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Deep Insight Section

RET point mutations in Thyroid Carcinoma

Rossella Elisei, Cristina Romei, Barbara Cosci, Eleonora Molinaro, Laura Agate, Valeria Bottici, Aldo Pinchera

Department of Endocrinology and Metabolism University of Pisa, Pisa, Italy (RE, CR, BC, EM, LA, VB, AP)

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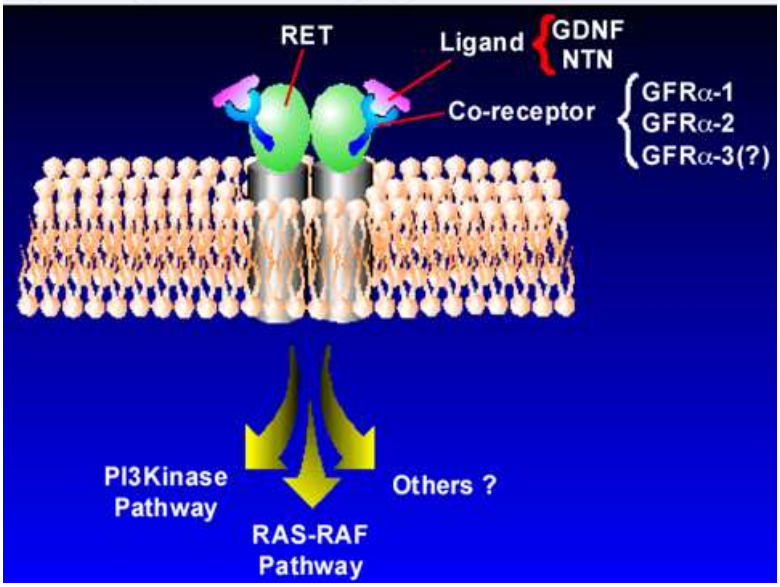
RET structure and function

The human RET gene maps on 10q11.2 and is composed by 21 exons with an estimated size of about 55 kb (Pasini et al., 1995). The RET gene encodes for a tyrosine kinase transmembrane receptor (Takahashi et al., 1988) characterized by 3 different domains: a) the extracellular domain, which contains the signal peptide, the cadherin-like region and the cysteine-rich region; b) the transmembrane domain; c) the intracellular portion containing the tyrosine kinase domain. RET is expressed in a variety of neuronal cell lineages including thyroid C cells and adrenal medulla. Although still debated, recently it has been reported that RET gene expression may also occur in follicular thyroid cells (Fludge et al., 2001). The physiological ligands of RET belong to the glial derived neurotrophic factors (GDNFs) family.

Four members of this family, neurturin, persephin, artemin and GDNF, have a specific trophic effect on RET (Robertson and Mason, 1997). The activation of RET is mediated by the interaction of the ligands with 4 co-receptors, GFRα1, 2, 3 and 4 (Jing et al., 1996; Treanor et al., 1996; Trupp et al., 1996; Klein et al., 1997; Buj-Bello et al., 1997; Milbrandt et al., 1998; Baloh et al., 1998). RET dimerization is the natural consequence of the formation of the ligand-coreceptor-receptor complex and is responsible for the activation of the kinase catalytic domain and of the signal transduction which induces cells proliferation through a complex network of second messengers (Marshall, 1995).

Fig 1: RET tyrosine kinase receptor protein activation

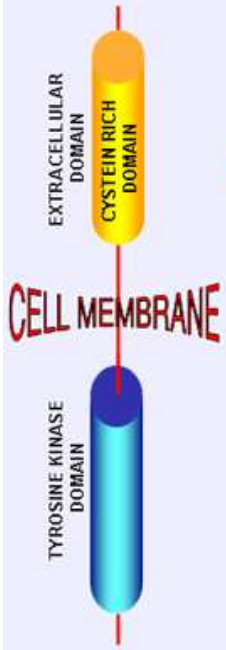


The oncogenic transformation of RET gene determines a constitutive activation of the receptor itself with the consequent induction of an uncontrolled cell proliferation and tumoral development (Fig. 1).
The oncogenic activation of RET in medullary thyroid carcinoma (MTC) occurs mainly through the substitutiton of a single nucleotide. The nature of the RET mutations determines the molecular mechanisms of its oncogenic activation. RET mutations involving the cystein reach region promote RET homodimerization via the formation of intermolecular disulfide bounds (Marshall, 1995; Santoro et al., 1995). Mutations affecting the region of RET coding for the intracellular catalytic portion appears to modify the substrate specificity of the tyrosine kinase activity leading to the constitutive activation of the receptor (Santoro et al., 1995; Songyang et al., 1995).

RET mutations in Multiple Endocrine Neoplasia type 2 (MEN 2)

MEN 2 is an autosomal dominant inherited disease characterized by the presence of MTC plus other endocrine tumors: pheochromocytoma (PHEO) and/or parathyroid adenomas (hyperPTH) in MEN 2A, PHEO and mucosal neurinomas in MEN 2B. MTC alone, lacking the association with other endocrine tumors, may also be inherited (FMTC) (Keiser et al., 1973; Cunliffe et al., 1970; Farndon et al. 1986). In these syndromes, MTC develops in nearly 100% of the affected individuals, while PHEO and hyperPTH develop in around 50% and 20% respectively.
In 1993 two independent groups discovered that germline point mutations of the RET proto-oncogene are causative events in MEN 2A (Mulligan et al., 1993) and in FMTC (Donis-Keller et al., 1993). One year later, also MEN 2B was associated with germline point mutations of the RET proto-oncogene (Eng et al., 1994; Carlson et al., 1994; Hofstra et al., 1994). Since then, a large number of publications have addressed the relationship between RET mutations and the clinical phenotype of MEN 2 patients and the clinical implication of screening MEN 2 family members for RET gene mutations. The most comprehensive study correlating the genotype to the phenotype of MEN 2 patients was published in 1996 by the International RET Mutation Consortium (Eng et al., 1996), which reported the results of a large cooperative survey including 477 pedigrees screened for the presence of germline RET proto-oncogene mutations. Germline RET point mutations were found in 92% of the whole group, including 95% of 79 families with MEN 2B, 98.0% of 203 families with MEN 2A and 88% of 34 families with FMTC. A specific mutation in exon 16, at codon 918 (ATG to ACG) was invariably associated with MEN 2B. In MEN 2A, several different cystein codons in exon 10 and 11 were affected, but codon 634 mutations were by far the most common, accounting for 85% of the cases. This mutation (mainly TGC to CGC) was also found to correlate significantly with the presence of PHEO and hyperPTH. In FMTC, the mutations were almost evenly distributed among the 5 cysteine codons 609, 611, 618, 620 and 634. Interestingly, mutations in the cystein rich domain (codons 609, 611, 618 and 620) are not only found in families with MEN 2A/FMTC but also in patients with Hirschprung's disease, a congenital malformation characterized by an absence of enteric galglia cells in the distal part of the colon, or patients having a combination of MEN 2 and Hirschprung's disease (Arighi et al., 2004; Takahashi et al., 1999; Sijmons et al., 1998).
The number and type of RET mutations have been grown over the last 10 years, especially after the introduction of RET genetic screening in the work up of all patients with MTC, both hereditary and apparently sporadic. As consequence of this more careful research, RET mutations have been found to be widely distributed not only among the 5 cysteine codons but also in other non-cysteine codons, such as codon 804 in exon 14, codon 883 and codon 891 in exon 15 and others (Jimenez et al., 2004; Niccoli-Sire et al., 2001). These widely spread mutations are mainly associated with FMTC phenotype (Niccoli-Sire et al., 2001; Elisei et al., 2007). Large series of MTC hereditary cases have shown a significant correspondence between specific RET mutations and both phenotype and the level of aggressiveness of the MTC tumor (Elisei et al., 2007; Machens et al., 2003; Machens et al., 2007; Frank-Raue et al., 2008; Machens et al., 2003) (Fig 2).
In particular M918T has been recognized as the most aggressive one, as demonstrated by the evidence that the majority of patients with this germline mutation (ie MEN 2B) usually die at young age while, at variance, mutations like Y791F (Frank-Raue et al., 2008) and A883T (Elisei et al., 2004) could never develop into MTC. The correlation between the type of mutation and the aggressiveness of the MTC has been confirmed also by several in vitro studies showing different degree of tumoral transforming activity (Carlomagno et al., 1997; Chappuis-Flament et al., 1998; Mise et al., 2006).
In about 4-10% of MEN 2A or FMTC patients and in about 95% of those with MEN 2B the germline RET mutation is a "de novo" mutation as demonstrated by the negative finding of the RET genetic analysis in the patients' parents. In these cases the mutation is usually located in the allele inherited from the patient's father (Schuffenecker et al., 1997).

Fig. 2 Genotype-phenotype correlations according to known germline mutations



MUTATED CODONS	EXONS	PHENOTYPE
533	8	FMTC
603	10	FMTC
609		FMTC/MEN 2A
611		FMTC/MEN 2A
618		FMTC/MEN 2A
620		FMTC/MEN 2A
630	11	FMTC
632/633/634		MEN 2A
634		MEN 2A
640		MEN 2A
641		MEN 2A
648		MEN 2A
768	13	FMTC
781		FMTC
790/791		FMTC
804	14	FMTC
844		FMTC
883	15	FMTC/MEN 2B
891		FMTC
904		FMTC/MEN 2B
912	16	MEN 2B
918		MEN 2B
922		MEN 2B

Clinical implication of RET genetic screening

The clinical implications of RET mutations in MEN 2 is the possibility to screen family members to find those who harbour the same germline mutation previously detected in a MTC index case. This allows the identification of the "gene carriers" when they are clinically unaffected or at an early stage of the disease, and to exclude "non gene carriers" from further testing for the rest of their life. Several series have confirmed the effectiveness of this approach. Gene carriers have been detected with a frequency ranging from 15.5% and 69.0% (Elisei et al., 2007; Pacini et al., 1995; Lips et al., 1994; Frilling et al., 1995; Frank-Raue et al., 1997; Skinner et al., 2005), in almost every decade of life, but more frequently at very young age.

Once a gene carrier is found, he/she must undergo clinical and biochemical examination to assess the presence or absence of clinical or pre-clinical disease. This includes screening of MTC by neck palpation, neck ultrasound and measurement of basal and pentagastrin-stimulated serum calcitonin, screening for hyperPTH by measurement of serum calcium and parathyroid hormone, and screening for PHEO by measurement of plasma or urinary cathecolamines. Since MTC is usually the first clinical manifestation of the disease, it is very rare to find no evidence of MTC in the presence of hyperPTH or/and PHEO. Nevertheless, screening for these conditions is mandatory to avoid the risk of surgical procedures in patients with undiagnosed PHEO. Whenever clinical or biochemical (elevated basal and/or pentagastrin-stimulated serum calcitonin) evidences of MTC are found, surgery is the treatment of choice. In this situation, the surgical procedure should be the same applied to any patient presenting with clinical MTC, i.e. total thyroidectomy and lymph node dissection. When a gene carrier is detected before the onset of clinical and biochemical manifestations, the decision of performing a prophylactic total thyroidectomy must be weighted against the possible morbidity of this procedure.

On the other hand, it is well known that permanent cure of MTC is only achieved when surgery is performed at an early stage, when the tumor is intrathyroidal. This has been demonstrated in old studies using biochemical screening programs (Wolfe et al., 1973) and more recently in several series of subjects operated on the basis of positive genetic screening (Lips et al., 1994; Pacini et al., 1994; Dralle et al., 1998). C-cell hyperplasia, a pre-malignant lesion, has been found by Gagel et al. (Gagel et al., 1995) in 4 children operated on the basis of a germline RET mutation, one of whom had microscopic MTC in addition. Out of 7 patients (7-28 years of age) treated by Learoyd et al (Learoyd et al., 1997) 3 had MTC and 4 had C-cell hyperplasia. In a series by Wells et al. (Wells et al., 1994), 13 of 21 family members carrying RET gene mutation were treated by surgery, including 6 with normal plasma calcitonin. Their age ranged from 6-20 years. All had C-cell hyperplasia with or without MTC. No patient had lymph node metastases and the post-operative stimulated plasma calcitonin was normal in all.

The practical recommendation that can be derived from the above considerations is that family members at-risk of hereditary MTC should be screened by genetic analysis as early as possible, to distinguish those with

or without RET mutation. The last can be reassured on their status and relieved from further follow-up. Those with the mutation should be considered for total thyroidectomy. The timing of surgery may change according to the results of the clinical and biochemical screening. In the presence of clinical or biochemical evidence of MTC, surgery will immediately be performed. If no evidence of disease is found, it is possible to go on with a prophylactic thyroidectomy, or to delay surgery while monitoring the calcitonin response to pentagastrin.

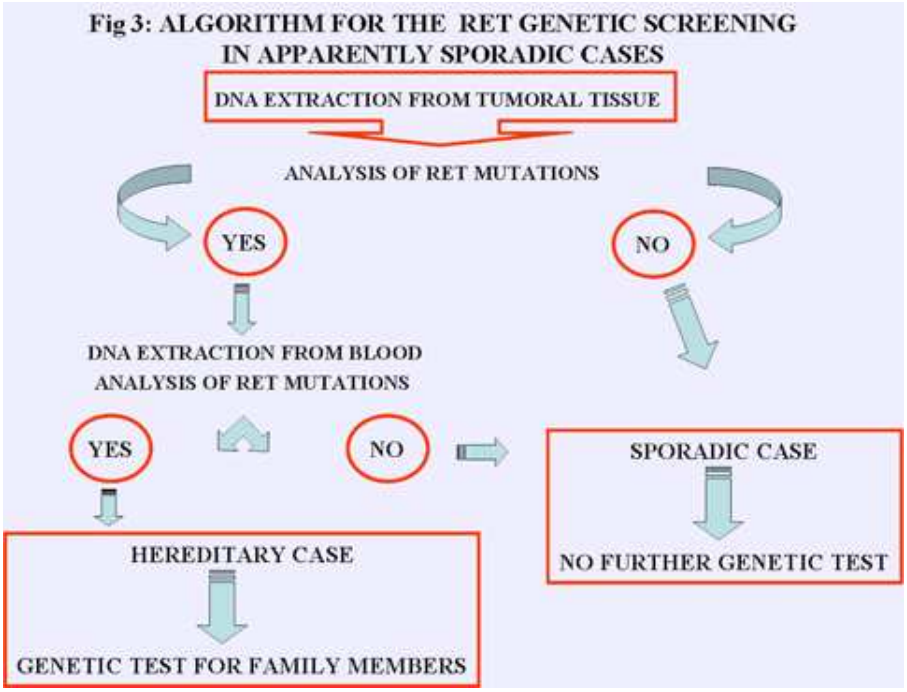
RET screening in patients presenting with apparently sporadic MTC

Screening sporadic MTCs for germline RET mutations may help in differentiating patients truly sporadic from those with unrecognized hereditary disease. The benefit of this procedure is evident for both the affected patients and their relatives, and for the unaffected patients. If an unexpected germline RET mutation is found the physician will be alerted on the possible coexistence or future development of adrenal or parathyroid disease. The screening may be extended to his/her first degree relatives, thus detecting additional gene carriers, usually in the preclinical phase of the disease. On the other hand, the negative germline RET mutated patient can be reassured on the sporadic nature of the disease, thus avoiding the need to screen his/her relatives.

The frequency of germline RET mutations in apparently sporadic cases ranges between 1.5% and 22.7% in different series (Schuffenecker et al.,
1997; Decker et al., 1995; Eng et al., 1995; Wohllk et al., 1996; Komminoth et al., 1995; Chiefari et al., 1998; Shirahama et al., 1998). In our series, 39 out of 485 patients (8%) presenting as sporadic cases, had a germline mutation in their constitutive DNA (Elisei et al., 2007). Five were MEN 2A (one “de novo”) and 34 were FMTC. Their recognition allowed the discovery of 45 additional family members carryng the mutation, unaware of their status. The systematic analysis of RET mutations in apparently sporadic MTC allowed us to identify 5 mutations never described up to now (A883T, M918V, S904F, V648I, M848T). In particular the A883T mutations was found associated to MTC only in its homozygous state (Elisei et al., 2004).

Screening of sporadic MTCs is better accomplished if the tumoral DNA is firstly tested. In case that a somatic RET mutation is found, the same mutation is searched in the blood DNA to ascertain whether the mutation is indeed an unexpected germline mutation. In this case the hereditary nature of the disease is certain. If not, the tumor is sporadic. In case that no mutation is found in the tumoral tissue, blood DNA analysis is not required: the case is very likely to be sporadic, although the existence of MEN 2 families in which the germline mutation is not found is reported in any series. An algorithm of the steps to follow for the RET genetic screening in apparently sporadic MTC is illustrated in Fig 3.

Somatic mutations of the RET proto-oncogene are found in sporadic MTC, with a frequency ranging from 23% to 70% (Eng et al., 1994; Hofstra et al., 1994; Elisei et al., 2007; Romei et al., 1996; Zedenius et al., 1994; Eng et al., 1995; Blaugrund et al., 1994). In most cases the mutation is a MEN 2B-like M918T mutation, but other codons may be involved (Eng et al., 1994; Eng et al., 1995).



Tab 1. Somatic mutations and deletions in sporadic MTC

Amino acid substitution	Nucleotide substitution	Reference
A876V	2627C>T	Uchino et al 1999
A883F	2647_2648GC>TT	Elisei et 2007
A919V	c.2756C>T	Uchino et al 1998
C630R	c.1888T>C	Bugalho et al 1997
C634A	c.?	Romei et al 1996
C634R	c.?	Elisei et 2007
C634T	c.?	Romei et al 1996
C634W	c.1902C>G	Elisei et 2007
C634Y	c.?	Elisei et 2007
D631_L633>E	c.1893_1898delCGAGCT	Musholt et al 1997
D631G	c.1892A>G	Shirahama et al 1998
D898_E901del	c.2694_2705del12	Uchino et al 1999
E632_A640>VRP	c.1895_1918>TGCGGC	Marsh et 1998
E632_C634>L	c.1895_1900delAGCTGT	Kimura et al 1995
E632_L633del	c.1894_1899delGAGCTG	Ceccherini et al 1997
E768D	c.2304G>C	Cho et al 2005
E884K	c.2650G>A	Uchino et al 1999
E901K	c.2701G>A	Uchino et al 1999
E921K	c.2761G>A	Dvoráková et al 2006
F612_C620del	c.1834_1860del27	Kalinin et al 1998
G592_G607del	c.1774_1821del48	Ceccherini et al 1997
G748C	c.2242G>T	Uchino et al 1999
G911D	c.2732G>A	Dvoráková et al 2006
M918T	c.2753T>C	Dvoráková et al 2006
P766S	c.2296C>T	Bugalho et al 1997
R908K	c.2723G>A	Uchino et al 1999
V591I	c.1771G>A	Dvoráková et al 2006
V778V	c.2334C>T	Uchino et al 1998

In addition to point mutations, a few cases of somatic gene deletions have been reported (Elisei et al., 2007; Romei et al., 1996; Ceccherini et al. 1997). Somatic RET point mutations are also found in nearly 10% of sporadic PHEO (Eng et al., 1994; Romei et al., 1996; Beldjord et al., 1995; Lindor et al., 1995; Eng et al., 1995) but not in hyperPTH (Romei et al., 1996; Padberg et al., 1995; Pausova et al. 1996; Uchino et al., 2000).

Few years ago, a correlation between the presence of a somatic RET mutation and a more aggressive phenotype of the sporadic MTC was reported by several groups (Romei et al., 1996; Zedenius et al., 1998; Zedenius et al., 1995; Schilling et al., 2001). We recently provided the evidence that MTC patients with a somatic RET mutation not only have a greater probability of unsuccessful treatment, but that they have a higher probability of dying from the disease, as demonstrated by their significantly worse 30-year survival rate with respect to that of patients without somatic RET mutations (Elisei et al., 2007).

Conclusions

Since 1993, year in which RET oncogene was demonstrated to be the causative event for MTC, several mutations have been described in MEN2 series and new mutations still continue to be discovered. A strict correlation between the type of mutation and the disease phenotype has been largely demonstrated in several studies during the last years and the RET genetic screening has been revealed as a very important diagnostic procedure for hereditary MTC. Finally RET somatic mutations have been shown to be an important bad prognostic indicator for sporadic MTC. For these reasons it appears evident that RET genetic screening is of great clinical relevance for its well established diagnostic and prognostic role. The possibility to employ new targeted therapy directed against RET mutated protein is the challenge of the near future and several tyrosine kinase inhibitors are under investigation in clinical trials (Schlumberger et al., 2008).

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