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Research Article

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Two Sequences Variations of the E768D Mutation Found in Familial Medullary Thyroid Carcinomas

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Abstract

Medullary thyroid cancer or MTC is present in sporadic form (75% of cases) and in familial form (25% of cases), in this latter situation, the MTC is a part of Multiple Endocrine Neoplasia type 2 (MEN 2). The MEN2 is divided into MEN2A, MEN2B and FMTC or isolated familial MTC. The MEN2 are rare hereditary disease, transmitted as an autosomal dominant mode, linked to mutations of the RET gene.

The discovery of a mutation in the RET proto-oncogene by molecular biology techniques in a index case of MTC confirms the diagnosis of familial and allows the genetic testing of healthy clinically related index case: those who carry the genetic mutation, will be offered prophylactic thyroidectomy before any biological or clinical manifestation.

The genetic analysis of the RET gene was performed by PCR / sequencing. The E768D mutation was found in the exon 13 of the RET gene in 2 differences sequences forms (GAG/GAC et GAG/GAT).
This mutation, already described, found in the FMTC.

Keywords: Multiple Endocrine Neoplasia Type 2, Medullary Thyroid Carcinoma, FMTC, Proto-oncogene RET, Mutation, Genetic Screening

Introduction

Medullary thyroid carcinoma or MTC is a C-cell tumor with secretion of calcitonin (CT).

MTC, which may be preceded by C-cell hyperplasia of the thyroid gland, has a very early metastatic spread that can be at the micro carcinoma stage [1].

There are two ways to reach the diagnosis of MTC before surgery: fine-needle aspiration and above all, CT [2-5]. It occurs sporadically in 75% and in a familial form in 25% of cases. These familial forms constitute Multiple Endocrine Neoplasia type 2 or MEN2. These are rare hereditary diseases, with autosomal dominant inheritance, almost complete penetrance and are characterized by the constant presence of medullary thyroid carcinoma (MTC) associated or not with the involvement of the adrenal and parathyroid medulla [6]. Behave FMTC, or familial isolated medullary thyroid carcinoma where MTC is the only clinical manifestation without other associated endocrine tumors, represents 35% of the MEN2 [7]. MEN2A or Sipple syndrome is the most common form (60% of MEN2) with medullary thyroid cancer often associated with pheochromocytoma and / or hyperparathyroidism [8]. MEN2B or Gorlin syndrome, the rarest form (5%) associated with MTC, pheochromocytoma, developmental abnormalities and mucosal neurons [9].

The predisposition gene for different forms of MEN2 is the proto-oncogene RET (rearranged during transfection), identified by Takahashi in 1985 and located on chromosome 10 (10q11.2) by Ishizaka in 1989 [10-14]. It consists of 21 exons which codes for a transmembrane receptor with tyrosine kinase activity [15-17].

This membrane receptor is composed of an N-terminal extracellular region with a cysteine-rich domain (CRD), a transmembrane region and a C-terminal intracellular region that contain two domains with tyrosine kinase activity [18].

The activation of the RET receptor proceeds in the following manner: a homodimeric ligand of the GFL family (GDNF Family Ligand) whether it be GDNF (Glial cell line-Derived Neurotrophic Factor), Neurturin, Artemin or Persephin linked to a specific GFRα receptor (GDNF family receptors-α1 to 4). The ligand-coreceptor binding in dimeric form causes the dimerization of the receptor RET and its activation. Germline mutations of this gene have been identified in 95% of FMTCs [10, 19]. The mutations found in the FMTC result in permanent activation of the receptor. These are point mutations or duplications, which sit in order of frequency at exons 10, 11, 13, 14, 15, 8 and 16 (the 7 most frequently mutated exons) and lead to a change of codon type false sense which affect specific coding regions of RET. These mutations result in the substitution of one amino acid for another.

Some mutations are FMTC specific and others are common to MEN2A and FMTC [10, 20].

The identification of these mutations by molecular biology techniques confirms the diagnosis of MEN2.

Molecular screening has taken an important place in family forms in search of relatives carrying the family mutation.

Materials and Methods

Blood samples from a male adult patient and a patient with medullary thyroid cancer without further clinical manifestations. Whole blood samples are taken on tube with EDTA.A request for a genotypic analysis was sent to us accompanied by a letter of consent and a sheet which gives the diagnosis, a clinical summary and a report of the biological exploration and imaging Subsequently samples of 11 relatives were sent to us (Table I).

DNA extraction Genomic DNA was extracted from peripheral blood leukocytes by the salt technique.

A decision algorithm was decided according to the order of the most frequently mutated exon. We start with the analysis of exons 10, 11 and 13 and if the mutation is not found, the analysis of exons14, 15, 8 and 16 will be performed.PCR conditions. The amplification of exon 13 of the RET proto-oncogene was performed using the following primers provided by Applied Bio Systems.

Exon 13F; 5' TGACCTGGTATGGTCATGGA 3'
Exon 13R; 5' GGAGAACAGGGCTGTATGGA 3'

The PCR conditions used are 4 µl of DNA (25 ng / µl), 2.5 µl µl of 10X PCR buffer; 1.25 µl of dNTPs (2 mM, 1.5 µl 25 mM MgCl 2, 1.25 µl of 2 mM dNTPs, 1.25 µl of 5 pmol / µl sense and antisense primers), 0.1 µl Taq polymerase Roche (5U / µI) and 13.15 µI of H2O.

The amplification is programmed as follows; an initial denaturation step of 5 minutes at 94° C., then 35 amplification cycles (comprising denaturation of one minute at 94° C., hybridization of one minute at 60° C. and elongation of one minute at 72° C.) followed by a final elongation step of 10 minutes at 72° C.

The amplicons are tested by electrophoresis in 2% agarose gel with Roche Size VII marker. The size of amplicon 13 is 250pb. Sequence PCR.The PCR products are purified on Millipore (Manu 30) plates and the sequence PCR reaction is carried out with 1.5 µl of purified PCR product; 0.8 µl of Big Dye terminator V1.1 (Applied Bio system); 3.6 µl of 5X buffer (V1.1) and 2 µl of sense or antisense primer (5 pmoI / µl).

The amplification is programmed with 25 cycles comprising a step of 30 seconds at 95° C. followed by a step of 4 minutes at 60° C. The sequence PCR products are purified to remove dNTPs, unincorporated free dideoxynucleotides and excess primers by a method based on the use of Sephadex G 50 gel on MultiScreen MAHV N45 plates (Millipore).Purified PCR sequence products are passed to the Applied Bio System 3130 Sequencer which performs capillary electrophoresis using POP 7 gel (Applied Bio systems).

Results and Discussion

The genotypic analysis of the two index cases with isolated medullary cancer found a mutation in the heterozygous state, due to a base substitution, at codon 768 of exon 13 of proto-oncogene RET. In the first index case, male, aged 50, the mutation found is due to a G> C transversion of c.2304 nucleotide and the GAG codon will give the GAC codon (Figure 1).

Whereas in the 40-year-old female index case, the mutation found is due to a G> T transversion of c.2304 nucleotide and therefore the GAG codon will give the GAT codon (Figure 2).

The GAG codon corresponds to glutamic acid represented by the letter E whereas the GAC and GAT codons correspond to the aspartic acid represented by the letter D. So in both cases, there is a change of glutamic acid at position 768 to aspartic acid. This germline mutation noted E768D was found in the FMTC [21]. It is considered specific FMTC where it is found in 8% [11].

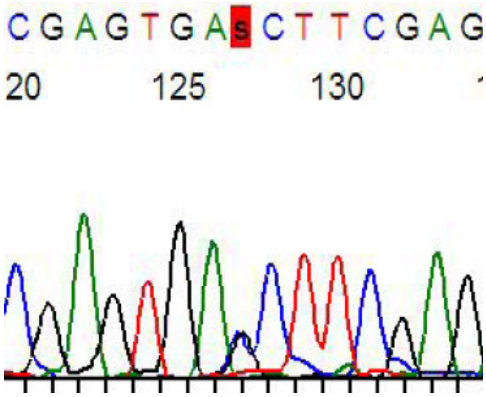

Figure 1: Chromatogram of the E768D mutation (GAG / GAC) found in exon 13.

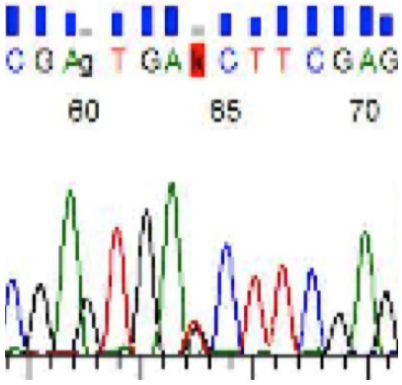

Figure 2: Chromatogram of the E768D mutation (GAG / GAT) found in exon 13.

Table I: List of related relatives

Family Number	Status	Sex	Age	Mutation
1	Index case: Father	M	50 years	Mut htz G768D (gag/gac)
	Child	F	20 years	Mut htz G768D (gag/gac)
	Child	M	18 years	RAS
	Child	M	17 years	RAS
	Child	M	15 years	Mut htz G768D (gag/gac)
	Child	M	13 years	RAS
	Child	F	12 years	RAS
	Child	F	07 years	RAS
2	Index case: Female	F	40 years	Mut htz G768D (gag/gat)
	Mother	F	69 years	Mut htz G768D (gag/gat)
	Sister	F	46 years	RAS
	Sister	F	39 years	RAS
	Sister	F	33 years	Mut htz G768D (gag/gat)

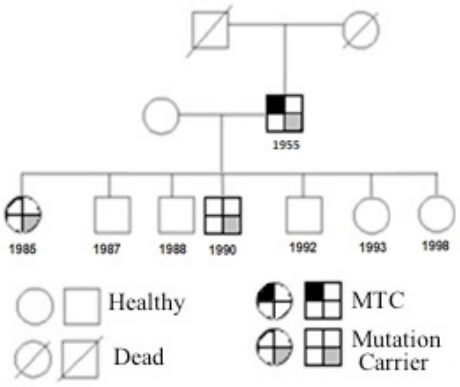

Figure 3: family tree of the family No. 1 studied.

After the mutation in the first index case was found, a study of the family was performed, 07 apparently healthy children benefited from genotypic analysis. 02 out of 07 children carry the E768D mutation (Figure 3).

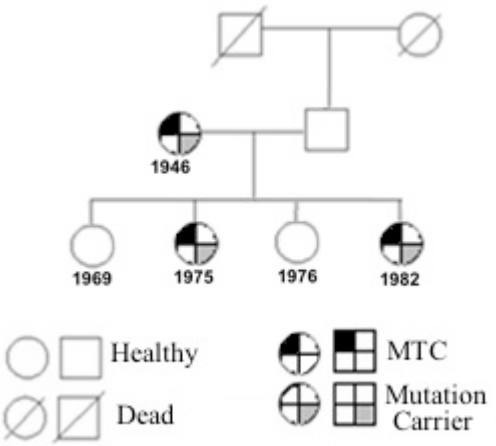

Figure 4: family tree of the N ° 2 family studied.

After the mutation in the 2nd index case was found, a family study was performed, the affected mother and sister and 2 apparently healthy sisters benefited from the genotypic analysis. 02 of the 04 are carriers of the E768D mutation (Figure 4).

Conclusion

Two index cases, which have isolated medullary thyroid carcinoma, carry a mutation in exon 13 of the RET gene, the E768D but in the form of two sequence variations. This mutation was detected using molecular biology techniques (PCR / sequencing). This discovery led to the diagnosis of familial MTC.

This mutation, already described, found only in the FMTC, exempts the carriers of a surveillance of the parathyroid glands and medulla-adrenal glands.11 relatives of both families benefited from genotypic analysis (Table I).02 children among the siblings of 07 children of the 1st family (Figure 3) are carriers of the family mutation and have benefited from a prophylactic thyroidectomy. Among the 04 relatives of the second family (Figure 4), the mother and a sister are affected and carry the mutationOther relatives who do not carry the mutation are considered to be free and thus are not supervised.

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Conflict of Interest

No conflict of interest related to the article exists.

References

1. Franc, B., & Modigliani, E. (1998). Le carcinome médullaire de la thyroïde: évolution des concepts. Archives d'anatomie et de cytology pathologiques, 46(1-2), 100-111.
2. Kini, S. R., Miller, J. M., Hamburger, J. I., & Smith, M. J. (1984). Cytopathology features of medullary carcinoma of the thyroid. Archives of pathology & laboratory medicine, 108(2), 156-159.
3. Karges, W., Dralle, H., Raue, F., Mann, K., Reiners, C., Grussendorf, M., & Brabant, G. (2004). Calcitonin measurement to detect medullary thyroid carcinoma in nodular goiter: German evidence-based consensus recommendation. Experimental and clinical endocrinology & diabetes, 112(01), 52-58.
4. Mirallié, E., Iacobone, M., Sebag, F., & Henry, J. F. (2004). Results of surgical treatment of sporadic medullary thyroid carcinoma following routine measurement of serum calcitonin. European Journal of Surgical Oncology (EJSO), 30(7), 790-795.
5. Iacobone, M., Niccoli-Sire, P., Sebag, F., De Micco, C., & Henry, J. F. (2002). Can sporadic medullary thyroid carcinoma be biochemically predicted? Prospective analysis of 66 operated patients with elevated serum calcitonin levels. World journal of surgery, 26(8), 886-890.
6. Santoro, M., Carlomagno, F., Romano, A., Bottaro, D. P., Dathan, N. A., Grieco, M., & Di Fiore, P. P. (1995). Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. Science, 267(5196), 381-383.
7. Farndon, J. R., Light, G. S., Dilley, W. G., Baylin, S. B., Small ridge, R. C., Harrison, T. S., & Wells Jr, S. A. (1986). Familial medullary thyroid carcinoma without associated endocrinopathies: a distinct clinical entity. Journal of British Surgery, 73(4), 278-281.
8. Sipple, J. H. (1961). The association of pheochromocytoma with carcinoma of the thyroid gland. The American journal of medicine, 31(1), 163-166.
9. Gorlin, R. J., Sedano, H. O., Vickers, R. A., & Červenka, J. (1968). Multiple mucosal neuromas, pheochromocytoma and medullary carcinoma of the thyroid—a syndrome. Cancer, 22(2), 293-299.
10. Hansford, J. R., & Mulligan, L. M. (2000). Multiple endocrine neoplasia type 2 and RET: from neoplasia to neurogenesis. Journal of Medical Genetics, 37(11), 817-827.
11. Van Heyningen, V. (1994). Genetics. One gene--four syndromes. Nature, 367(6461), 319-320.
12. Hofstra, R. M., Landsvater, R. M., Ceccherini, I., Stulp, R. P., Stelwagen, T., Luo, Y., & Buys, C. H. (1994). A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature, 367(6461), 375-376.
13. Takahashi, M., Ritz, J., & Cooper, G. M. (1985). Activation of a novel human transforming gene, ret, by DNA rearrange-

ment. Cell, 42(2), 581-588.
14. Ishizaka, Y., Itoh, F., Tahira, T., Ikeda, I., Sugimura, T., Tucker, J., & Nagao, M. (1989). Human ret proto-oncogene mapped to chromosome 10q11. 2. Oncogene, 4(12), 1519-1521.
15. Hu, I. (1993). Structural analysis of the human ret proto-oncogene using exon trapping. Oncogene, 8, 2575-2582.
16. Myers, S. M., Eng, C., Ponder, B. A., & Mulligan, L. M. (1995). Characterization of RET proto-oncogene 3'splicing variants and polyadenylation sites: a novel C-terminus for RET. Oncogene, 11(10), 2039-2045.
17. Takahashi, M. A. S. A. H. I. D. E., & Cooper, G. M. (1987). Ret transforming gene encodes a fusion protein homologous to tyrosine kinases. Molecular and cellular biology, 7(4), 1378-1385.
18. Takahashi, M. A. S. A. H. I. D. E., & Cooper, G. M. (1987). Ret transforming gene encodes a fusion protein homologous to tyrosine kinases. Molecular and cellular biology, 7(4), 1378-1385.
19. Airaksinen, M. S., & Saarma, M. (2002). The GDNF family: signaling, biological functions and therapeutic value. Nature Reviews Neuroscience, 3(5), 383-394.
20. CHIKOUCHE, A., BESSI, N. O., HABAK, N., BOUDISSA, M., SEMROUNI, M., MESKINE, D., ... & GRIENE, L. du cancer médullaire de la thyroïde à Alger.
21. Eng, C., Smith, D. P., Mulligan, L. M., Healey, C. S., Zvelebil, M. J., Stonehouse, T. J., & Ponder, B. A. (1995). A novel point mutation in the tyrosine kinase domain of the RET proto-oncogene in sporadic medullary thyroid carcinoma and in a family with FMTC. Oncogene, 10(3), 509-513.