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 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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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.
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 kindreds 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.
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 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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