DOI: 10.31793/1680-1466.2025.30-1.51

Determination of mutations in the *RET* gene for the diagnosis of medullary thyroid carcinoma syndromes

I.I. Komisarenko[1], B.B. Guda[1], I.V. Kroups'ka[2], A.V. Mazov[2], R.V. Gulkovskyi[2], G.V. Gerashchenko[2], V.M. Pushkarev[1], N. I. Levchuk[1], N.Ya. Kobrynska[1]

[1] State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine»

[2] Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine

Abstract. Medullary thyroid carcinoma (MTC) arises from non-epithelial parafollicular cells. MTC can be hereditary (25%) or sporadic (75%). Activating germline REarranged during Transfection (*RET*) mutations are found in 95-98% of hereditary MTC, while somatic *RET* mutations are present in 25-40% of sporadic MTC. **Aim.** The aim of the study was to determine *RET* gene mutations in codons 634 and 918, which are associated with the highest risk of MTC. **Material and methods.** Whole blood was obtained by standard venipuncture. Genomic DNA was isolated using the EliGene® Blood DNA Isolation kit. DNA amplification was performed by polymerase chain reaction (PCR) using primers specific for the regions of exons 10, 11 and 16 of the *RET* gene, which may contain mutations. The next-generation sequencing (NGS) for genomic DNAs from each sample was performed using the Ion Ampliseq Cancer Hotspot Panel v2 following the manufacturer's instructions. **Results.** Among all the tested samples, heterozygous p.M918T mutation was detected only in sample No. 2. As an outcome of the sequencing of the genomic DNA of 7 patients diagnosed with MTC, four different pathogenic inherited mutations of the *RET* gene were identified in four patients. **Conclusions.** Determination of mutations in the *RET* gene is a promising approach for establishing a diagnosis in the treatment of hereditary forms of MTC and allows planning further directions of therapy. The PCR-restriction fragment length polymorphism (PCR-RFLP) method makes it possible to detect individual mutations in the *RET* and other genes, associated with the highest risk of MTC (M918T, C634F and A883F). Given the population frequencies of occurrence of various pathogenic variants in the *RET* gene in Ukraine, the (PCR-RFLP) method can become an effective screening tool. NGS allows analyzing the entire sequence of the *RET* gene and other genes, and detecting a much larger spectrum of variants, including previously undescribed ones.
Keywords. Medullary thyroid carcinoma, *RET* gene mutations, next-generation sequencing.

VERTE

MTC arises from non-epithelial parafollicular cells of the neural crest, which produces the peptide calcitonin. MTC can be hereditary (25%) or sporadic (75%). In hereditary forms, MTC is a major component of the syndrome of multiple endocrine neoplasia (MEN) type 2 (MEN 2) together with other endocrine tumors. The *RET* proto-oncogene that located on chromosome 10q11.2 and includes 21 exons is often mutated at MTC. Activating germline *RET* mutations are found in 95-98% of hereditary MTC, while somatic *RET* mutations are present in 25-40% of sporadic MTC [1-3]. All *RET* mutations in MTC result in ligand-independent constitutive activation, but the mechanism of action differs depending on the mutation. For example, the *RET* C634R (p.Cys634Arg) mutation in exon 11 (a mutation in the cysteine-rich region of the extracellular domain) leads to disulfide bond formation of *RET* homodimers and subsequent constitutive activation, whereas the *RET* M918T (p.Met918Thr) mutation – in exon 16 (a mutation in the intracellular tyrosine kinase domain) leads to autophosphorylation of tyrosine kinase domain. In addition, different *RET* mutations have different transforming activities. For example, the *RET* M918T mutation has a higher transforming activity than other mutations [4].

MEN is a group of inherited clinical syndromes that affect various endocrine glands. Three different types of MEN syndromes can occur (MEN 1, MEN 2A, and MEN 2B). MEN syndromes are rare, occur in people of all ages, and affect both sexes equally. MEN 1, OMIM 131100 is a rare inherited disorder that can include combinations of more than 20 endocrine and non-endocrine tumors [5, 6]. MEN 1 is characterized by neoplastic transformation of the parathyroid glands, pancreatic islets, anterior pituitary, and gastrointestinal tract. In MEN 2 syndromes, medullary thyroid cancer almost always (95%) develops, which may be associated with pheochromocytoma and/or multiple parathyroid adenomas with hyperparathyroidism (up to 25% in MEN 2A) [6].

Combinations of endocrine neoplasias result in 3 syndromes: MEN 2A, MEN 2B and familial MTC. The clinical course of MTC differs considerably in the three syndromes. It is highly aggressive in MEN 2B, almost indolent in most patients with familial MTC and with variable aggressiveness in patients with MEN 2A. Activating germline point mutations of the *RET* proto-oncogene are present in 98% of families with MEN 2. There is a strong genotype-phenotype correlation and the specific *RET* mutation may be responsible for the aggressiveness of the clinical course. The treatment of choice for primary MTC is total thyroidectomy with neck lymph node dissection. The prognosis of MEN 2 depends on the aggressiveness of the MTC and, therefore, on the success of the initial treatment [3, 7].

Distant metastases develop in 15-20% of patients, and retrospective series have reported a 10-year survival rate of 10-40% from the time of first metastasis [8, 9]. Lymph node metastases are common in MTC, with distant metastases being rare. The most common sites of metastasis are the lungs, liver, and bones. Another study reported extremely rare metastases of MTC to the bone marrow and adrenal glands [10].

Material and methods

Permission to conduct the research was obtained from the Bioethics Commission of the State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine» (SI «IEM NAMS»). All patients signed an informed consent to conduct scientific research with postoperative biomaterials. Whole blood samples were obtained in the Surgical Department of (SI «IEM NAMS»). The final diagnosis was obtained from the Laboratory of Morphology of the Endocrine System of (SI «IEM NAMS») and the CSD Laboratory.

The study was conducted in the department of cell signaling systems of the Institute of Molecular Biology and Genetics of the NAS of Ukraine and the department of fundamental and applied problems of endocrinology.

Whole blood was obtained by standard venipuncture, frozen and stored at -25 °C until use. Genomic DNA was isolated using the EliGene® Blood DNA Isolation kit according to the manufacturer's recommendations. The quantity and quality were assessed using a Nanodrop spectrophotometer.

In vitro DNA amplification. Genomic DNA amplification was performed by PCR using primers specific for the region of exons 10 and 11, which may contain a mutation at codon 634, and the region of exon 16, which may contain a mutation in codon 918 of the *RET* gene. For this purpose, the following primer pairs were used: forward – (1) GGGGGATTAAAGCTGGCTAT, reverse – (2)

TGGTAGCAGTGGATGCAGAA (for the region with the mutation in codon 634), forward – (3) GTGTCTACAGCACTCCTCTGG, reverse – (4) TGTAACCTCCACCCCAAGAGA (for the region with the mutation in codon 918). The expected length of the PCR product is 988 and 228 bp, respectively.

The reaction mixture with a total volume of 25 µl contained: 2.5 µl of 10X «DreamTaq» buffer, 0.5 µl of dNTP (10 mM), 2 µl of specific primers (5 µM), 0.5 µg of genomic DNA, 0.65 units of DNA polymerase («DreamTaq») and 19 µl of nuclease-free water. PCR conditions: DNA denaturation – 98°C, 1 min, followed by 35 cycles with denaturation at 95°C, 30 sec, hybridization – 60°C, 30 sec, elongation – 72°C, 1 min, as well as a final elongation stage at 72°C, 5 min. The PCR reaction was performed on a Flex Cycler DNA amplifier («Analytik Jena», Germany).

Analysis of single nucleotide substitutions in the RET gene by RFLP analysis. To determine the mutation c.2753T>C (NM_020975.6) in codon 918 of the *RET* gene, the PCR product of the 16th exon was hydrolyzed with the restriction endonuclease FokI (Thermo Fisher Scientific, USA) or the 10th and 11th exons with the restriction endonuclease HhaI (Thermo Fisher Scientific, USA) to determine the mutation c.1902 C>G (NM_020975.6) mutation in codon 634 of the *RET* gene. The reaction mixture with a total volume of 25 µl contained: amplified DNA fragment (20 of 25 µl of the reaction mixture after DNA amplification from the previous reaction), 2.5 µl of 10X buffer and 1 unit of the corresponding endonuclease. Next, the reaction mixture was incubated for 2 h at 37°C and subsequently electrophoretic separation of DNA fragments was performed in agarose or polyacrylamide gels (PAGE).

DNA electrophoresis in agarose and PAGE. Electrophoretic separation of DNA in agarose or PAGE was performed according to generally accepted methods. Briefly, for agarose gel electrophoresis, a 1.5% agarose gel was prepared in 1xTAE (Tris-acetate) buffer. For DNA visualization, ethidium bromide (EtBr) was added to the agarose gel at a rate of 0.5 mg/ml. DNA samples were mixed with six-fold loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol) and added to the gel wells. The size of DNA fragments was determined by DNA markers (GeneRuler 1kb DNA Ladder, «Thermo Fisher Scientific», USA). As an alternative, a 12% PAGE was used to separate DNA fragments.

NGS. The NGS of genomic DNAs from each sample was performed using the Ion Ampliseq Cancer Hotspot Panel v2, which covers approximately 2800 mutational hotspot regions from 50 cancer-related genes following the manufacturer's instructions [11]. In brief, 10 ng of genomic DNAs were used to construct barcoded DNA libraries using an Ion Ampliseq Library Kit Plus («Thermo Fisher Scientific», USA). Quantification of the final libraries was performed using an Ion Library TaqMan™ Quantitation Kit («Thermo Fisher Scientific», USA) on the QuantStudio™ 5 Real-Time PCR System («Thermo Fisher Scientific», USA), following the manufacturer's protocols. The obtained libraries were diluted to ~70 pM and then pooled together for further processing. Template preparation was performed on the Ion Chef System («Thermo Fisher Scientific», USA) using the Ion 540 Kit-Chef («Thermo Fisher Scientific», USA). Samples were loaded onto an Ion 540 Chip («Thermo Fisher Scientific», USA) for sequencing using the Ion GeneStudio S5 Plus Sequencer («Thermo Fisher Scientific», USA). Data were processed and analyzed through the Ion Reporter pipeline [12].

Sanger sequencing. The cycle sequencing analysis of the PCR products of samples positive for *RET* gene mutations was performed with the above-mentioned primers 1,2,3 and 4. The PCR products were purified from residual primer mix using Exonuclease I and Shrimp Alkaline Phosphatase («Thermo Fisher Scientific», USA) according to the manufacturer's protocol. Cycle sequencing was performed using BigDye™ Terminator v3.1 Cycle Sequencing Kit («Thermo Fisher Scientific», USA) according to manufacturer instructions. Bidirectional sequencing was performed on the Applied Biosystems™ 3500 Genetic Analyzer («Thermo Fisher Scientific», USA). Sequencing traces were analyzed by Applied Biosystems SeqScape Software v2.5 («Thermo Fisher Scientific», USA).

Results and discussion

In order to identify hereditary pathogenic variants of the *RET* gene, we conducted a molecular genetic study of biomaterial from 7 operated patients diagnosed with MTC using PCR-RFLP and NGS (**Table 1**). The average age of the patients was 37.5 years (14 - 52 years).

VERTE

Table 1. Characteristics of patients with MTC who underwent surgery

#	Operation data	Gender	Birth data	Age	Diagnosis	Form
1	18.08.2022	w	29.08.1984	37	MTC	Sporadic
2	30.08.2022	m	20.03.2006	21	MTC	MEN 2B
4	19.09.2022	w	18.06.1977	45	MTC	Familial
7	25.11.2022	m	29.12.1992	29	MTC	Familial
12	16.10.2023	w	22.05.1995	14	MTC	Familial
14	21.03.2024	m	19.04.1975	48	MTC	Familial
16	08.05.2024	w	04.11.1971	52	MTC	Familial

Note. The number represents the individuality of the patient (a total of 17 individuals were analyzed).

Study design. **Fig. 1** and **Fig. 2** show the sequences of both PCR products studied, the recognition sites for restriction endonuclease and mutations.

Detection of mutations in exons 11 and 16 of the RET gene by PCR followed by restriction fragment length polymorphism analysis. The C634W mutation, located in exon 11 of the *RET* gene, is a transversion – a single-nucleotide substitution of cytosine for guanine. The result of this substitution is the emergence of an additional recognition site for the HhaI restriction endonuclease. Therefore, in individuals without a single-nucleotide substitution C>G, after hydrolysis of a PCR product with a length of 989 base pairs (bp), we expect to detect 2 fragments with a length of 779 and 210 bp, and the PCR product with the mutation should be hydrolyzed into 3 fragments – 779, 129 and 81 bp. In heterozygous carriers of a single-nucleotide substitution, we expect to detect fragments of 779, 210, 129 and 81 bp.

The presence of amplification products and separation of PCR fragments of the *RET* gene product, after hydrolysis with the specific restriction endonuclease HhaI, was analyzed by electrophoresis in a 2% agarose gel **(Fig. 3)**.

In all 7 analyzed samples, according to the PCR-RFLP analysis data, after hydrolysis by the specific restriction endonuclease HhaI, 2 fragments with a length of 779 and 210 bp were observed (as can be seen from Fig. 3), which is characteristic only for wild-type homozygotes, i.e., we did not detect 129 bp PCR the C634W mutation of the *RET* gene.

The M918T mutation, localized in exon 16 of the *RET* gene, is a transition – a mononucleotide substitution of thymine for cytosine. The result of this substitution is the emergence of a recognition site for the FokI restriction endonuclease. Therefore, in individuals without a mononucleotide substitution T>C, after hydrolysis of the PCR product with a length of 228 bp should remain intact, and the PCR product with the mutation should be hydrolyzed into 2 fragments – 153 and 75 bp. In heterozygous carriers of the mononucleotide substitution, we expect to detect fragments of 228, 153 and 75 bp.

The presence of amplification products and separation of PCR fragments of the *RET* gene product

GGGGGATTAAAGCTGGCTATGGCACCTGCAACTGCTTCCCTGAGGAGGAGAAGTGCTTCTGCGAGCCCGAAGACATCCAGGGTGAGTGGGTGGCGGCCGGGACCACCACCACCTCCCA
GCCCCACAGAGGTCTCAACAGCACATCTGAGGTCCCAACAAGGGAGGAAATTGCTGGGAGGCGAGTGGGCCCCATGAAACTTCCCTCCCTCCCTCTGGGCCTCTGTTACTCCACCCAGG
AGAGGGGCCAGGGCCCCTGTAAAGTGTCTTCTGGCCATAAGTTCTATGATGGACAGGCCAGAAAAGCAGTTCTTCCACCAAACAACTTGTCAGCCTGACAAGTCACTGTCCCTGTGACCA
TGCAGCTGGGACCCACCCAGGAACACATTTCAAGGTCAGCAGGTATGGTGGGTTGCACAGCCACACTGACTACACTCAGGGGTGCTGTTCTGCCTGAGCATAGGGACACGTTTCTGTCAT
TGAGTTTTCTGGTATTATATAGCCCTACGTCCCTAGCCACTTAGCATTTTCATAAAGAAAATGCCAAAGACATTTGGAACAGAGGAAAATTTTGACCTCCCCTGCCAGCCCTCCAGTGCCAG
CTGGTGTAATGAGCACAGCCTCTGCTGTGTGACCTTGGCAGGCTGCTCAGCCTCTCTGAGCCTCTGTCTCCATCTGTAAGAGGGCAATAGTGGTCTAGGAGGGGGCAGTAAATGGCAGTA
CCCATGCTCGATGGGGTGTTCTCAGGCCTTCCCACACCTCCATGGCCACTTCCCAGCTGGCGCGGACACGGCAGGCTGGAGAGCCATGAGGCAGAGCATACGCAGCCTGTACCCAGTGG
TGCCGAGCCTCTGGCGGTGCCAAGCCTCACACCACCCCCACCCACAGATCCACTGTGCGACGAGCTGTGgCGCACGGTGATCGCAGCCGCTGTCCTCTTCTCCTTCATCGTCTCGGTGCTG
CTGTCTGCCTTCTGCATCCACTGCTACCA

Fig. 1. PCR product sequence of exons 10 and 11 (Seq: https://www.ncbi.nlm.nih.gov/nuccore/171184431) with HhaI restriction sites highlighted. The C634W mutation site is indicated by a lowercase g.

GTGTCTACAGCACTCCTCTGGTTACTGAAAGCTCAGGGATAGGGCCTGGCCTTCTCCTTTACCCCTCCTTCCTAGAGAGTTAGA
GTAACTTCAATGTCTTTATTCCATCTTCTCTTTAGGGTCGGATTCCAGTTAAATGGAtGGCAATTGAATCCCTTTTTGATCATATCT
ACACCACGCAAAGTGATGTGTAAGTGTGGGTGTTGCTCTCTTGGGGTGGAGGTTACA

Fig. 2. PCR sequence of exon 16 product with FokI restriction site highlighted. The M918T mutation site is indicated by a lowercase t.

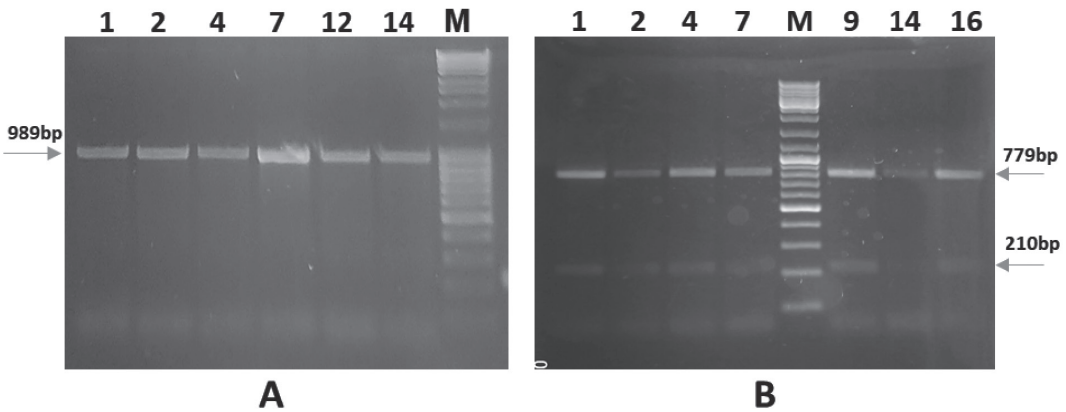

Fig. 3. Electrophoregram of the separation of fragments of the PCR product of exon 11 (primer 1 and 2) of the *RET* gene, before (**A**) and after (**B**) cleavage by the specific restriction endonuclease HhaI, in a 2% agarose gel: M is a molecular weight marker (with a step of 100 bp).

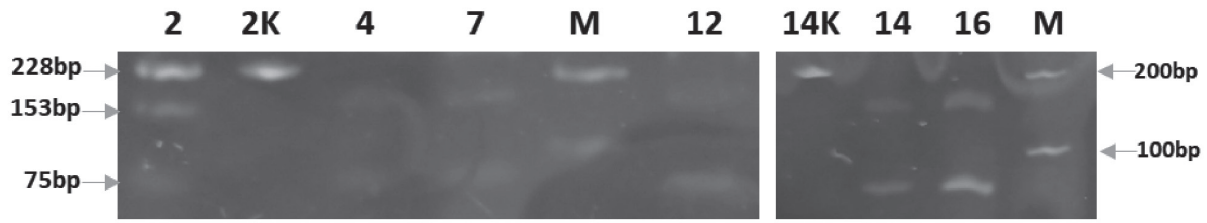

Fig. 4. Electrophoregram of the separation of fragments of the exon 16 (primers 3 and 4) PCR product of exon 16 (primers 3 and 4) of the *RET* gene, before and after hydrolysis by the specific restriction endonuclease FokI, in 12% PAGE: M – molecular weight marker (with a step of 100 bp); 2K and 14K – control without the addition of restriction endonuclease; 1, 4, 7, 12, 14 and 16 – individuals with the homozygous TT genotype; 2, – individual with the heterozygous TC genotype.

after hydrolysis by the specific FokI restriction endonuclease was analyzed by electrophoresis in a 12% PAGE **(Fig. 4)**.

Among all the tested samples, only sample No. 2 retains an intact 228bp fragment, although restriction products of 153bp and 75bp are also present, which indicates the presence of a heterozygous p.M918T mutation, i.e. only in one of the two alleles of the gene, in the maternal or paternal.

Detection of RET gene mutations by next-generation semiconductor ion sequencing. We performed molecular genetic analysis of the genomic DNA of 7 previously mentioned patients diagnosed with MTC, also by next-generation semiconductor ion sequencing (Ion Torrent, Thermo Fisher Scientific, USA) using the Ampliseq Hotspot Cancer Panel (Thermo Fisher Scientific, USA), in order to implement its use in a format suitable for clinical practice. The Ampliseq Hotspot Cancer Panel primer set allows analysis of 5 loci of the *RET* gene coding sequence, including the most common clinically relevant variants.

As an outcome of the sequencing of the genomic DNA of 7 patients diagnosed with MTC, four different pathogenic inherited mutations of the *RET* gene were identified in four patients **(Table 2)**.

Table 2. Description of the *RET* gene mutations that were identified by NGS

#	Gender	Nucleotide substitution	Amino acid substitution	Exon	dbSNP ID
2	m	c.2753T>C	p.Met918Thr	16	rs74799832
4	w	c.1853G>A	p.Cys618Tyr	10	rs79781594
7	m	c.1900T>C	p.Cys634Arg	11	rs75076352
16	w	c.1860C>G	p.Cys620Trp	10	rs79890926

The presence of all four detected mutations was further confirmed by direct Sanger sequencing of the PCR products of the 10th and 11th exons (primers 1 and 2), which may contain mutations in codons 618, 620, 634, and the 16th exon (primers 3 and 4), which may contain a mutation in codon 918 of the *RET* gene **(Fig. 5)**.

According to the Sanger sequencing of PCR products, the presence of all four detected *RET* gene mutations in the heterozygous state was confirmed.

We therefore detected the M918T mutation (c.2753T>C, p.Met918Thr) in exon 16 of the *RET*

VERTE

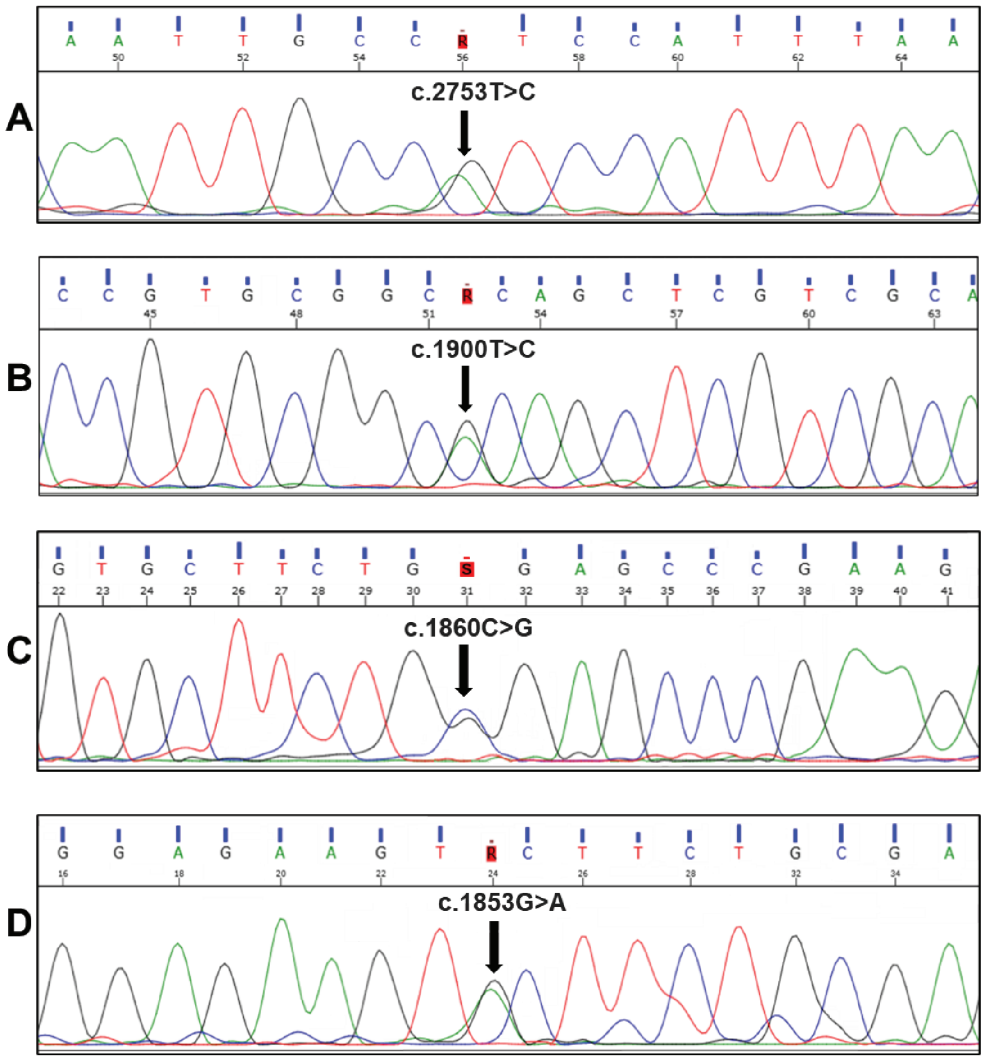

Fig. 5. Sanger sequencing chromatogram of PCR products of the 10th and 11th exon loci (B, C, D) and the 16th exon locus (A) of the *RET* gene in DNA samples of patients diagnosed with MTC. A – patient #2, B – patient #7, C – patient #16, D – patient #14.

gene in the DNA sample of patient #2 by all three applied molecular genetic methods. In the DNA sample of patient #7, we detected the C634R mutation (c.1900T>C, p.Cys634Arg) by NGS but not by PCR-RFLP because the restriction endonuclease Hhal that was selected allows detection of different mutations in codon 634, namely C634W (c.1902C>G, p.Cys634 Trp). In the present study, a *RET* gene mutation was detected in four out of seven patients, yielding a detection rate of 57%. The employment of the PCR-RFLP method enabled the identification of a single mutation; however, the utilisation of NGS facilitated the detection of four pathogenic hereditary mutations in the *RET* gene.

More than 20 *RET* gene mutations associated with the development of MTC have now been identified, which occur in different populations with different frequencies. In MEN 2A, the most common mutations are present in exon 10 (codons 609, 611, 618 and 620) or exon 11 (codons 630 and 634). In particular, mutations in codon 634 account for ~87% of all *RET* mutations implicated in MEN 2A, with p.Cys634Arg and p.Cys634Tyr being the most frequent events (52.5% and 25.4%, respectively) [13]. In contrast, in MEN 2B and in most sporadic MTCs, *RET* mutations are located in exons 15 and 16 (codons 883, 891, and 918) [14]. Different *RET* mutations are associated with different age of onset and aggressiveness of MTC, as well as the presence/absence of other endocrine tumors accompanying syndromic MTC [15, 16].

Most *RET* germline variants in familial MTC are identified in both extracellular codons (other than codon 634) and intracellular codons (768, 790,

and 791 in exon 13, 804 in exon 14, or 891 in exon 15) [17]. Mutations in exons 8, 10, 11, 13, 14, and 15 overlap with those identified in MEN 2A [18]. The American Thyroid Association (ATA) has developed a risk classification system for MTC. The highest risk category includes patients with MEN 2B syndrome and the *RET* M918T mutation. Patients with the C634F and A883F mutations are considered high risk. A statistically significant correlation was found between the presence of *RET* mutations (especially M918T) and advanced disease stage, higher T category, and the presence of lymph node and distant metastases [2, 6].

For those at the highest risk, total thyroidectomy with central compartment lymph node dissection should be performed within the first year of life. Preventive thyroidectomy performed in children with confirmed RET mutations can prevent the development of MTC and its complications, which is the main cause of mortality in this group of patients [6, 15].

Conclusions

Determination of mutations in the *RET* gene is a promising approach for establishing a diagnosis in the treatment of hereditary forms of MTC and allows planning further directions of therapy. With proper monitoring planning and responsible attitude on the part of the patient, this makes it possible to implement an early diagnostic strategy that allows diagnosing a potential oncological disease at an early stage or avoiding its development.

The PCR-RFLP method makes it possible to detect individual mutations in the *RET* and other genes, for example, the most common and associated with the highest risk of MTC (M918T, C634F and A883F). Given the population frequencies of occurrence of various pathogenic variants in the *RET* gene in Ukraine, the PCR-RFLP method can become an effective screening tool. At the same time, NGS allows analyzing the entire sequence of the *RET* and other genes and detecting a much larger spectrum of variants, including previously undescribed ones.

References

1. Ciampi R, Romei C, Cosci B, Vivaldi A, Bottici V, Renzini G, et al. Chromosome 10 and RET gene copy number alterations in hereditary and sporadic Medullary Thyroid Carcinoma. Mol. Cell. Endocrinol. 2012;348:176-182.
2. Ciampi R, Romei C, Ramone T, Prete A, Tacito A, Cappagli V, et al. Genetic Landscape of Somatic Mutations in a Large Cohort of Sporadic Medullary Thyroid Carcinomas Studied by Next-Generation Targeted Sequencing. iScience. 2019 Oct 25;20:324-336. doi: 10.1016/j.isci.2019.09.030.
3. Dabelić N, Jukić T, Fröbe A. Medullary Thyroid Cancer – Feature Review and Update on Systemic Treatment. Acta Clin Croat. 2020 Jun;59(Suppl 1):50-59. doi: 10.20471/acc.2020.59.s1.06.
4. Barletta JA, Nosé V, Sadow PM. Genomics and Epigenomics of Medullary Thyroid Carcinoma: From Sporadic Disease to Familial Manifestations. Endocr Pathol. 2021 Mar;32(1):35-43. doi: 10.1007/s12022-021-09664-3. Epub 2021 Jan 25.
5. Pieterman CRC, Valk GD. Update on the clinical management of multiple endocrine neoplasia type 1. Clin Endocrinol (Oxf). 2022 Oct;97(4):409-23. doi: 10.1111/cen.14727.
6. Тронько МД, Гуда ББ. Орфанні ендокринні захворювання: сучасні тенденції хірургічного лікування в Україні. Ендокринологія. 2022;27(4):287-301 (Tronko, B.B. Guda M.D. Orphan endocrine diseases: current trends and the state of their surgical treatment in Ukraine. Endokrynolohiya. 2022;27(4):287-301). Ukrainian. doi: 10.31793/1680-1466.2022.27-4.287.
7. 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. doi: 10.1155/2012/705036.
8. Angelousi A, Hayes AR, Chatzellis E, Kaltsas GA, Grossman AB. Metastatic medullary thyroid carcinoma: a new way forward. Endocr Relat Cancer. 2022 May 31;29(7):R85-R103. doi: 10.1530/ERC-21-0368.
9. Zhang Y, Zheng WH, Zhou SH, Gu JL, Yu Q, Zhu YZ, et al. Molecular genetics, therapeutics and RET inhibitor resistance for medullary thyroid carcinoma and future perspectives. Cell Commun Signal. 2024 Sep 28;22(1):460. doi: 10.1186/s12964-024-01837-x.
10. Ebrahimi P, Payab M, Shariati A, Alipour N, Nozheh A, Tavangar SM, et al. Unusual Presentation of Metastatic Medullary Thyroid Cancer Involving Bone Marrow, Kidneys, and Adrenal Gland: A Literature Review Based on a Case Report. Cancer Rep (Hoboken). 2024 Oct;7(10):e70022. doi: 10.1002/cnr2.70022.
11. Watanabe T, Nanamiya H, Kojima M, Nomura S, Furukawa S, Soeda S, et al. Clinical implication of oncogenic somatic mutations in early-stage cervical cancer with radical hysterectomy. Sci Rep. 2020 Oct 30;10(1):18734. doi: 10.1038/s41598-020-72518-1.
12. Roy S, Coldren C, Karunamurthy A, Kip NS, Klee EW, Lincoln SE, et al. Standards and Guidelines for Validating Next-Generation Sequencing Bioinformatics Pipelines: A Joint Recommendation of the Association for Molecular Pathology and the College of American Pathologists. J Mol Diagn. 2018 Jan;20(1):4-27. doi: 10.1016/j.jmoldx.2017.11.003.
13. Mucha L, Leidig-Bruckner G, Frank-Raue K, Bruckner T, Kroiss M, Raue F. German study group for rare thyroid cancer. Phaeochromocytoma in multiple endocrine neoplasia type 2: RET codon-specific penetrance and changes in management during the last four decades. Clin Endocrinol (Oxf). 2017 Oct;87(4):320-326. doi: 10.1111/cen.13386.
14. Elisei R, Tacito A, Ramone T, Ciampi R, Bottici V, Cappagli V, et al. Twenty-Five Years Experience on RET Genetic Screening on Hereditary MTC: An Update on The Prevalence of Germline RET Mutations. Genes (Basel). 2019 Sep 10;10(9):698. doi: 10.3390/genes10090698.
15. Wells SA Jr, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid. 2015 Jun;25(6):567-610. doi: 10.1089/thy.2014.0335.
16. Raue F, Bruckner T, Frank-Raue K. Long-Term Outcomes and Aggressiveness of Hereditary Medullary Thyroid Carcinoma: 40 Years of Experience at One Center. J Clin Endocrinol Metab. 2019 Oct 1;104(10):4264-4272. doi: 10.1210/jc.2019-00516.
17. Salvatore D, Santoro M, Schlumberger M. The importance of the RET gene in thyroid cancer and therapeutic implications. Nat Rev Endocrinol. 2021 May;17(5):296-306. doi: 10.1038/s41574-021-00470-9.
18. Martins RS, Jesus TT, Cardoso L, Soares P, Vinagre J. Personalized Medicine in Medullary Thyroid Carcinoma: A Broad Review of Emerging Treatments. J Pers Med. 2023 Jul 13;13(7):1132. doi: 10.3390/jpm13071132.

VERTE

Abbreviations

MEN (1, 2A, 2B) – multiple endocrine neoplasia (type 1, 2A, 2B)
MTC – medullary thyroid carcinoma
NGS – next-generation sequencing
PAGE – polyacrylamide gel
PCR – polymerase chain reaction
RET – REarranged during Transfection
RFLP – restriction fragment length polymorphism

Визначення мутацій в гені *RET* для діагностики синдромів медулярної карциноми щитоподібної залози

І.І. Комісаренко[1], Б.Б. Гуда[1], І.В. Крупська[2], А.В. Мазов[2], Р.В. Гулковський[2], Г.В. Геращенко[2], В.М. Пушкарьов[1], Н.І. Левчук[1], Н.Я. Кобринська[1]

[1]ДУ «Інститут ендокринології та обміну речовин ім. В. П. Комісаренка НАМН України»

[2]Інститут молекулярної біології і генетики НАН України

Резюме. Медулярна карцинома щитоподібної залози (МТС) виникає з неепітеліальних парафолікулярних клітин (С-клітин). МТС може бути спадковою (25%) або спорадичною (75%). Активуючі зародкові мутації *RET* виявляють у 95–98% спадкових МТС, тоді як соматичні мутації *RET* присутні в 25–40% спорадичних МТС. **Мета**. Метою дослідження було визначення мутацій гена *RET* у кодонах 634 і 918, які асоціюються з найвищим ризиком МТС. **Матеріали та методи**. Цільну кров отримували стандартною венепункцією. Геномну ДНК виділяли за допомогою набору EliGene® Blood DNA Isolation kit. Ампліфікацію ДНК проводили методом ПЛР з використанням праймерів, специфічних для ділянок екзонів 10, 11 і 16 гена *RET*, які можуть містити мутації. Секвенування наступного покоління для геномних ДНК кожного зразка було виконано за допомогою Ion Ampliseq Cancer Hotspot Panel v2 згідно з інструкціями виробника. **Результати**. Серед усіх досліджених зразків лише зразок № 2 має гетерозиготну мутацію p.M918T. У результаті секвенування геномної ДНК 7 пацієнтів із діагнозом МТС у чотирьох пацієнтів було виявлено чотири різні патогенні спадкові мутації гена *RET*. **Висновки**. Визначення мутацій у гені *RET* є перспективним підходом для встановлення діагнозу при лікуванні спадкових форм МТС і дозволяє планувати подальші напрямки терапії. Метод PCR-RFRP (polymerase chain reaction-restriction fragment length polymorphism) дозволяє виявити окремі мутації в гені *RET* та інших генах, пов'язаних із найвищим ризиком МТС (M918T, C634F і A883F). Враховуючи популяційні частоти різних патогенних варіантів гена *RET* в Україні, метод PCR-RFRP може стати ефективним інструментом скринінгу. Секвенування наступного покоління дозволяє проаналізувати всю послідовність гена *RET* та інших генів і виявити набагато більший спектр варіантів, включаючи раніше неописані.

Ключові слова. Медулярна карцинома щитоподібної залози, мутації гена RET, секвенування наступного покоління.

For citation: Komisarenko II, Guda BB, Kroups'ka IV, Mazov AV, Gulkovskyi RV, Gerashchenko GV, Pushkarev VM, Levchuk NI, Kobrynska NYa. Determination of mutations in the *RET* gene for the diagnosis of medullar thyroid carcinoma syndromes. Endokrynologia. 2025;30(1):51-59. DOI: 10.31793/1680-1466.2025.30-1.51.

Correspondence address: Pushkarev Volodymyr Mykhaylovych, pushkarev.vm@gmail.com. SI «V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine», 69, Vyshgorodska st., Kyiv 04114, Ukraine.

Information about the authors: Komisarenko Ihor Ihorovych – Researcher of the Department of Orphan Endocrine Diseases and Endocrine Surgery, SI «V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine», ORCID: 0000-0002-1808-667X; Guda Bohdan Bohdanovich, Dr. Sci. (Medicine), Head of the Scientific and Practical Department of Orphan Endocrine Diseases and Endocrine Surgery, SI «V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine», ORCID: 0000-0002-9181-0679; Kroups'ka Iryna Volodymyrivna – PhD, Senior Research, of the Department of Cell Signaling, Institute of Molecular Biology and Genetics of the NAS of Ukraine, ORCID: 0000-0002-6821-6225; Mazov Andriy Valeriyovytch – PhD student of the Department of Cell Signaling, Institute of Molecular Biology and Genetics of the NAS of Ukraine, ORCID: 0009-0006-2163-3845; Gulkovskyi Roman Vladislavovich – Cand. Sci. (Biology), Senior Research Fellow of the Department of Molecular Oncogenetics, Institute of Molecular Biology and Genetics of the NAS of Ukraine, ORCID: 0000-0002-2632-5634; Gerashchenko Ganna Volodymyrivna – Doct. Sci. (Biology), Senior Research, Leading Research Fellow of the Department of Molecular Oncogenetics, Institute of Molecular Biology and Genetics of the NAS of Ukraine, ORCID: 0000-0002-4700-5736; Pushkarev Volodymyr Mykhaylovych, Dr. Sci. (Biology), Senior Research Fellow, Chief Researcher of the Department of Fundamental and Applied Problems of Endocrinology, SI «V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine», ORCID: 0000-0003-0347-7771; Levchuk Nataliia Ivanivna, Cand. Sci. (Biology), Senior Scientist, Leading Research Fellow of the Department of Fundamental and Applied Problems of Endocrinology, SI «V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine», ORCID: 0000-0003-0482-5176; Kobrynska N.Ya., Cand. Sci. (Medicine), surgeon-endocrinologist, Head of the Consulting Polyclinic Department, SI «V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine», ORCID: 0000-0001-8698-9793.

Personal contribution: Komisarenko I.I., Guda B.B., Kobrynska N.Ya. – collection of tumor tissues, data analysis, writing the text; Kroups'ka I.V., Mazov A.V., Gulkovskyi R.V., Gerashchenko G.V., Levchuk N.I. – conducting experiments; Pushkarev V.M., Gulkovsky R.V., Gerashchenko G.V., Levchuk N.I., – analysis of literary sources, writing and editing the text, design and translation of the article.

Funding: the article was prepared within the budget funding of the National Academy of Medical Sciences of Ukraine according to the plan of research work of the SI «V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine» and Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine.

Declaration of ethics: the authors have declared no conflicts of interest or financial obligations.

Article: received January 29, 2025; revised February 19, 2025; accepted March 05, 2025; published March 30, 2025.

Для цитування: Комісаренко ІІ, Гуда ББ, Крупська ІВ, Мазов АВ, Гулковський РВ, Геращенко ГВ, Пушкарьов ВМ, Левчук НІ, Кобринська НЯ. Визначення мутацій в гені *RET* для діагностики синдромів медулярної карциноми щитоподібної залози. Ендокринологія. 2025;30(1):51-59. DOI: 10.31793/1680-1466.2025.30-1.51.

Адреса для листування: Пушкарьов Володимир Михайлович, pushkarev.vm@gmail.com; ДУ «Інститут ендокринології та обміну

речовин ім. В.П. Комісаренка НАМН України», вул. Вишгородська, 69, Київ 04114, Україна.

Відомості про авторів: Комісаренко Ігор Ігорович – науковий співробітник відділу ендокринних орфанних захворювань та ендокринної хірургії ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України», ORCID: 0000-0002-1808-667X; Гуда Богдан Богданович – д-р мед. наук, керівник науково-практичного відділу орфанних ендокринних захворювань та ендокринної хірургії ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України», ORCID: 0000-0002-9181-0679; Крупська Ірина Володимирівна, канд. біол. наук, старша наукова співробітниця відділу сигнальних систем клітини Інституту молекулярної біології і генетики НАН України, ORCID: 0000-0002-6821-6225; Мазов Андрій Валерійович, аспірант відділу сигнальних систем клітини Інституту молекулярної біології і генетики НАН України, ORCID: 0009-0006-2163-3845; Гулковський Роман Владиславович – канд. біол. наук, старший науковий співробітник відділу молекулярної онкогенетики Інституту молекулярної біології і генетики НАН України, ORCID: 0000-0002-2632-5634; Геращенко Ганна Володимирівна – докторка біол. наук, старша наукова співробітниця відділу молекулярної онкогенетики Інституту молекулярної біології і генетики НАН України, ORCID: 0000-0002-4700-5736; Пушкарьов Володимир Михайлович – д-р біол. наук, старш. наук. співроб., головний науковий співробітник відділу фундаментальних та прикладних проблем ендокринології ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України», ORCID: 0000-0003-0347-7771; Левчук Наталія Іванівна – канд. біол. наук, старш. наук. співроб., провідна наукова співробітниця відділу фундаментальних та прикладних проблем ендокринології ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України», ORCID: 0000-0003-0482-5176. Кобринська Наталія Яремівна – канд. мед. наук, лікар хірург-ендокринолог, завідувачка консультативно-поліклінічного відділення ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України», ORCID 0000-0001-8698-9793.

Особистий внесок: Комісаренко І.І., Гуда Б.Б., Кобринська Н.Я. – збір тканин пухлин, аналіз даних, написання тексту; Крупська І.В., Мазов А.В., Гулковський Р.В., Геращенко Г.В., Левчук Н.І. – проведення експериментів; Пушкарьов В.М., Гулковський Р.В., Геращенко Г.В., Левчук Н.І. – аналіз літературних джерел, написання і редагування тексту, оформлення та переклад статті.

Фінансування: стаття підготовлена в рамках бюджетного фінансування НАМН України за планом науково-дослідних робіт ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України» та Інституту молекулярної біології і генетики НАН України.

Декларація з етики: автори задекларували відсутність конфлікту інтересів і фінансових забов'язань.

Стаття: надійшла до редакції 29.01.2025 р.; перероблена 19.02.2025 р.; прийнята до друку 05.03.2025 р.; надрукована 30.03.2025 р.