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Analysis of a family that carries the mutation c.1900T>C on codon 634 of the RET gene: the need to achieve standard management in medullary thyroid carcinoma

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ABSTRACT

INTRODUCTION: Hereditary medullary thyroid carcinoma (MTC) represents 25% of MTC cases, and genetic screening is recommended once an index case is identified. OBJECTIVES: To report the clinical condition of MTC patients in a family with multiple endocrine neoplasia type 2A and to describe the mutation of *RET* proto-oncogene and its penetrance. MATERIALS AND METHODS: Observational, cross-sectional study, with data from MTC patients' electronic records, between January 2013 and December 2020. Clinical and demographic data were collected, as well as information on management and treatments. A genetic evaluation was performed in the index case and family members to investigate mutations in the *RET* gene. RESULTS: Of the 15 members included, 11 developed MTC, of whom six were male. MTC was diagnosed in two individuals from the first generation at the ages of 62 and 58 years. The median age at diagnosis in the second and third generations were 30 and 13 years, respectively. Only one member of the third-generation underwent prophylactic thyroidectomy at eight years old following evidence of the c.1900T>C mutation on codon 634 of the *RET* gene. Other patients underwent surgical treatment before the *RET* gene sequencing due to its unavailability at the time. The penetrance in the family was 90%. CONCLUSION: This study demonstrated the limitations of the approach taken on a Brazilian family with suspected hereditary MTC due to the unavailability of sequencing for the *RET* gene. The c.1900T>C mutation of the *RET* gene was identified in the family and the penetrance was 90%.

1 INTRODUCTION

Precision medicine is defined by treatments that are tailored to the individual needs of patients, based on the genetics, biomarkers, phenotypes, and psychological characteristics. With more objective diagnoses, it has become possible to stratify patients and come up with more precise interventions over the course of an illness, which has occurred in familial forms of medullary thyroid carcinoma (MTC) [1].

MTC represents 5% of sporadically occurring thyroid cancers and the familial form accounts for around 20-25% of cases, with an autosomal dominant inheritance pattern[2-4]. According to the World Health Organization (WHO), an estimated 580,000 new cases of MTC occurred worldwide in 2020, with 30,000 cases in Brazil[5]. Research has shown that approximately 7-10% of patients diagnosed with sporadic MTC have a germline mutation in the *RET* proto-oncogene [6]. The familial form occurs as part of multiple endocrine neoplasia type 2 (MEN 2A in 95% of cases and MEN 2B in 5%)[7].

Studies have demonstrated that germline mutations of the *RET* proto-oncogene, located on the long arm of chromosome 10 at position 11.21 (10q11.21) (Figure 1), have been associated with MEN 2 disorders (MEN 2A and MEN 2B), as well as MTC, as previously mentioned[8-15]. MTC is typically caused by single nucleotide polymorphisms (SNPs) in the genetic sequence (point mutations). However, deletions and insertions may also occur[16,17].

MEN has two main variants with their own characteristics, MEN 2A and MEN 2B. MEN 2A has been shown to be associated with MTC (present in 95% of cases), pheochromocytoma (50%), hyperplasia/parathyroid adenoma (20%), and the presence of the mutation of *RET* (>98%)[4,13]. MEN 2B, meanwhile, is clinically associated with MTC (100%) and pheochromocytoma (75%) in individuals with Marfanoid habitus and diffuse intestinal ganglioneuromatosis[18]. It is considered the most aggressive form and has a mutation of the *RET* in over 95% of cases [4,14,18].

The mutations of the *RET* proto-oncogene have been classified into four risk levels by the American Thyroid Association (2009), related to the aggressiveness of MTC. The mutation of codon 634 is fitted into the C risk level, which involves a high risk of aggressive MTC. It is

recommended that such patients be referred for prophylactic total thyroidectomy at five years of age and that gene sequencing be used to monitor family members[17,19,20].

The mutation on codon 634 of the *RET* gene is associated with almost complete penetrance of MTC at early ages. Once it is identified in a family, genetic analysis should be offered to all first-degree relatives as soon as possible, a Grade A recommendation of the American Thyroid Association (ATA)[15,19]. The National Comprehensive Cancer Network (NCCN) and ATA recommend the total thyroidectomy of patients affected by this mutation at age five or as soon as it is identified, if the patient has more than five years old[19,20].

This article aims to report the clinical condition of MTC patients belonging to a single family with MEN 2A in the thyroid cancer outpatient clinic of the West Paraná Union to Study and Combat Cancer (UOPECCAN) in Cascavel, Paraná, Brazil, as well as to describe the mutation of the *RET* proto-oncogene and its penetrance in this family.

2 MATERIALS AND METHODS

This is an observational, cross-sectional study, with a qualitative and retrospective longitudinal approach analyzing data from the electronic medical records of patients affected by MTC. They were attended at the UOPECCAN between January 2013 and December 2020.

This research was submitted to and approved by the Committee for Ethics in Research on Human Subjects of the Assis Gurgacz University Center, Cascavel, by positive ruling No. 4,252,838, registered on the *Plataforma Brasil* under protocol No. 35771620.0.0000.5219. The ethics committee exempted this study from the requirement of a Free and Informed Consent Form since it consists of a review of electronic records, which were accessed under the Data Use Consent Form jointly with UOPECCAN, and the confidentiality of the information was maintained by the authors.

The index case of one of the families with MTC and their family members who were already being monitored by UOPECCAN were included in the study, as well as family members of the first,

second, and third degrees, who were not being monitored by the oncology department. Family members whose direct ancestor did not have a mutation were excluded from the analysis.

The data mining was carried out in the electronic medical records in the TASY system of the Ciro Antônio Kreuz Study and Research Center of UOPECCAN, where clinical and demographic data were collected, as well as information on treatments, stratification with imaging tests, anatomopathological data from thyroidectomy tissue and the progression of patients in the family that were treated in the institution.

The clinical and demographic data used consisted of age, sex, age when diagnosed, cytopathological result under the Bethesda System for the FNA of the nodule, presence of metastasis, occurrence of pheochromocytoma and hyperparathyroidism, genetic sequencing findings, and anatomopathological results.

During treatment, the levels of serum calcitonin (CT) from pre- and post-operative FNA were assessed. The laboratory tests were analyzed with respect to chemiluminescence, with normal values of CT of <8.4 pg/mL in men and <5 pg/mL in women.

The genetic testing of the family was carried out using Sanger sequencing with Big Dye Terminator in an ABI 3131XL Genetic Analyzer, with genetic data analyzed on the presence of mutations in the *RET* proto-oncogene in the eight exons tested (5, 8, 10, 11, 13, 14, 15, and 16). Due to the lack of an available sequence of the *RET* gene at the time of the diagnosis of the first generation of the family described in this manuscript, testing was only carried out after surgical treatment in those cases.

The data were grouped and analyzed using descriptive statistics, including the arithmetic mean, standard deviation, frequencies, and the mean, minimum, and maximum values.

3 RESULTS

We identified 17 members of a single family, distributed into three generations (Table 1). Individuals 3 and 4 did not have recorded clinical and demographic data because they died prior to

the beginning of the study, and it was not possible to access the information of their medical records since they were not attended at UOPECCAN. It was, therefore, possible to carry out genetic testing on 15 members of the family (Figure 2).

Excluding the two members of the first generation who were not evaluated (individuals 3 and 4), the age at MTC diagnosis was 62 years in the case of patient 1 and 58 in the case of patient 2. The median age of MTC diagnosis by histopathology of those affected in the second generation (individuals 6, 8, 9, and 11) was 30 years old, with a minimum age of 19 and a maximum of 34. For the third generation (individuals 12, 13, and 15), the diagnosis was made between 10 and 14 years of age, with a median of 13. All of the patients underwent surgical treatment for MTC before the sequencing of the *RET* gene due to sequencing being unavailable at the time of evaluation. The exception was patient 14, who underwent prophylactic thyroidectomy at the age of eight after a mutation was found on codon 634 of the *RET* gene.

Assuming that individual 3 also had MTC, 11 members of the family developed this neoplasia or had the potential for it to emerge, of whom six were male.

The average time during which patients were monitored at UOPECCAN was 3.9 years, though this figure varied between 10 months and six years. The initial screening of the family was carried out by measuring serum CT and ultrasound scans of the thyroid for six years until it was possible to gain access to genetic sequencing through a collaboration with an endocrinology research center. The genetic evaluation allowed targeted actions to be carried out on members 7 and 10 of the second generation, as well as on members 14, 16, and 17 of the third generation (Figure 2).

Table 1 shows the cytopathological findings using the Bethesda system for the members of the family who underwent thyroid nodule FNA. The procedure was carried out on nine patients who were being monitored in the institution, and the most frequent result was Bethesda category II (N=3), followed by Bethesda III (N=2), Bethesda V (N=2), Bethesda IV (N=1), and Bethesda I (N=1).

The index case (patient 1) was sent to UOPECCAN in 2013 at the age of 61 for assistance due to a papillary thyroid carcinoma diagnosed at age 30 in a different clinic. The condition had evolved with the emergence of the lymph nodes on the left and right sides of the cervical chain. FNA was

performed with inconclusive results. However, given that the ultrasound results indicated a suspected lymph node metastasis, the patient underwent a modified radical lymphadenectomy of the lateral cervical lymph node chains. Subsequent anatomopathological findings showed lymph node metastases of MTC.

Two months after MTC diagnosis in the index case, patient 6 sought attention at UOPECCAN after an anatomopathological diagnosis of MTC was made in another service. This gave rise to the hypothesis of hereditary MTC in the family, and the family members of the two patients were invited to undergo evaluation in the institution. Since the sequencing of the *RET* gene was not available at that time in our oncological service, the family members were monitored by measuring CT and carcinoembryonic antigen (CEA), in addition to FNA of the thyroid node when present.

Patient 11 arrived at the outpatient triage of the institution in 2015 due to nodules on both sides of the thyroid and did not communicate his family history of MTC to the medical team that assisted him. The FNA of the nodules resulted in two lesions with cytopathology of Bethesda II and one Bethesda IV lesion on the left lobe of the thyroid. He, therefore, underwent total thyroidectomy and lymphadenectomy along the left recurrent nerve chain, the pathological anatomy of which indicated the presence of MTC without lymph node metastases.

Given the family history of MTC, family member 5, who had a thyroid nodule with a Bethesda II cytopathological finding, in addition to normal serum CEA and CT, expressed the wish to undergo thyroidectomy due to genetic sequencing being unavailable at the time. The anatomopathological result was negative for malignancy.

Patient 13, aged 13, had a nodule measuring 0.5 cm, classified as Bethesda category II using FNA and a serum CT of 8 pg/mL (normal < 5 pg/mL). The examination was repeated one year later, with the result once again Bethesda II. However, the amount of CT in the FNA needle wash-out fluid was over 5,000 pg/mL, compared to a serum CT of 100 pg/mL. The patient underwent thyroidectomy after the presence of pheochromocytoma or hyperparathyroidism was ruled out, and the anatomopathological finding documented the presence of MTC.

Ultrasound monitoring and serum CT and CEA measurements were initiated on patient 14 at the age of six, which were in the normal range. This patient underwent *RET* gene sequencing 18 months later, which showed a mutation on codon 634. As a result, she underwent total prophylactic thyroidectomy, and the anatomopathological findings showed a histologically normal thyroid gland.

Genetic sequencing of the family, except for patient 14, was performed in a retrospective manner. It was only possible to access this exam due to the agreement in place with the Molecular and Translational Endocrinology Laboratory (MTEL) of the Federal University of São Paulo (UNIFESP). In total, 15 members of the family were tested (members 3 and 4 could not be assessed since they were already deceased), with 10 family members (1, 2, 6, 8, 9, 11, 12, 13, 14, and 15) exhibiting the presence of the mutation c.1900 T>C on the *RET* gene (Figure 2). The age at which genetic testing was carried out ranged from eight to 66 years, with a median age of 34 years. The penetrance of the mutation on codon 634 was 90% in the family since it was possible to prevent the disease from emerging in one family member by performing prophylactic thyroidectomy.

To date, five of the 10 patients with the mutation on the *RET* gene developed pheochromocytoma, and primary hyperparathyroidism was observed in two individuals. No other pathologies associated with MEN 2A, such as cutaneous lichen amyloidosis or Hirschsprung's disease, were observed.

4 DISCUSSION

MTC is a rare and aggressive malignant neoplasm of the thyroid. The prevalence of hereditary MTC does not vary by sex since it is a dominant autosomal disease. This is reflected in the family described in this article, in which 54.5% of the patients affected by hereditary MTC, or with the potential for MTC to develop, were male[21]. With that said, it is possible to infer that patient 3 had the potential for MTC phenotypes to emerge, given that the mutation of the *RET* gene was observed in two of her three children. It was not possible to analyze patient 4 because his clinical and demographic data were missing, as well as his death before this study began and the lack of any offspring, which made a retrospective genetic analysis impossible.

The consensus recommendation of the ATA is that once an index case is identified where the individual is a carrier of the mutation on codon 634 of the *RET* gene, the family members should undergo genetic assessment immediately or, in the case of infants, once they reach the age of 3-5. Children with the mutation on codon 634 should begin monitoring with an ultrasound scan and serum CT beginning at age three, and prophylactic thyroidectomy should be carried out no later

than age five since the serum CT levels help to inform the extent and timing of surgery[7]. In this study, the index case was diagnosed with MTC in his sixth decade of life, while patient 2 was diagnosed at the age of 58. The descendants had a median age at anatomopathological diagnosis of 30 in the second generation and 11.5 in the third. The median age at which genetic sequencing was carried out was 35 in the second generation and 13 in the third. The members of the first generation only underwent sequencing in their sixties.

In this cohort, three patients in the third generation did not have the opportunity to undergo genetic sequencing before receiving surgical treatment, which allowed confirmation of MTC after anatomopathological analysis. Only three patients of the third generation, who were being monitored through the measurement of serum CT and thyroid ultrasounds, were able to have their treatment directed by the genetic sequencing results 18 months after the beginning of outpatient assistance. Among these patients, two were released from monitoring because they did not have the mutation on the *RET* gene, while one patient underwent prophylactic thyroidectomy after the mutation c.1900 T>C on the *RET* gene was confirmed. The diagnosis of the third generation, both pathological and genetic, was later than recommended by the ATA consensus. This pattern is seen elsewhere in Brazil, as reported in the BrasMEN study, which found that the median age at MTC diagnosis in patients with the mutation on codon 634 was 28 years[22].

The treatments described highlight the importance of carrying out genetic sequencing once the index case is identified, as the monitoring of family members with ultrasound scans and serum CT measurements are not effective at identifying MTC at an early stage. In the cohort examined in this study, only 28.5% (N=2/7) of the MTC patients had a positive diagnosis of the carcinoma through FNA prior to the operation. This may have occurred due to the known difficulty in characterizing the cytopathology of MTC, in addition to the technical difficulty of puncturing a micronodule, which can result in the needle puncturing adjacent, histologically normal thyroid tissue[23].

Although sequencing is well-established in the literature, its availability is limited in Brazilian oncology centers, meaning that our institution was unable to carry out suitable triage from the beginning, leading to non-assertive approaches. This was the case for patient 5: out of fear of having the same pathology as her family members, she opted to undergo thyroidectomy despite testing negative for MTC prior to the operation. Later, she was found not to have the mutation on the *RET* gene.

Another improper situation occurred in a patient from the same family, who exhibited bilateral thyroid nodules with a cytopathological finding of Bethesda IV. This individual underwent total thyroidectomy, with histopathology showing MTC. However, the removal of the lymph nodes along the recurrent nerve chain did not occur during surgery since he did not indicate the family history of the illness. MTC is known to be the "only thyroid cancer where a total thyroidectomy and prophylactic central neck dissection is routinely recommended" as it is a highly aggressive disease[19,24]. In addition to this, the patient was observed to have pheochromocytoma, which may have caused peri-operational instability in the total thyroidectomy procedure. This highlights the importance of genetic sequencing and the active search for family members once a mutation has been identified in the index case since this can prevent improper surgical approaches and enable the monitoring of neoplastic diseases that co-occur with MTC, as recommended in the literature[7, 19, 25-27].

This study identified the c.1900 T>C mutation on the *RET* proto-oncogene in members of the same family, with 90% penetrance of MTC, 50% of pheochromocytoma, and 20% of hyperparathyroidism. These frequencies are similar to those identified in studies in the 1990s and early 2000s, in which phenotypic expressions of hereditary MTC were observed in 95% of cases, pheochromocytoma in 50%, and hyperparathyroidism in 20%[13,28]. It is important to note that the studies cited above were carried out before or shortly after the beginning of genetic testing to monitor thyroid carcinomas, which demonstrates the delay in these technologies reaching Brazilian oncology centers. At an early genetic diagnosis, the patient can undergo surgery prior to the emergence of MTC, which prevents the phenotypical expression of the disease, consistent with the recommendations of the most recent guidelines in the area[7,26].

The cancer management guidelines of the Brazilian Unified Health System (SUS) do not even mention protocols for the management of MTC patients[29]. This makes the need for innovation clear, as well as the need to bring Brazilian management protocols in line with international recommendations, which would result in the creation or designation of a national center for genetic analysis to provide large-scale assistance. The testing of the family described in this study, late as it was, was only possible due to the involvement of scientists and a partnership between the research center of UOPECCAN and the MTEL (UNIFESP). Other laboratories like MTEL do exist in Brazil, which are run by specialized teams and help take a more assertive approach to genetic diseases. However, there is a need for a task force to make the required investments and protocols that would

allow genetic sequencing to be available throughout the country. This project is of significant value in managing diseases like hereditary MTC, where genetic sequencing allows for decisive changes in management during the natural history of the disease, which can improve the assertiveness of the relevant surgical procedures, as well as saving costs for the transportation, medical consultations, laboratory exams, and imaging for family members who do not have the mutation[7].

Currently, the cost of genetic testing is considered high, which may be the main reason why it is not performed by the SUS. However, in the long term and at scale, the cost of preventive medicine becomes minuscule due to the improvements it brings about in the flow of patients who use the healthcare system while also eliminating the stigma associated with the disease, increasing patients' quality of life. As noted in a study by Iriart (2019), medicine "is going through a process of 'molecularization,' with some areas, such as oncology, undergoing profound transformations thanks to the incorporation of new knowledge and technology." Such progress in medicine, mainly in the oncology field, is extremely important in helping to manage oncological and genetic diseases[30].

One of the limitations of this study was the small sample size, leading to conclusions based on a limited group of patients. Even so, it was possible to demonstrate the significant challenges these patients faced in decision-making when seeking greater diagnostic accuracy prior to a surgical operation, as well as the non-assertive approach taken in the absence of genetic sequencing, demonstrating that genetic testing is essential when there is a suspicion of MTC. Another limitation was the absence of clinical and demographic data of some patients belonging to the family due to their death prior to the start of this study, which impeded the accurate description of their phenotype and made it impossible to carry out genetic sequencing. However, it was possible to presume the disease by a retrospective analysis of their offspring.

The authors of this article suggest that other oncology centers provide reports on difficulties they have experienced in the field of precision medicine, with the purpose of fostering a discussion on the importance of genetic sequencing in determining an approach and demonstrating the reality of the access to such technologies within the Brazilian public network, which currently does not make them available. This situation goes against a basic principle of the SUS, namely universal access.

5 CONCLUSIONS

This study shows the difficulty of limitations of clinical observation in a Brazilian family with suspected hereditary MTC due to the unavailability of sequencing for the *RET* gene, which demonstrates the importance of the latter in clinical and surgical approaches for familial forms of MTC. The c.1900T>C mutation of the *RET* gene was confirmed, phenotypically characterized by MEN 2A, which had a penetrance of 90% in the family described.

6 ACKNOWLEDGMENTS

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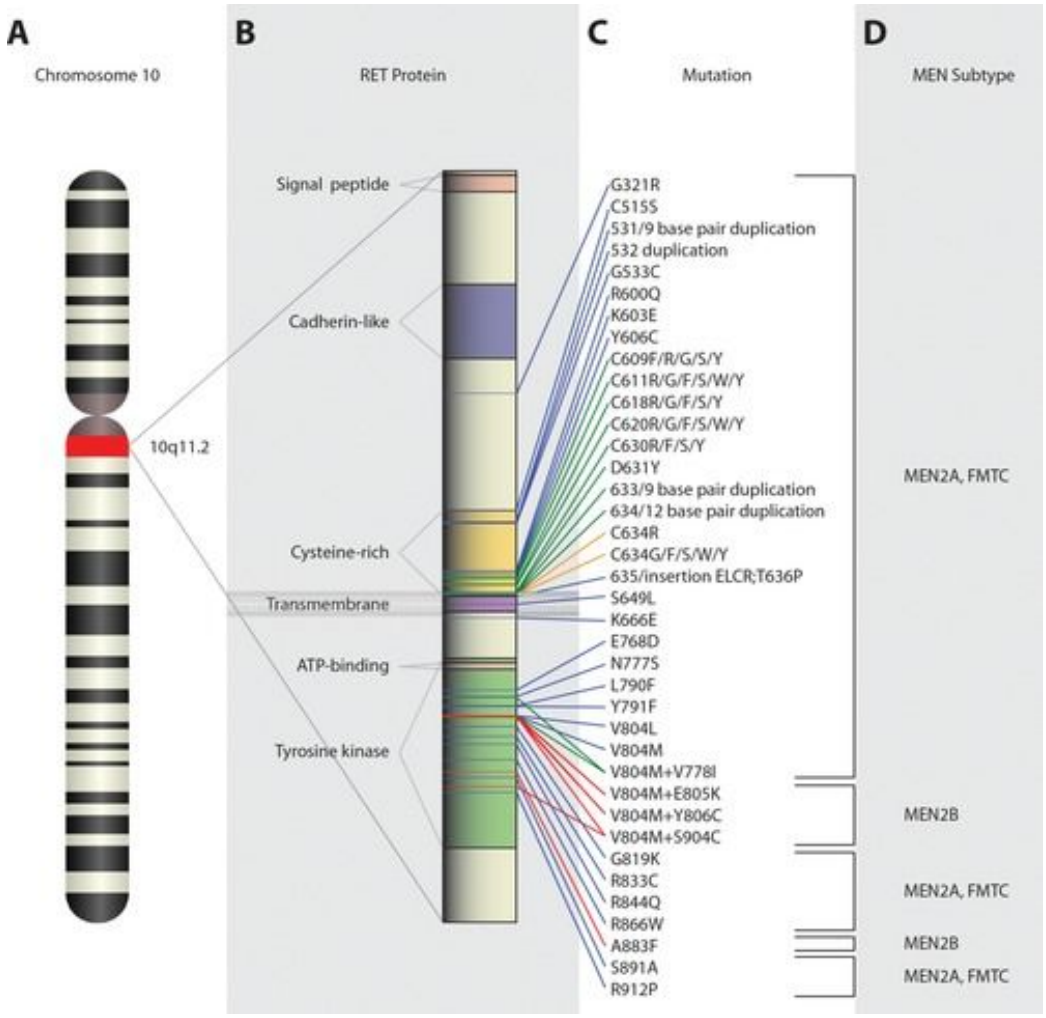
7 CONFLICTS OF INTEREST

The researchers declare that they do not have any conflict of interest related to this article.

FIGURE 1: The chromosomal position of the RET proto-oncogene that causes MEN2



(A) The RET proto-oncogene is comprised of 21 exons located on chromosome 10 (10q11.2) and decodes a transmembrane tyrosine kinase receptor. (B) The protein RET is composed of three functional domains, including a domain that links to the extracellular ligand, an extracellular domain, and a cytoplasmic tyrosine kinase domain. The extracellular domain contains a signaling peptide that is cleaved, four cadherin repeats, and one cysteine-rich region, which is critical for the formation of the disulfide bond necessary for dimerization. (C) The known loci of RET mutations are highlighted in accordance with the risk classification of the American Thyroid Association (blue = classification A, green = classification B, orange = classification C, red = classification D). (D) The mutations are grouped by the MEN subtype they cause.
Source: Adapted from Krampitz and Norton, 2014.[11]

Table 1: Clinical and demographic data

Pt	S	A.D	A.S.T.	FNA	N.S.	Staging	Metastasis	PHEO	HYPERPARA	Genetic test	Age at genetic test	Testing prior to diagnosis?
1	M	62	61	---	---	pTxN1bM1	BONES, LIVER, AND LUNG	YES	NO	POSIT.	66	NO
2	M	58	58	III	0.6 to 1.5 cm	pT1bN0Mx	NO	YES	NO	POSIT.	59	NO
5	F	**	35	II II	0.8 cm	histologically normal	NO	NO	NO	NEGAT.	37	**
6	F	28	33	---	1.2 to 4.8 cm	pT3N0Mx	NO	NO	NO	POSIT.	37	NO
7	M	**	**	NP	**	**	**	NO	NO	NEGAT.	35	**
8	F	32	32	III	1 to 1.7 cm	pT1bN0Mx	NO	NO	YES	POSIT.	34	NO
9	M	34	36	V	1.8 to 2.6 cm	pT2N0Mx	NO	YES	NO	POSIT.	39	NO
10	F	**	31	NP	**	**	**	NO	NO	NEGAT.	32	**
11	M	19	19	IV	0.7 to 1 cm	pT1aN0Mx	NO	YES	YES	POSIT.	23	NO
12	M	10	10	II	0.4 cm	pT1aN0Mx	NO	NO	NO	POSIT.	13	NO
13	F	14	12	II	0.5 cm	pT1aN0Mx	NO	YES	NO	POSIT.	14	NO
14	F	8	6	NP	**	histologically normal	NO	NO	NO	POSIT.	8	YES
15	M	13	12	II V	0.4 to 0.5 cm	pT1aN0Mx	NO	NO	NO	POSIT.	16	NO
16	F	**	11	I	0.3 to 0.4 cm *	**	**	NO	NO	NEGAT.	13	**
17	F	**	**	NP	**	**	**	NO	NO	NEGAT.	9	**

Table 1. Pt = Patient/ S = Sex/ A.D. = age at diagnosis / A.S.T. = Age at start of treatment at UOPECCAN / FNA = cytopathological findings under the Bethesda system from nodules on which fine needle aspiration was performed / N.S. = Maximum and minimum nodule size or size of sole nodule from histopathology (thyroidectomy was not performed on patient 16, though they did have nodules of under 0.4 cm on the ultrasound/ Imaging = Imaging per the TNM classification / Metastases = Topography of metastases / PHEO = occurrence of pheochromocytoma / HYPERPARA = occurrence of hyperthyroidism / Genetic test = Result of genetic test / POSIT. = Positive / NEGAT. = Negative / Age at genetic test = age at which genetic test results received / Testing prior to diagnosis? = whether genetic sequencing was performed prior to anatomopathological diagnosis / --- = no data / NP = Not performed / ** = Parameter does not apply to the patient.*
Source: the authors (2021).

Figure 2: Family tree

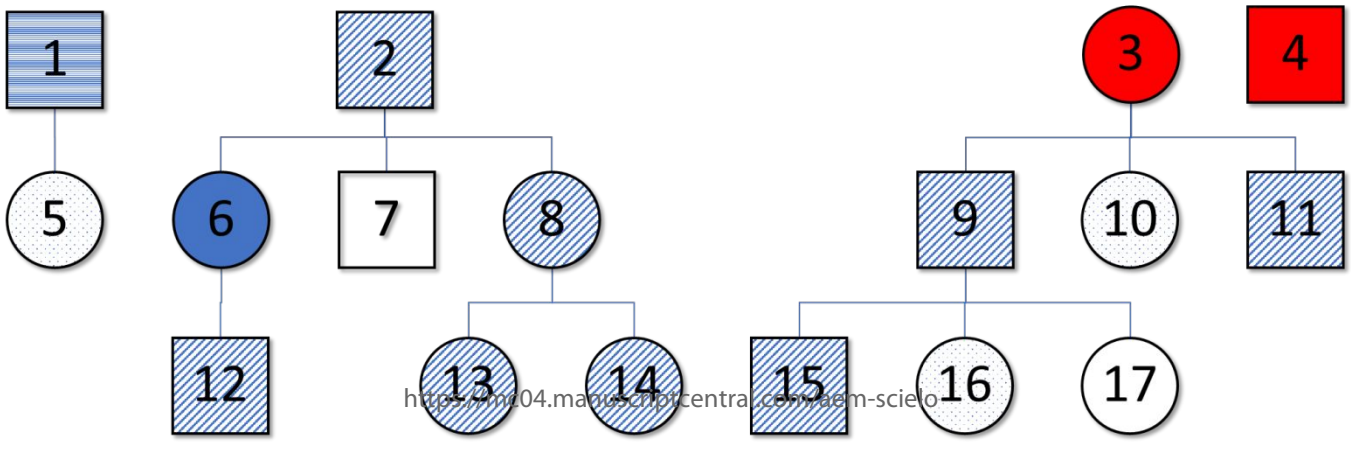


Figure 1: Family tree. Square: male. Circle: female. Red fill: patient deceased prior to the start of data collection. Blue fill: Mutation of the RET proto-oncogene confirmed through genetic sequencing, confirmation of MTC via anatomopathological analysis, and patient of the institution. Blue horizontal hatching: all the conditions of blue fill + index case. Blue diagonal hatching: patients who underwent clinical evaluation at the invitation of the institution + mutation of RET proto-oncogene confirmed by genetic sequencing. White fill: patients with wild type RET proto-oncogene confirmed by genetic sequencing, no occurrence of MTC, and no clinical evaluation performed. Blue dots: patients with wild type RET proto-oncogene confirmed by genetic sequencing, no occurrence of MTC, and who did undergo clinical evaluation.
Source: the authors (2021)

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