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Case Report

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A Case of MEN2A Associated with a C634R De-novo Mutation of the RET Gene at Algiers

Ammar Chikouche[1,2*], Nadia Ould Bessi[1,3], Nawal Habak[1,3] and Mebarek Boudissa[1,4]

[1]*Department of medicine, Faculty of Medical Sciences, Algiers University 1, Algeria*

[2]*Laboratory of Biochemistry, Pierre and Marie Curie Center, Algiers, Algeria*

[3]*Department of pharmacy, Faculty of Medical Sciences, Algiers University 1, Algeria*

[4]*Department of Endocrinology, Pierre and Marie Curie Center, Algiers, Algeria*

[*]**Corresponding author:** Ammar Chikouche, Department of medicine, Faculty of Medical Sciences, Algiers University 1, Algeria, **Tel:** 00213555696816; **Email:** chikouchea@gmail.com

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Abstract

Introduction

Multiple endocrine neoplasia type 2A (MEN2A) is characterized by the association of medullary thyroid carcinoma (MTC) with pheochromocytoma and hyperparathyroidism. MEN2A represents 60% of MEN2 which is also subdivided into MEN2B (5%) and medullary thyroid cancer of the family (FMTC) which represents 35%. MEN2A is a rare hereditary disease, with autosomal dominant inheritance. Specific mutations of the RET protooncogene are associated with this disease.

Case Presentation and methods

The index case was an 18-year-old female patient with medullary thyroid cancer, without other clinical abnormalities. A blood sample taken from an EDTA tube was sent to the molecular biology laboratory accompanied by a request for genotypic analysis of the RET gene. A family pedigree was established and a consent form for DNA analysis was signed by the index case, his parents and his sister. DNA extraction was performed by the salt method. The genetic study concerned exon 11. It was carried out by amplification of the exon by polymerase chain reaction (PCR), followed by sequencing on ABI 3130 Applied Biosystems®.

After finding the mutation in the index case; this mutation has been searched in relatives, including father, mother and sister.

Results and Discussion

The index case carried a mutation in exon 11 of the RET protooncogene. This mutation causes the replacement of cysteine at codon 634 with an arginine (C634R). The apparently healthy father; mother and only sister did not carry the mutation. C634R mutation has been described and found specifically in cases of MEN2A. This is why; the search for a pheochromocytoma should be carried out as well as a biological and medical monitoring. Only the clinically affected patient is a carrier of the RET gene mutation. Neither the father, the mother, nor the sister are carriers of it. This means that this C634R mutation is de novo.

Conclusion

Our work found a de novo mutation specific to MEN2A, which allows us to reassure parents and to provide a clinical and biological care and genetic counseling for the index case. The genetic study will be done only for his children.

Keywords: MEN2A; RET; De novo mutation; C634R

Introduction

Medullary thyroid cancer (MTC) is a rare cancer that develops at the expense of parafollicular C cells of thyroid. There are two forms, a sporadic form in the majority of cases and a familial form which is integrated into multiple endocrine neoplasia type 2 (MEN2) [1].

MEN 2 is an autosomal dominant syndrome of MTC [**2**], which is divided into three clinical subtypes: MEN2A, MEN2B and familial medullary thyroid cancer (FMTC) [3-6].

MEN2A is characterized by the presence of MTC (in 100% of cases), associated with a pheochromocytoma (found in 50-60% of cases) and hyperplasia of the parathyroid glands (present in 20 -30% of cases).

In MEN2B, there is a MTC associated with a pheochromocytoma, a marfanoid syndrome and neurogangliomatosis of the intestinal tract, but there is no involvement of the parathyroid gland.

In FMTC, medullary thyroid carcinoma is the only clinical manifestation of the disease [7].

In addition, MTC is sporadic in 75% of cases and it is familiar in 25% of cases [8].

Each of these syndromes shows specific mutations of the RET protooncogene [9-12].

The RET protooncogene is located on chromosome 10q11.2 [**13**] .It codes for a membrane tyrosine kinase receptor (RET receptor) expressed in cells derived from the neural crest [14].

This RET receptor has an extracellular domain, a trans-membrane domain and an intracellular domain. It binds a neurotrophic factor called GDNF (glial derived neurotrophic factor), itself bound to a receptor (GDNF-R) which is attached to the cytoplasmic membrane by a glycosylphosphatidyl-inositol intermediate. These three components form a complex that leads to the transmission of mitogenic signals [15-17].

Most RET mutations found in MEN 2A (95%) were found in exons 10 and 11, which encodes the extracellular domain of the receptor.

These are missense mutations that affect one of the codons corresponding to a cysteine residue positioned in the cysteine-rich domain in NH2 terminal extremity region [9,10,12,18,19].

FMTC patients carry mutations at the same codons resulting in substitutions with different amino acids. They may also be carrying mutations in codons in other exons of the RET gene.

MEN 2B is almost exclusively associated with mutation at codon 918 in exon 16 [9-12,18,19] and an A883F mutation in exon 15 [20].

It has been suggested that the cysteine residues of the extracellular domain of the wild RET receptor form intramolecular disulfide bridges. The pathogenic mechanism implies that in the MEN2A, the unpaired cysteine residues of the mutant RET receptor form intermolecular disulfide bridges. Switching from one cysteine to another amino acid will automatically cause a cysteine to be unpaired. This will cause the unpaired cysteine of a RET monomer to automatically pair with a cysteine opposite another RET monomer. These conformational changes followed by dimerization of the receptor will lead to activation of the Tyrosine Kinase domain [21]. This constitutive RET activation dimerization is independent of the ligand [2,22]. All MEN 2A cases reported to date have the same mechanism of activation [2].

The discovery of a mutation of the RET protooncogene in a MTC index case by molecular biology techniques confirms the diagnosis of familial form and allows the genetic screening of clinically healthy relatives; Those who carry The genetic abnormality, before any biological or clinical manifestation, will be offered a prophylactic thyroidectomy. Clinical and biological monitoring of the index case and relatives carrying the mutation will also be proposed.

We describe here a case of MEN 2A caused by a mutation of the RET gene: a transition C to T at position 634, this causes the substitution of a cysteine into an arginine in exon 11. Interestingly, the mutation is a de novo mutation.

Case Report and methods

Case Index

An 18-year-old woman admitted at the endocrinology department at the Pierre and Marie Curie Center in Algiers, for the exploration of a right lobe thyroid nodule evolving for two years. The diagnosis of MTC was made on the results of Fine needle aspiration cytology in favor of a malignancy and the biological examinations (Preoperative basal serum Thyrocalcitonin = 981.19 pg/ml, Preoperative CEA: 85.88 ng/ml). The patient had a total thyroidectomy with cervical lymph node dissection. The anathomopathological study revealed a histology and immunohistochemistry in favor of a MTC. The Postoperative Thyrocalcitonin, performed three months later decreased to 13.10 pg/ml. The investigation for hyperparathyroidism was negative (normal phospho-calcium balance, normal PTH) and that of pheochromocytoma also (negative methoxylated derivatives).

A genetic investigation was then launched. A pedigree has been traced (Figure 1). She comes from a family composed of a sister aged 22 years; a father aged 63 and a mother aged 58 years old. No family history of MTC was found.

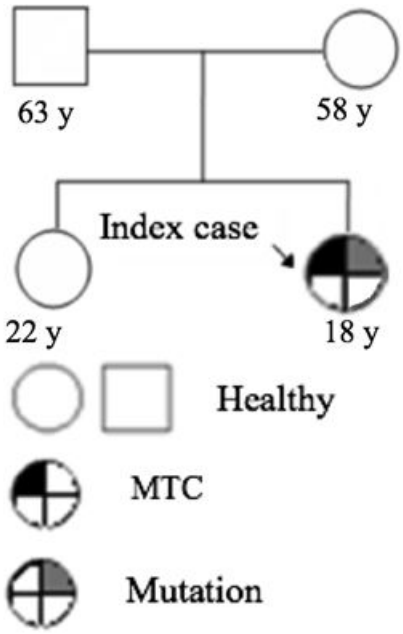

Figure 1: Pedigree of family

DNA extraction, PCR and mutation detection

The sampling was carried out on the index case, after informed consent, on two EDTA tubes. The genomic DNA was extracted from white blood cells according to the salt method. Exon 8, 10, 11, 13, 14, 15 and 16 had to be studied. The frequency argument led to look for the mutation first in the 10 and 11. We began with exon 11. By finding the mutation, the research was not carried out in the other exons.

It was performed by amplification of the exon by PCR, followed by direct sequencing using Sanger method in an automated Sequencer (ABI 3130 Applied Biosystems®)

The oligonucleotide primers used to amplify the exon 11 of the RET gene were designed on the flanking intronic sequences and is:

11F 5'-3 ': CAGAGCATACGCAGCCTGTA

11R 5'-3 ': ACACAGCGCCCTATGGAAAT

PCR amplification were carried out in 25 µl reaction containing 4 µl of DNA (25 ng / µI), 2.5 µI of 10 × PCR buffer; 1.25 µI of dNTPs (2 mM), 1.5 µI of $MgCl_2$ (25 mM), 1.25 µI of dNTP (2 mM), 1.25 µl of sense and antisense primer solution (5 µmol/µl), 0.1 Ml Taq polymerase Roche (5U / µI) and 13.15 µI H_2O. Fragments were amplified as follow: An initial denaturation step of 5 minutes at 94°C, followed by 35 cycles of amplification (comprising one minute denaturation at 94°C, one minute annealing at 60°C (for exon 11) and elongation of 1 minute at 72°C) followed by a final elongation step of 10 minutes at 72°C. The PCR amplification product was tested on a 2% agarose gel and the strips visualized by staining with ethidium bromide.

The PCR products were purified on Millipore plates (Manu 30) and the sequence PCR reaction was carried out with 1.5 µl of purified PCR

product; 0.8 µl of Big Dye terminator V1.1 (Applied Biosystem), 3.6 µl of 5X buffer (V1.1) and 2 µl of sense or antisense primer (5 pmoI / µl).

The amplification was programmed with 25 cycles including a step of 30 seconds at 95°C, followed by a step for 4 minute at 60°C.

After purification by G50 gel filtration, the sequence PCR products were separated by capillary electrophoresis using the Sanger method in an ABI Prism 3130 automated sequencer (Applied Biosystem Division).

The presence of the mutation was detected by comparison with the reference sequence.

Results and Discussion

The analysis of exon 11 revealed a transition from a thymine to a cytosine at c.1900T> C (Figure 2). This replacement of T by a C leads to the substitution of a cysteine by an arginine at codon 634 at exon 11.

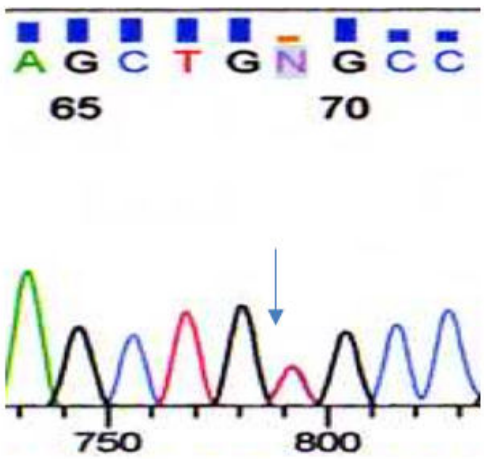

Figure 2: Heterozygous mutation C634R (TGC / CGC) in Exon 11

The 18-year-old patient carried a heterozygous mutation; Moreover, this patient also carried, at the same exon a heterozygous polymorphism (SNP), G691S (GGT/AGT), which causes a glycine to be changed to a serine at codon 691 with a sequence variation c. 2071G> A, and a transition from G to A.

The genotypic analysis of the relatives showed no mutation in parents and sister (Table 1).

Patient	Age (years)	Mutation	Polymorphism
Index case	18	C634R	Heterozygous G691S
Father	63	No mutation	/
Mother	58	No mutation	Homozygous G691S
Sister	23	No mutation	Heterozygous G691S

Table 1: Laboratory finding for the Family genotypic analysis results.

In the father's, no mutation or sequence variation (no polymorphism) was found.

In the mother's, the mutation C634R was not found but the polymorphism G691S (GGT / AGT) was found in homozygous form. In the sister, the mutation C634R was not found but the polymorphism G691S (GGT/AGT) was also found in heterozygous form.

Discussion

We reported the identification of a case of medullary thyroid cancer associated with a mutation of the RET gene. It is a missense mutation found at codon 634 (TGC/CGC) at exon 11, encoding a cysteine of the extracellular part of the RET protein, called Cys634Arg or C634R [**9**, **23**].

This C634R mutation is specific to conventional MEN2A cases [9,23].

Indeed, this mutation has never been found in the FMTC, in the literature. This result established the diagnosis of MEN2A [24,25]. This mutation, which was found in the index case but was not found in the relatives, is considered to be a de novo mutation, and has also been reported by Tessitore et al [26], Schuffenecke et al[27,29].

Conclusion

We have described a specific mutation of MEN2A, C634R, at exon 11 of the RET gene, in an 18-years-old woman with an isolated CMT. Genetic screening in relatives, the father, mother and sister does not find this mutation, they can be reassured. This mutation is de novo and research in other relatives is not indicated except for the children of this patient. This patient should be regularly monitored for the possible appearance of a pheochromocytoma and/or a hyperparathyroidism.

The authors declare no conflicts of interest.

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