REVIEW

RET codon 609 mutations: a contribution for better clinical managing

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Medullary thyroid carcinoma currently accounts for 5–8% of all thyroid cancers. The clinical course of this disease varies from extremely indolent tumors that can go unchanged for years to an extremely aggressive variant that is associated with a high mortality rate. As many as 75% of all medullary thyroid carcinomas are sporadic, with an average age at presentation reported as 60 years, and the remaining 25% are hereditary with an earlier age of presentation, ranging from 20 to 40 years. Germline *RET* proto-oncogene mutations are the genetic causes of multiple endocrine neoplasia type 2 and a strong genotype-phenotype correlation exists, particularly between a specific *RET* codon mutation and the (a) age-related onset and (b) thyroid tumor progression, from C-cell hyperplasia to medullary thyroid carcinoma and, ultimately, to nodal metastases. *RET* mutations predispose an individual to the development of medullary thyroid carcinomas and can also influence the individual response to RET protein receptor-targeted therapies. *RET* codon 609-point mutations are rare genetic events belonging to the intermediate risk category for the onset of medullary thyroid carcinoma. A large genealogy resulting in a less aggressive form of medullary thyroid carcinoma is associated with the high penetrance of pheochromocytoma and has been reported in the literature. In this short review article, we comment on our previous report of a large multiple endocrine neoplasia type 2A kindred with the same Cys609Ser germline *RET* mutation in which, conversely, the syndrome was characterized by a slightly aggressive, highly penetrant form of medullary thyroid carcinoma that was associated with low penetrance of pheochromocytoma and primary hyperparathyroidism.

KEYWORDS: Pheochromocytoma; MEN2A; Medullary Thyroid Carcinoma; Hereditary Thyroid Cancer.

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INTRODUCTION

Medullary thyroid carcinoma (MTC) currently accounts for 5–8% of all thyroid cancers. It arises from the parafollicular C-cells originating from the neural crest that are incorporated into the thyroid during fetal development. The clinical course of MTC varies from extremely indolent tumors that can go unchanged for years to extremely aggressive variants that are associated with a high mortality rate.

The relationship between proto-oncogene *RET* mutations and MTC is one of the best examples of translational medicine. *RET* mutations predispose an individual to the development of parafollicular C-cell hyperplasia and, subsequently, to the onset of MTC. Most of the sporadic MTC cases harbor a somatic *RET* mutation that can be correlated with disease aggressiveness. Up to 20–30% of the subjects with an apparently sporadic MTC have a germline mutation (1).

Hereditary MTC can occur as three major subtypes: multiple endocrine neoplasia type 2A (MEN2A), multiple endocrine neoplasia type 2B (MEN2B), or familial MCT (FMTC). When the *RET* mutation presents at the germline level, the disease, either FMTC or MEN2, is transmitted as an autosomal-dominant trait with complete penetrance. In MEN2A, in addition to MTC (100% of cases), patients develop pheochromocytoma (PHEO) and primary hyperparathyroidism (HPT) in approximately 50% and 20% of cases, respectively. The MEN2B phenotype is characterized by the presence of mucosal neuromas and marfanoid habitus as well as MTC (100% of cases) and PHEO (50% of cases) (1).

In this syndrome, the phenotype-genotype correlation is strong. Thus, different codon mutations along the *RET* oncogene predispose individuals to different ages of onset and disease aggressiveness. Also, the specific *RET* codon mutation can influence decisions about surgical prophylactic thyroid intervention.

Complete penetrance, autosomal-dominant transmission, strong phenotype-genotype correlation, and a relatively simple genomic structure are the key points of the success of *RET*, as an example, in translational medicine. For these reasons, the detection of germline *RET* mutations is now broadly accepted for the clinical evaluation of subjects with MTC.

The *RET* gene is localized on chromosome 10, band 10q11.2; it is comprised of 21 exons and encodes for the RET transmembrane receptor with intrinsic tyrosine kinase (TK) activity and is stimulated by interaction with different

No potential conflict of interest was reported.

growth factors belonging to the glial-derived neurotrophic factor family. In animal models, RET is essential for the development of the sympathetic and parasympathetic enteric nervous systems and the kidneys.

In 98% of MEN2A families, germline mutations affect the cysteine-rich extracellular domain by converting a cysteine into another amino acid, and by determining RET spontaneous dimerization and activation. These mutations are located in codon 634 (exon 11) or codons 609, 611, 618, and 620 (exon 10). The most common mutation, accounting for 80% of MEN2A families, affects codon 634, and in this codon a particular point mutation, when a cysteine is substituted for an arginine, accounts for 50% of all MEN2A cases. Approximately half of FMTC kindred are due to germline mutations in exon 10 (codon 618 and 620), but some FMTC families are caused by mutations in exon 11 (codons 630, 631 or 634). Interestingly, the cysteine-arginine substitution in the *RET* 634 codon has never been found in FMTC families. In an increasing number of FMTC cases, germline mutations have been reported in exons 13 (codons 768, 790 and 791), 14 (codon 804 and 844), and 15 (codon 891), which are located in the TK domain, thereby interfering with intracellular ATP binding. In approximately 95% of patients with MEN2B, a single mutation that converts methionine to threonine at codon 918 (exon 16) has been identified (1). This mutation causes alterations in the substrate recognition pocket of the TK catalytic core. Other rare intracellular mutations that are associated with MEN2B involve codon 882 (exon 15).

There is a close relationship between genotype and phenotype. Thus, more aggressive phenotypes have been noticed in cases carrying mutations in the extracellular portion of RET rather than the intracellular portion. Furthermore, the average age at diagnosis for MEN2 patients with C-cell hyperplasia and RET extracellular domain mutations is 8.3 years, whereas the average age at diagnosis is of 11.2 years in patients with RET intracellular domain mutations. In patients with node-negative MTC, the average age at diagnosis is 10.2 years for patients with the associated extracellular domain mutations and 16.6 years for patients harboring intracellular domain mutations (2).

The strong genotype-phenotype correlation and the age-related progression of MTC based on the type of *RET* mutation has enabled researchers to identify different classes of risk regarding MTC penetrance and aggressiveness. Thus, a ''codon-directed'' appropriate timing for surgery in *RET* mutation carriers has been defined. Patients with level 3 mutations (codons 883, 918 and 922) are at the highest risk of developing aggressive MTC, while patients with level 2 mutations (codons 611, 618, 620 and 634) are at intermediate risk and patients with level 1 mutations (codons 768, 790, 791, 804 and 891) are at the lowest risk (3).

The *RET* codon 609 mutation

RET mutations in codon 609 are extremely rare genetic events in patients with MEN2A (<1% of all reported cases) and were initially considered as level 1 mutations (4). However, after the publication of a family pedigree carrying the *RET* 609 cysteine-to-glycine substitution, in which a *RET* mutation carrier was diagnosed with MTC at 5 years of age (5), some authors shifted the *RET* codon 609 mutations from risk level 1 to risk level 2 (6).

One way to overcome the relatively poor information available regarding the genotype-phenotype correlation in patients with the *RET* codon 609 mutation is to take into account descriptions of large, affected families and registry studies with sufficiently large numbers of individuals with the *RET* 609 codon mutation.

Several large families with *RET* codon 609 mutations have been reported in the literature. In the family reported by Kinlaw et al. (7), carriers of the *RET* codon 609 cysteine-to-serine substitution were characterized by the low penetrance of MTC and the high penetrance of PHEO. Calva et al. (8) described 16 affected patients belonging to a 38-member genealogy with a RET Cys609Tyr mutation. The phenotype of these subjects was characterized by MTC in nine out of 16 affected cases, lymph node metastasis in six out of nine cases, parathyroid adenoma in one out of 16 cases; however, PHEO was not found in this family.

We have also described a 5-generation, 48-member family with MEN2A syndrome that also harbored the RET Cys609Tyr mutation (9). Furthermore, a large registry study of individuals carrying *RET* exon 10 mutations, which also includes this family, was published by Frank-Raue et al. (10).

Description of the Italian RET C609S pedigree

The proband was a 36-year-old man. He had a 12-mm hypoechogenic, highly vascularized thyroid node of indeterminate cytology and a serum calcitonin (CT) level of 76 pg/ml. His family history was remarkable in terms of MTC and PHEO, but a previous genetic test on one of his affected relatives had failed to identify known any *RET* mutations.

Urinary metanephrines and adrenal computed tomography revealed no biochemical or radiological signs of PHEO; serum PTH, calcium and phosphorus levels were also within the normal ranges. The patient underwent a total thyroidectomy and central neck dissection, and the histological diagnosis was MTC without lymph node involvement (T1mN0Mx).

His genealogy included five already diagnosed cases of MTC. Two subjects had isolated MTC, three had lymph-node-positive MTC, and one had liver metastasis. Two out of five patients had PHEO (which was the first clinical sign of MEN2A syndrome in one case).

RET analysis, which was reconsidered in the proband, revealed a codon 609 mutation (TGC609TCC) that lead to a cysteine-to-serine substitution, Cys609Ser. This mutation was also confirmed in the members of his family already known to be affected. Another 24 family members underwent genetic testing for this *RET* mutation, revealing nine carriers (Figure 1). Two at-risk individuals refused genetic investigations.

Phenotypic characterization of the gene mutation carriers

In short, clinical investigation revealed that none of the patients had a palpable thyroid node. None of the nodes were larger than 10 mm in diameter, presented with a suspected echographic pattern on ultrasound or had hypertension; one patient had two episodes of nephrolithiasis as the first clinical sign of the syndrome. Serum CT measurements revealed that the older subjects, one of whom was an 86-year-old woman, had the highest basal CT levels, while those of the younger subjects were low or unresponsive to pentagastrin. None of the patients had biochemical or radiological evidence of PHEO. All patients who underwent thyroid surgery demonstrated isolated MTC and/or C-cell hyperplasia.

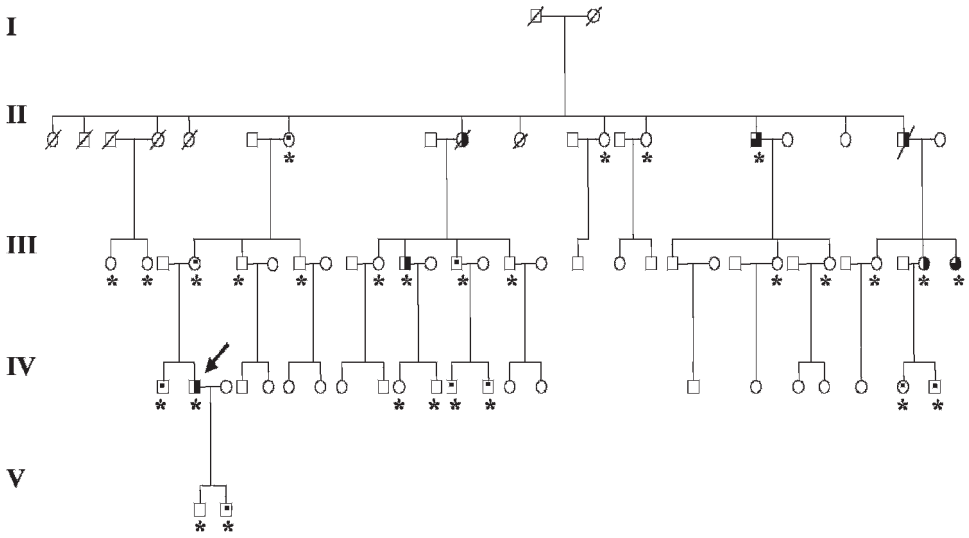

Figure 1 - Pedigree analysis. The proband is indicated by the black arrow. Deceased patients are identified by diagonal lines. Subjects with signs of MEN2A syndrome are indicated by black shading, which covers half of the pedigree symbol for patients with MTC alone and three-quarters of the symbol for patients with MTC plus PHEO. All patients who underwent genetic testing are marked with an asterisk. After genotyping, gene mutation carriers are identified by the square shape of their respective pedigree symbols.

Phenotype-genotype correlations in RET 609 carriers and recommendations

The 1999 consensus statement on MEN recommended that codon 609 *RET* mutations should be considered as risk level 1, for which there are no unequivocal clinical management guidelines. Some clinicians recommend a prophylactic total thyroidectomy by the age of 5 years, others by the age of 10 years, whereas others advocate surgery as soon as routine pentagastrin-stimulated test findings become abnormal (3).

Indeed, 609 *RET* mutations are quite rare genetic events at the onset of MEN2A and few affected families have been described in the literature (7,9,10,11,12). One family was comprised a 5-year-old boy who harbored a Cys609Gly substitution that is associated with an invasive MTC at final histology and warranting the inclusion of such mutations in the intermediate risk category (5,6). On the other hand, *in vitro* data obtained from transfected NIH3T3 cells seem to suggest that 609 mutations have a smaller capacity for neoplastic transformation than other level 2 *RET* mutations (13). Only one large family carrying a Cys609Ser mutation was reported by Kinlaw et al. and, regarding its clinical aspects, this genealogy revealed an unusual phenotype characterized by a scarcely penetrant, non-aggressive MTC (20%) and a tendency for cases to present with PHEO rather than MTC (7). We described a kindred with the same *RET* mutation. Based on the large number of affected subjects with this specific mutation in this family who had undergone total thyroidectomy, we agree that the Cys609Ser mutation carries a level 1 risk: we saw no MTC develop before the age of 17 years, no lymph node metastases up to 30 years of age and no distant metastases up to 60 years of age. There was also an 86-year-old mutation carrier who did not undergo surgery. Moreover, we saw no anticipation effect, which is by no means rare in MEN2A kindreds carrying level 1 mutations, leading to MTC onset at an earlier age in each successive generation (14).

MTC was highly penetrant in our family (75% of cases) and was the first sign of disease in all but two cases (one presented with PHEO and another presented with the clinical consequences of primary HPT); the penetrance of PHEO and primary HPT was low. Furthermore, the paper by Frank-Raue et al. (10) was a very large registry study on *RET* exon 10 mutations. In this study, the 45 carriers of *RET* codon 609 mutations, each with different cysteine substitutions (including 15 individuals belonging to the Italian C609S pedigree), demonstrated a less aggressive MTC but a 50% penetrance of PHEO (10).

In conclusion, the results obtained in this study of large genealogies may be very helpful for establishing a better genotype-phenotype correlation. In particular, the results we obtained by analyzing this very large MEN2 kindred suggest that carriers of the Cys609Ser *RET* mutation can be considered as risk level 1, for which non-aggressive clinical management may be indicated. Thus, as also reported by Frank-Raue et al. (10), in these cases, prophylactic total thyroidectomy may be postponed until after 5 years of age if careful yearly monitoring of stimulated calcitonin levels is implemented.

AUTHOR CONTRIBUTIONS

Mian C has actively contributed to the clinical and molecular studies on patients and to the drafting of the article. Sartorato P has contributed to the review of the literature on the topic and to the drafting of the article. Barollo S and Zane M have performed the genetic studies. Opocher G has coordinated the clinical and molecular studies conducted on patients and planned the drafting of the review.

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