. V. Johnson, “Endocrine surgery in children,” Springer, Berlin, Germany, 2017.
[2] F. Raue and K. Frank-Raue, “Update multiple endocrine neoplasia type 2,” Familial Cancer, vol. 9, no. 3, pp. 449–457, 2010.
[3] S. A. Wells, S. L. Asa, H. Dralle et al., “Revised American thyroid association guidelines for the management of medullary thyroid carcinoma,” Thyroid, vol. 25, no. 6, pp. 567–610, 2015.
[4] M. Santoro, F. Carломagno, I. D. Hay et al., “Ret oncogene activation in human thyroid neoplasms is restricted to the papillary cancer subtype,” Journal of Clinical Investigation, vol. 89, no. 5, pp. 1517–1522, 1992.
[5] Y. Ishizaka, S. Kobayashi, T. Ushijima, S Hirohashi, T Sugimura, and M Nagao, “Detection of retTPC/PTC transcripts in thyroid adenomas and adenomatous goiter by an RT-PCR method,” Oncogene, vol. 6, pp. 1667–1672, 1991.
[6] L. M. Mulligan, J. B. J. Kwok, C. S. Healey et al., “Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A,” Nature, vol. 363, no. 6428, pp. 458–460, 1993.
[7] L. F. Reynolds and C. Eng, “RET mutations in multiple endocrine neoplasia type 2 and Hirschsprung disease,” The Journal of Pediatrics, vol. 7, no. 6, pp. 702–709, 1995.
[8] C. Eng, L. M. Mulligan, D. P. Smith et al., “Mutation of the RET protooncogene in sporadic medullary thyroid

**Figure 2: Flow diagram of RET mutations and the ATA risks for 45 Iranian patients affected with medullary thyroid carcinoma.**
carcinoma,” *Genes, Chromosomes and Cancer*, vol. 12, no. 3, pp. 209–212, 1995.

[9] A. B. Nunes, M. C. L. Ezabella, A. C. Pereira, J. E. Krieger, and S. P. A. Toledo, “A novel Val648Ile substitution in RET protooncogene observed in a Cys634Arg multiple endocrine neoplasia type 2A kindred presenting with an adrenocorticotropic-producing pheochromocytoma,” *Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 12, pp. 5658–5661, 2002.

[10] M. Nasiri Aghdam, M. R. Abbaszadegan, A. Tafazoli, M. Aslzare, and Z. Mosavi, “Presence of the RET Cys634Tyr mutation and Gly691Ser functional polymorphism in Iranian families with multiple endocrine neoplasia type 2A,” *Hormones*, vol. 15, no. 1, pp. 65–72, 2016.

[11] M. Hedayati, I. Nabipour, N. Rezaei-Ghaleh, and F. Azizi, “Germline RET mutations in exons 10 and 11: an Iranian survey of 57 medullary thyroid carcinoma cases,” *Medical Journal of Malaysia*, vol. 61, pp. 564–569, 2006.

[12] M. Hedayati, M. Z. Yeganeh, S. S. Eslami, S. R. Barez, L. H. Rad, and F. Azizi, “Predominant RET germline mutations in exons 10, 11, and 16 in Iranian patients with hereditary Medullary thyroid carcinoma,” *Journal of Thyroid Research*, vol. 2011, Article ID 264248, 6 pages, 2011.

[13] P. Kheiroddin, S. Z. Rasihashemi, M. A. Estiar et al., “RET gene analysis in patients with medullary thyroid carcinoma,” *Clinical Laboratory*, vol. 62, pp. 871–876, 2016.

[14] A. A. Ghazi, M. Bagheri, A. Tabibi et al., “Multiple endocrine neoplasia type 2A in an Iranian family: clinical and genetic studies,” *Archives of Iranian Medicine*, vol. 17, pp. 378–382, 2014.

[15] L. M. Mulligan, C. Eng, C. S. Healey et al., “Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC,” *Nature Genetics*, vol. 6, no. 1, pp. 70–74, 1994.

[16] M. Hedayati, M. Zarei Yeganeh, and S. Sheikholeslami, “Hotspot mutational spectrum of RET proto-oncogene in hereditary medullary thyroid carcinoma,” *Tumour Biology*, vol. 36, no. 7, pp. 5225–5231, 2015.

[17] I. Berard, J.-L. Kraimps, F. Savagner et al., “Germline-sequenced variants S836S and L769L in the RET 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, pp. 150–152, 2004.

[18] F. Lesueur, A. Cebrian, M. Robledo et al., “Polymorphisms in RET and its coreceptors and ligands as genetic modifiers of multiple endocrine neoplasia type 2A,” *Cancer Research*, vol. 66, no. 2, pp. 1177–1180, 2006.