Genetic Analysis of Medullary Thyroid Cancer in Algiers, Algeria

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ABSTRACT

Medullary thyroid cancer or MTC occurs sporadically (75% of cases) and familial form (25% of cases) which, in the latter situation, is part of Multiple Endocrine Neoplasia type 2 (MEN2). The MEN2 are subdivided into MEN2A, MEN2B and FMTC or isolated family MTC. MEN2 are rare inherited conditions, transmitted in the autosomal dominant mode, linked to mutations in the RET gene. We report the results of the genotypic study carried out at the CPMC in Algiers, in 209 index cases with medullary thyroid cancer or MEN2. 24.40 % of index cases are classified as MEN2, 47 of which carry germline mutations in the RET gene. Mutations in the seven (07) different exons of the RET gene have been found.

Keywords: *Multiple Endocrine Neoplasia Type 2, Medullary Thyroid Carcinoma, FMTC, Protooncogene RET, Mutation*

Introduction

Medullary thyroid cancer (MTC) is a C-cell tumor with calcitonin secretion (CT). MTC, which may be preceded by hyperplasia of thyroid C cells (Franc and Modigliani, 1998), has very early metastatic spread which can occur in the microcarcinoma stage. There are two ways of arriving at a diagnosis of MTC before the procedure: fine needle aspiration (Kini *et al*., 1984) and above all CT dosage whose levels exceed 10pg/ml (Karges *et al*., 2004; Mirallié *et al*., 2004; Niccoli *et al*., 1997).

It occurs sporadically in 75% and in familial form in 25% of cases. These familial forms constitute Multiple Endocrine Neoplasia type 2 or (MEN2). These diseases are rare hereditary, with autosomal dominant transmission (Santoro *et al*. 1995), and almost complete penetrance. They are

characterized by the constant presence of medullary thyroid cancer (MTC) associated or not with tumors of adrenal medullas and parathyroids. These different forms are:

FMTC or familial isolated thyroid medullary cancer (Farndon *et al*., 1986) where MTC is the only clinical manifestation without any other associated endocrine tumor represents 35% of MEN2.

MEN 2A or Sipple syndrome is the most frequent form (60% of MEN2) involves medullary thyroid cancer often associated with pheochromocytoma and / or hyperparathyroidism (Sipple, 1961).

MEN 2B or Gorlin syndrome, the rarest form (5%) which combines MTC, a pheochromocytoma, developmental abnormalities and mucous neurons (Gorlin *et al*., 1968).

The predisposition gene for different forms of MEN2 (Hansford and Mulligan, 2000; van Heyningen, 1994; Hofstra *et al*., 1994) is the RET (rearranged during transfection) proto-oncogene, identified by Takahashi in 1985 (Takahashi *et al*., 1985) and located on chromosome 10 (10q11.2) by Ishizaka in 1989 (Ishizaka *et al*., 1989). It consists of 21 exons (Kwok *et al*., 1993; Myers *et al*., 1995) which codes for a transmembrane receptor with tyrosine kinase activity (Takahashi and Cooper, 1987).

This membrane receptor is made up of an N-terminal extracellular region with a cysteine-rich domain (CRD), a transmembrane region and a C-terminal intracellular region which contain two domains with tyrosine kinase activity (Arighi *et al*., 2005).

Activation of the RET receptor proceeds as follows: a homodimeric ligand of the GFL family (GDNF Family Ligand) whether it is GDNF or (Glial cell line-Derived Neurotrophic Factor), Neurturine, Artemin or Persephine binds to a specific GFRα receptor (GDNF family receptors-α1 to 4). The ligand-coreceptor bond in dimeric form results in the dimerization of the RET receptor and its activation (Airaksinen and Saarma, 2002).

Germline mutations of this gene have been identified in 95% of FMTC (Hansford and Mulligan, 2000). The mutations found in the FMTC cause a permanent activation of the receptor.

These are point mutations or duplications, which sit in order of frequency at the level of exons 10, 11, 13, 14, 15, 8 and 16 (the 7 most frequently mutated exons) and cause a change of codon type false sense which affect specific coding regions of RET. These mutations lead to the substitution of an amino acid for another. Some mutations are specific to FMTC and others are common to MEN2A and FMTC (Hansford and Mulligan, 2000; GTE, 2006). The identification of these mutations by molecular biology techniques confirms the diagnosis of MEN2.

Objectives of Our Study

Research in the patients diagnosed with medullary thyroid cancer, using molecular biology techniques, a mutation in exons of the RET gene which confirms the diagnosis of MEN2.

Materials and Methods

We received blood samples from 209 patients diagnosed with MTC (medullary thyroid cancer) isolated or MEN2.174 were isolated MTC, 32 were MEN2A and 03 were MEN2B. The whole blood samples taken on a tube with EDTA were sent to us by different endocrinology services in the Algiers region. A request for genotypic analysis was sent to us accompanied by a letter of consent and a file in which the diagnosis is made, a clinical summary and a report of the biological exploration and imaging.

Genomic DNA was extracted from leukocytes in peripheral blood by the salt technique. The genetic study was carried out by PCR amplification followed by purification on a millipore plate, then a sequence PCR followed by purification by gel filtration and sequencing. A decision algorithm was decided according to the order of the most frequently mutated exon.

If the index case is a MTC or MEN2A, we start with the analysis of exons 11, 10 and 13 and if the mutation is not found, the analysis of exons 14, 15, 8 and the 16 will be performed. If this is a case of MEN2B we move on to the study of exons 16, if there is nothing, we study the exon 15.

Results and Discussion

The study focused on the 209 index cases of isolated MTC or MEN2. We found that among the 174 cases of isolated MTC, 158 had no mutation and 16 were carriers of the RET gene mutation in heterozygous form. Among the 32 cases of MEN2A diagnosed, 28 were carriers of the RET gene mutation in heterozygous form and 04 were not carriers of deleterious mutations of the RET gene.

The 3 MEN 2B cases were all carriers of the same mutation specific to MEN2B. According to the diagnosis on arrival (Table 1)

Table 1: Distribution of patients.

Pathology	Number
MTC	174
MEN2A	32
MEN2B	3
Total	209

Distribution of the different forms of MEN2 compared to all MTCs according to the genotypic study of all Index cases (Table 2).

Table 2: Presence or no of mutation of RET gene and distribution of MTC sporadic and MEN2.

Pathology		Mutation of RET	Number	Frequency	
MTCs		No	158	75.59%	
MEN2	FMTC	Yes	16	24.40%	7.65%
	MEN2A	Yes	32		15.32%
	MEN2B	Yes	3		1.43%
Total			209		

According to our data, our patients (209) are divided into 158 sporadic MTCs and 51 family MTCs or MEN2 (which represents 24.40%). Distribution of the different forms of MEN2 (Table 3)

Table 3: Distribution of MEN2.

Pathology	Number	Frequency	Literature frequency
FMTC	16	31.37%	35%
MEN2A	32	62.74%	60%
MEN2B	3	5.88%	5%
Total MEN2	51		

These MEN2 are divided into 03 MEN2B (5.88%), 16 FMTC (31.37%) and 32 MEN2A (62.74%). These percentages are almost equivalent to those in the literature (Table 3). The nature of the mutations found in all patients MTCs and MEN2 are (Table 4).

Table 4: Nature of mutation and frequency in specific MEN2.

Pathology	Sequence variation	Number	% of mutation in the specific MEN2
MTCs	No mutation		-
	Mut htz C618Y (tgc / tac)	1	6,25
	Muthtz C634F (tgc / ttc)	1	6,25
	Mut htz C634Y (tgc / tac)	6	37,5
FMTC	Muthtz G768D (gag / gat)	3	18,75
	Muthtz G768D (gag / gac)	1	6,25
	Muthtz L790F (ttg / ttt)	1	6,25
	Muthtz V804M (gtg / atg)	1	6,25
	Muthtz S891A (tcg / gcg)	2	12,5
	Muthtz C634R (tgc / cgc)	15	46,87
	Mut htz C634Y (tgc / tac)	11	34,37
MEN2A	Muthtz C620R (tcg / cgc)	1	3,125
	Muthtz C634G (tgc / ggc)	1	3,125
	No classic mutations	4	12,5
MEN2B	M918T	3	100

Among the 174 MTC, 16 were carriers of germline mutations, C634Y in 6 cases (37,5%), E768D in 03 cases was found as GAG / GAT form (18,75%) and in 01 cases in GAG / GAC form (6,25%), S891A in 02 cases (12,5%), C618Y in 01 case, C634F in 01 case, L790F in 01 case, and V804M in 01 case (6,25%).

Among the 32 MEN2A patients, 28 carried mutations known and described in the literature, C634R in 15 cases (46,87%), C634Y in 11 cases (34,37%), C620R in 01 case and C634G in 1 case (3,125%) and 04 cases were not carriers of germline mutation. And one mutation M918T, was found in the 3 MEN2B patients, this described mutation is specific for MEN2B.

Then 47 genetic mutations of the RET gene were found in our series. These germline mutations are found in heterozygous form.

12 different mutations have been found. These mutations are known and described in the literature.

Among these 12 mutations (Fig. 1):

02 are specific for MEN2A, C634R (TCG / CGC) and C634G (TCG / GGC).

01 is specific to MEN2B, M918T (ATG / ACG).

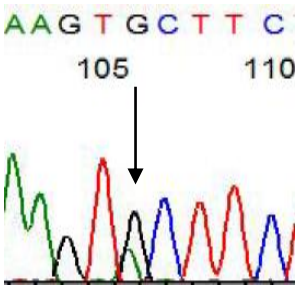
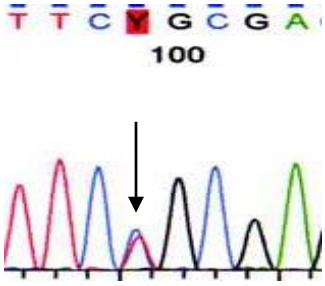
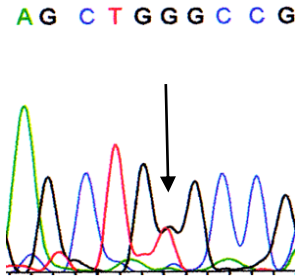
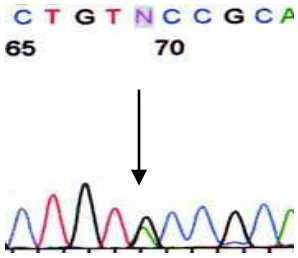
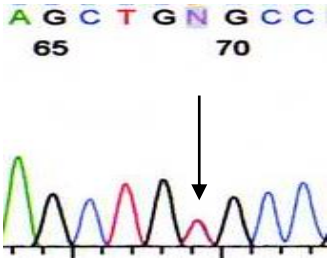
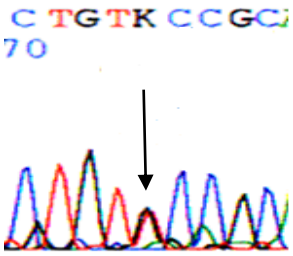
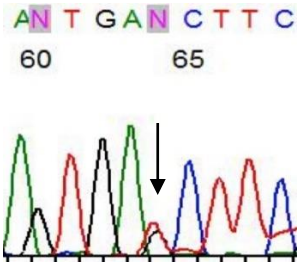
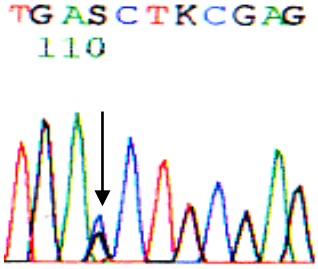
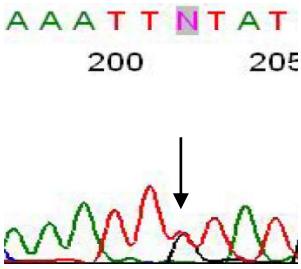
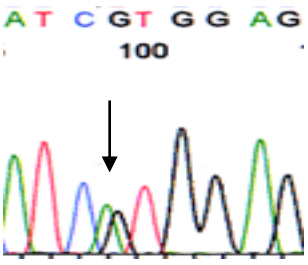
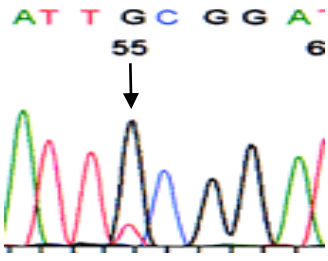
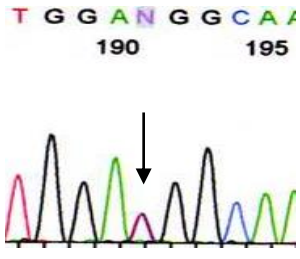
04 are specific to FMTC, E768D (GAG / GAC and GAG / GAT), V804M (GTG / ATG) and S891A (TCG / GCG).

05 are common to MEN2A and FMTC, C618Y (TGC / TAC), C620R (TCG / CGC), C634F (TGC / TTC), C634Y (TGC / TAC) and L790F (TTG / TTT).

Conclusion

The different mutations found in our series of Algerian populations are known and listed in the literature. The mutations most frequently found in MEN2A are the C634R in 46, 87% and the C634Y in 34, 37% of cases. In cases of MTC Familial, the predominant mutations are C634Y at 37,5%. The M918T mutation found in the 03 cases of MEN2B, is the most frequently found in the literature. The search for the family mutation in relatives of an index case of MEN2 is necessary for the early management of cases carrying this mutation, since prophylactic thyroidectomy represents the only effective therapeutic measure in the treatment of MTC.

Figure 1

		
Exon 10: C618Y (TGC/TAC)	Exon 10: C620R (TGC/CGC)	Exon 11: C634G (TGC/GGC)
		
Exon 11: C634Y (TGC/TAC)	Exon 11: C634R (TGC/CGC)	Exon 11: C634F (TGC/TTC)
		
Exon 13: E768D (GAG/GAT)	Exon 13: E768D (GAG/GAC)	Exon 13: L790F (TTG/TTT)
		
Exon 14: V804M (GTG/ATG)	Exon 15: S891A (TCG/GCG	Exon 16: M918T (ATG/ACG)

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