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Pediatric Medullary Thyroid Carcinoma

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Abstract

Medullary thyroid carcinoma (MTC), which originates from thyroid parafollicular C cells, accounts for 3 to 5% of thyroid malignancies. MTC occurs either sporadically or in an inherited autosomal dominant manner. Hereditary MTC occurs as a familial MTC or as a part of multiple endocrine neoplasia (MEN) type 2A and B syndromes. A strong genotype-phenotype correlation has been observed between hereditary MTC and germ-line “gain of function” mutations of the *RET* proto-oncogene. Most cases of pediatric MTC are hereditary whereas sporadic MTC is rare in children and is usually diagnosed in adults. Therefore, MTC in children is most often diagnosed in the course of a familial genetic investigation. The standard treatment of MTC mainly requires surgery involving total thyroidectomy and central neck node dissection before extrathyroidal extension occurs. To prevent MTC development in hereditary syndromes, prophylactic thyroidectomy is performed in presymptomatic patients. An appropriate age at which the surgery should take place is determined based upon the data from genotyping, serum calcitonin measurements, and ultrasonography. For the treatment of advanced MTC cases, the broad spectrum receptor tyrosine kinase inhibitors vandetanib and cabozantinib, which also inhibit *RET*, are used although they are not always effective.

Keywords

Medullary thyroid carcinoma; *RET*; MEN2A; MEN2B

1. INTRODUCTION

Thyroid cancer is the most common endocrine neoplasia which accounts for about 1% of human cancers. MTC originates from calcitonin-producing cells (C-cells) of the thyroid gland and accounts for 3–5% of thyroid cancers [1]. MTC is relatively slow-growing tumor but, if metastasized or relapsed, it becomes very aggressive causing more than 13% of all thyroid cancer-related mortality [2]. In the United States, about 1200 new MTC cases are diagnosed every year [3]. MTC has an incidence in children of 0.03 per 100 000 population per year with a fairly equal female to male ratio [4, 5]. MTC occurs either sporadically or in an inherited autosomal dominant manner. In adults, sporadic MTC accounts for 65–75% of

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MTC, but in children, sporadic MTC is very rare; the vast majority of MTC diagnosed in the childhood is hereditary [4].

Hereditary MTC occurs as a familial MTC (FMTC) or as a part of multiple endocrine neoplasia (MEN) type 2A and B syndromes, wherein other endocrine glands are also affected. MTC diagnosed during childhood almost always results from a dominantly inherited or *de novo* activating mutations in the *RET* proto-oncogene, which encodes the RET receptor tyrosine kinase [6–9]. Advances in predictive genetic testing for *RET* mutations have enabled early diagnosis of hereditary MEN syndromes and prophylactic thyroidectomy in presymptomatic patients to prevent MTC. The early onset of MTC in hereditary syndromes makes it an important endocrine disease that is increasingly managed by pediatric providers [10–12]. In this review, we discuss the etiology of pediatric MTC and currently available therapeutic modality for the cancer.

2. STRUCTURE AND FUNCTION OF RET

RET encodes a receptor tyrosine-kinase which is expressed in the neural crest-derived cell types, including thyroid parafollicular cells, neuronal cells, and adrenal medullary chromaffin cells. In these cell types, *RET* plays a central role in regulating cell proliferation, growth, differentiation, migration and survival [13]. In humans, *RET* is localized on the chromosome 10 and contains 21 exons [14]. After alternative splicing at the 3’ end, *RET* transcripts encode three protein isoforms with distinct C-terminal ends that contain either 9 (RET9), 51 (RET51), or 43 (RET43) amino acids [15]. RET exon 19 is present in all transcripts and its differential splicing at the 3’ end produces distinct transcripts wherein exon 19 is either unspliced, spliced to exon 20, or spliced to exon 21 [16]. All three resulting RET isoforms commonly contain a tyrosine (Tyr1062) whose phosphorylation is critical for their activation [17]. The major RET isoforms *in vivo* are RET9 and RET51, which consist of 1072 and 1114 amino acids, respectively, and are usually co-expressed [18].

RET consists of an extracellular ligand binding domain, a trans-membrane domain, and an intracellular kinase domain (Figure 1). The extracellular domain includes four cadherin-like repeats and a highly conserved cysteine-rich region, which is located near the cell membrane. The transmembrane domain is required for the dimerization of RET. The intracellular domain consists of two tyrosine-kinase subdomains, TK1 and TK2, which contain multiple tyrosine residues that are phosphorylated during receptor activation and are required for the activation of different downstream signaling pathways of RET [19, 20]. The ligands for RET are the glial cell line-derived neurotrophic factor (GDNF) family proteins, including GDNF, neurturin, artemin, and perseptin. Activation of RET also requires the formation of a heterodimeric complex recruiting a GDNF-family receptor alpha (GFRα) [21]. When unbound by a ligand, RET is monomeric, unphosphorylated, and inactive. When a ligand and the GFRα co-receptor bind to the extracellular domain of RET, RET undergoes dimerization and autophosphorylation of the tyrosine residues in their kinase domains. This generates the docking sites for their downstream effectors that contain the Src Homology 2 domain [20]. For example, GDNF-mediated stimulation of RET results in activation of the pathways regulated by phosphatidylinositol 3-kinase (PI3K) and different mitogen-activated protein kinases (MAPKs), including the extracellular regulated kinases (ERKs), c-Jun

amino-terminal protein kinases (JNKs), the p38 MAPK and the big MAP kinase (BMK1) ERK5 [22, 23].

RET is one of the first receptor tyrosine-kinases (RTKs) that have been found to play a role in neoplasia, being most well-known as a key etiological factor for thyroid cancer [6, 24]. Activating mutations of *RET* abnormally enhance RET activity and can trigger tumorigenesis in certain organs although the exact underlying mechanisms are as of yet unclear. Gain-of-function *RET* mutations mainly occur in two different ways. First, mutations of the six cysteine residues (Cys609, 611, 618, 620, 630, and 634) in the extracellular domains can promote RET dimerization via disulfide bonds and result in constitutive ligand-independent activation of RET [25]. Second, mutations affecting the tyrosine kinase domains can also confer ligand-independent catalytic activity to monomeric RET [26]. These RET mutants exhibit different patterns of autophosphorylation and altered substrate specificity [26–28]. Indeed, activation of different downstream signaling pathways is associated with different clinical features of *RET* mutant thyroid cancers, as observed in MEN2 syndromes discussed below [19]. Intriguingly, loss-of-function mutations are also detected in RET. For example, the Hirschprung disease, a congenital disorder of neural crest development is caused by a loss-of-function *RET* mutation [29]. Of note, the Hirschprung disease is closely associated with MEN2A, demanding a genetic screening for MEN2A for children with familial Hirschsprung's disease [30].

A strict correlation exists between specific *RET* mutations and the onset of hereditary MTC (Table 1) [31, 32]. The detailed and up-to-date information of *RET* sequence variations can be obtained from the MEN2 RET database (www.arup.utah.edu/database/MEN2/MEN2_welcome.php), which also contains links to selected MEN2 literature reviews, gene and protein information, and *RET* reference sequences [32].

3. MEN TYPE 2 SYNDROMES

The MEN2A subtype, accounting for 90–95% of the MEN type 2 cases, is a highly penetrant, autosomal dominant endocrine tumor syndrome characterized by the development of MTC in >90% of RET mutation carriers [33]. In association with MTC, MEN2A patients typically feature bilateral pheochromocytoma and multiple tumors of parathyroid glands [34]. In addition, rare variants of MEN2A are also associated with cutaneous lichen amyloidosis and Hirschsprung disease [35, 36]. Patients with MEN2A usually have mutations in the extracellular cysteine-rich region of the RET tyrosine kinase receptor, usually in exon 10 (codons 609, 611, 618 or 620) or exon 11 (codon 634) (Table 1, Figure 1) [31, 37]. More than 80% of MEN2A patients exhibit a specific substitution, i.e., Cys634Arg, on exon 11. MTC is generally the first manifestation of MEN2A syndrome and develops during early childhood, usually before age six and sometimes before age two [38].

The MEN2B subtype accounts for approximately 5–10% of the MEN type 2 cases. MEN2B patients typically feature early coincident onsets of MTC, pheochromocytoma, and gastrointestinal mucosal ganglioneuromas [39]. Visible physical symptoms include mucosal neuromas of lips (bumpy lips) and tongue, and asthenic marfanoid body habitus [36, 40]. MEN2B patients usually have mutations in the tyrosine kinase domain 2 (TK2) in the

intracellular region of RET, which almost always (>95%) lead to a single substitution, i.e., Met918Thr, in exon 16 (Figure 1) [31, 37]. De novo mutations, which usually occur on the paternal allele, are also common in MEN2B [41]. MEN2B is characterized by the early development of an aggressive form of MTC in all affected individuals, typically during the first year of life [38]. MEN2B phenotype is often not apparent at early childhood. Thus, apart from the genetic testing of *RET* mutations in children born to a parent with MEN2B, early diagnosis of MEN2B remains challenging [42]. Individuals with MEN2B are likely to develop metastatic MTC at an early age if they do not undergo prophylactic thyroidectomy before age one. Without this intervention, the average survival expectancy in MEN2B carriers is about 21 years [40].

FMTC is considered as the least aggressive clinical variant of MEN2A with decreased penetrance and/or delayed onset of the other endocrine pathologic manifestations [39, 43]. Similarly to sporadic cases, familial MTCs are isolated and are not associated with other endocrine tumors. Patients with FMTC harbor mutations similar to MEN2A in either the extracellular or intracellular region of the RET tyrosine kinase receptor [6, 44]. The onset of FMTC is relatively late, not appearing until the second or the third decade of life, and its penetrance is lower than the MTC caused by MEN2A and MEN2B [31, 39, 45, 46]. Therefore, it is often difficult to determine FMTC based upon a family history and only careful genetic screening can distinguish between inherited and sporadic forms of MTC [24].

4. DIAGNOSIS AND TREATMENT

MTC cells secrete the polypeptide hormone, calcitonin, and the glycoprotein carcinoembryonic antigen (CEA), and these are used as diagnostic biomarkers for MTC [47]. MTC is most commonly diagnosed by immunohistochemical staining of fine-needle aspiration of a new thyroid nodule for calcitonin, chromogranin A, or CEA [48]. Serum calcitonin is the primary biochemical marker used for detection, staging, postoperative management, and prognosis for MTC patients [49]. However, in very rare cases, certain MTC cells do not secrete calcitonin, which makes diagnosis and patient follow-up difficult [50]. Symptoms of MTC include neck pain, a palpable neck mass, and/or diarrhea resulting from hypercalcitoninemia [51].

The clinical course of MTC in MEN2 patients is variable and is determined by the codon specific mutations [45]. In hereditary form, an age-related progression of malignant disease is observed, with lymph-node and distant metastases being typically detected years after the onset of tumorigenesis [52]. Metastatic spread to cervical and regional lymph nodes (i.e., parathyroid, paratracheal, jugular chain, and upper mediastinum) or to distant sites including the liver, lungs, or bone is common and is frequently present in individuals with a palpable thyroid mass or diarrhea [53]. Positive lymph-node status and higher stage at diagnosis predict lower disease-free survival and higher mortality [5, 54–56].

MEN2 is one of few hereditary cancer syndromes for which predictive genetic testing is recommended at childhood. Genetic testing for hereditary MTC syndromes has had an enormous impact on reducing the incidence of MTC in the families affected by these

hereditary syndromes [57, 58]. Genetic counseling is indicated for all children diagnosed with MTC and others who either carry or are at risk of inheriting a *RET* mutation. Children of patients with MEN2B should undergo *RET* analysis at birth, and children of patients with MEN2A or FMTC should undergo *RET* analysis before age six [59, 60]. Even 6–10% of apparently sporadic cases of MTC demonstrate *de novo* germ-line *RET* mutations, thus making genetic testing worthwhile in all patients with MTC [61]. In very rare cases, *RET* mutations are not found despite clear familial MTC. Thus, all children with an affected parent in this setting retain a 50% risk of MTC, and surgical decisions must rely solely on clinical testing.

The standard treatment for MTC is surgical removal of all thyroid tissue including the posterior capsule [39, 62]. Early thyroidectomy in all MEN2 patients can change the course of disease, either in a preventive or a curative fashion [63]. The American Thyroid Association Guidelines Task Force has classified mutations based upon a model that uses the genotypephenotype correlations to rank the mutations into risk levels for the development of aggressive MTC from the lowest “A” to the highest “D” (Table 1) [39]. This classification may be used to predict phenotype, to recommend the timing of prophylactic thyroidectomy and the extent of surgical intervention, and to begin biochemical screening for pheochromocytoma and hyperparathyroidism.

The ages at which the prophylactic thyroidectomy is recommended for the children tested positive for the *RET* gene mutation are as follows: ages 0–1 for *RET* mutations that carry the highest risk for aggressive metastatic MTC at young ages, i.e., classified as “ATA-D”; before age 5 for *RET* mutations that carry a lower, yet still high risk of aggressive MTC at any age, i.e., classified as “ATA-C”; after age 5 for *RET* mutations that carry a lower risk of aggressive MTC, i.e., classified as “ATA-B” or “ATA-A,” so long as the affected children have no other clinical signs of MTC development [39]. There is ongoing debate on what age the thyroidectomy should be recommended for FMTC patients. Some clinical institutes suggest the prophylactic surgery at age 10–15, depending upon the exact mutation and family history, while recommending yearly test of calcitonin levels prior to deciding the surgery [64]. These guidelines continue to be modified as more data become available [65]. Indeed, the ATA management guideline for MTC has been very recently revised [66]. In the setting of a prophylactic thyroidectomy, the lymph-nodes are not routinely removed since metastases are not expected to occur at this stage [54]. In the case of clinically apparent MTC, whether sporadic or hereditary, thyroidectomy and concomitant central and compartment-oriented lateral neck dissection should be performed to increase clinical outcomes. Primary hyperthyroidism is rare during childhood; therefore, parathyroidectomy is usually avoided, particularly during a prophylactic procedure. Dissection and autotransplantation of parathyroid tissue is not typically performed at the time of thyroidectomy unless there is enough biochemical evidence for hyperparathyroidism [39]. Thyroidectomy in children is usually associated with a higher rate of complications, such as recurrent laryngeal nerve injury and hypoparathyroidism, as compared to the surgery in adults [67]. Therefore, pediatric thyroidectomy must be performed by highly experienced thyroid surgeons [62].

For individuals with a *RET* mutation who have not had a thyroidectomy, annual biochemical screening of calcitonin levels is recommended and, if the results are abnormal, immediate thyroidectomy is required [68]. Annual serum calcitonin screening should begin at age six months for children with MEN2B and at age 3–5 for children with MEN2A or FMTC [39]. After the surgery, patients require careful surveillance for disease recurrence. Biochemical evidence of disease recurrence includes elevation of calcitonin and CEA levels [69]. All individuals who have undergone thyroidectomy need thyroid hormone replacement therapy along with annual screening for pheochromocytoma and hyperparathyroidism depending upon the *RET* mutation present in the patients [70].

There are not many therapeutic options for MTC other than surgery. MTC does not respond well to radiation therapy or the standard cytotoxic chemotherapeutic agents, including doxorubicin, dacarbazine, capacitabine, and 5-fluorouracil [43, 71]. Of note, the mechanism-based targeted therapies that inhibit RET and other receptor tyrosine kinases have become available for the treatment of surgically inoperable progressive MTC. These include the multi-kinase inhibitors, vandetanib (ZD6474, Caprelsa™) and cabozantinib (XL-184, Cometriq™), which have been recently approved by the US Food and Drug Administration [72, 73]. A recent phase I/II trial of vandetanib in children with MTC reported partial responses in 47% patients [74]. In general, the drug efficiency and the primary side effects, i.e., diarrhea, rash, headache, hypertension, and nausea, were similar between children and adults [75]. Phase III trial of cabozantinib demonstrated a 28% response rate in adults with significant adverse effects [76]. Therefore, there is a critical need for more effective therapies for patients with advanced MTC. Characterization of additional molecular pathways responsible for MTC development may allow the discovery of therapeutic targets that can be exploited to induce reduction of tumor size, disease stabilization, and symptomatic improvement [77–82].

5. CONCLUSION

MTC and the MEN type 2 syndromes are rare but significant endocrine diseases that are increasingly encountered by pediatricians. Our understanding of MTC has been greatly increased by the discovery of *RET* and the genotype–phenotype relationship of its various oncogenic mutations. Genetic tests according to the established guidelines should be performed whenever diagnosis of MTC is made. Due to limited adjuvant treatment options, adequate surgical treatment is critical for initial control of the disease and prophylactic thyroidectomy is recommended for children with MEN2A and MEN2B at an early age, sometimes during infancy. Emerging newer treatments are expected to better treat this rare but life-threatening malignancy.

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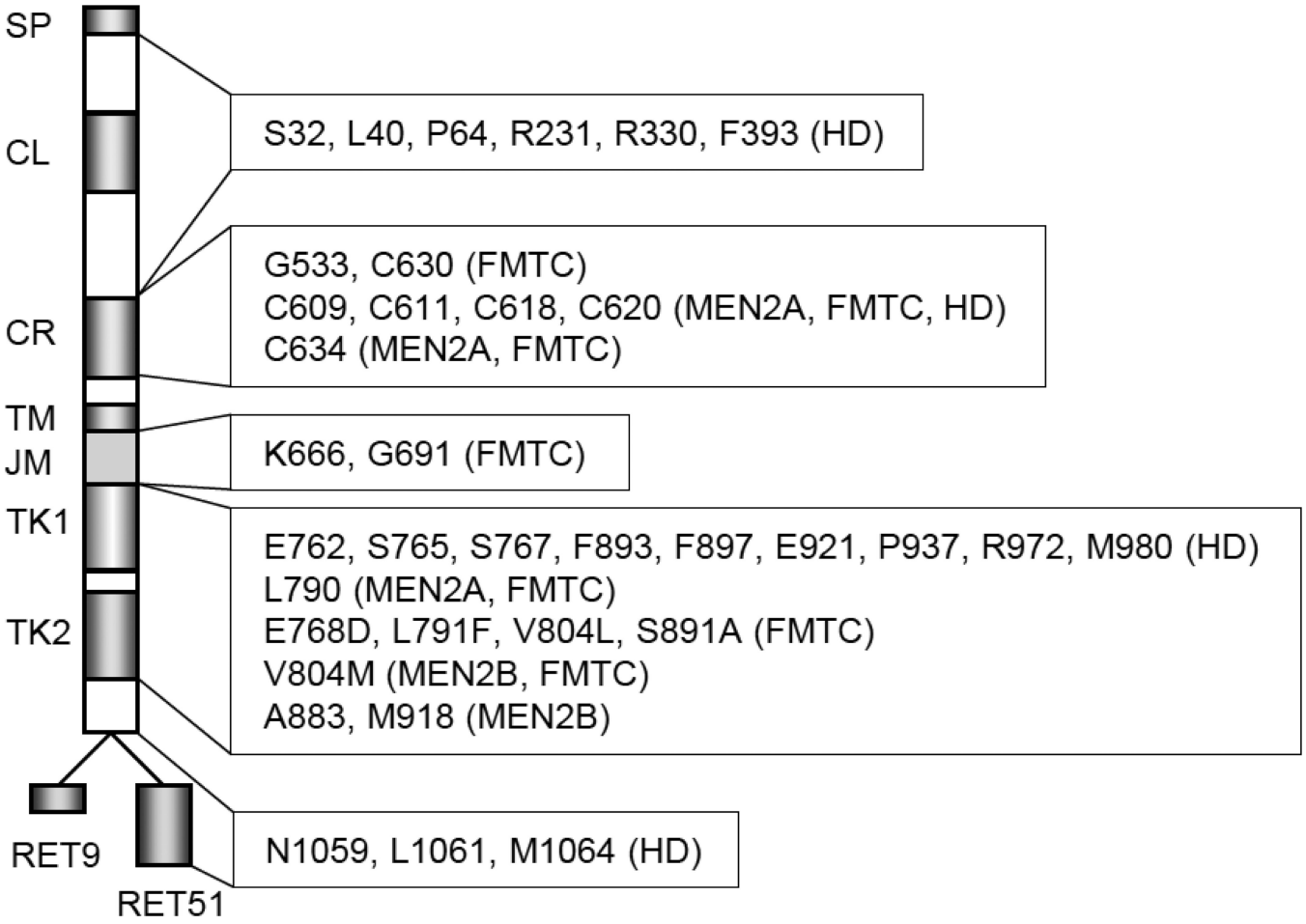

Figure 1. Structure of the RET receptor and germline point mutations of *RET* in different diseases

Depicted are different RET domains/motifs, including signal peptide (SP), cadherin-like (CL), cysteine-rich (CR), transmembrane (TM), juxtamembrane (JM), and tyrosine kinase (TK). *RET* mutations associated with multiple endocrine neoplasia type 2A (MEN2A), MEN2B, familial medullary thyroid carcinoma (FMTC), and Hirschsprung's disease (HD) are also indicated.

Table 1

Genotype, Risk Level, and Earliest Age of Detection and Onset of Hereditary MTC. Data compiled from the ATA Medullary Thyroid Cancer Management Guidelines [39] and updated from the ARUP Institute for Clinical and Experimental Pathology MEN2 RET online database [32]. The RET mutations are ranked from the highest “D” to the lowest “A” risk levels for the development of aggressive MTC in patients, according to the ATA classification. (na - patient age information is not available).

Mutation	Exon	ATA Risk Group	MEN2 Phenotype	Age of MTC Onset
M918T	16	D	2B	0.17
A883F	15	D	2B	10
V804M + E805K	14	D	2B	50
V804M + Y806C	14	D	2B	23
V804M + S904C	14/15	D	2B	18
C634R/G/F/S/W/Y/L	11	C	2A, FMTC	1.4/3/7/8/3/0.8/na
C609F/R/G/S/Y	10	B	2A, FMTC	18/27/4/15/14
C611R/G/F/S/W/Y	10	B	2A, FMTC	na/28/14/47/14/6
C618R/G/F/S/Y	10	B	2A, FMTC	8/9/5/9/25
C620R/G/F/S/W/Y	10	B	2A, FMTC	6/22/27/14/37/18
C630R/F/S/Y	11	B	2A, FMTC	1/na/39/22
D631Y	11	B	2A, FMTC	30
633/9 base pair duplication	11	B	2A, FMTC	56
V804M + V778I	13/14	B	2A, FMTC	26
G321R	5	A	2A, FMTC	61
V292M	5	A	2A	13
531/9 base pair duplication	8	A	2A, FMTC	19
532 duplication	8	A	FMTC	na
C515S	8	A	2A, FMTC	35
G533C	8	A	2A, FMTC	21
R600Q	10	A	2A, FMTC	46
K603Q	10	A	2A, FMTC	35
Y606C	10	A	FMTC	58
635/insertion ELCR; T636P	11	A	2A, FMTC	9
S649L	11	A	2A, FMTC	29
K666E	11	A	2A, FMTC	35
A750P	12	A	2A	na
E768D	13	A	2A, FMTC	22
N777S	13	A	2A, FMTC	60
L790F	13	A	2A, FMTC	16
Y791F	13	A	2A, FMTC	5
V804L/M	14	A	2A, FMTC	12/6
R833C	14	A	FMTC	59

Mutation	Exon	ATA Risk Group	MEN2 Phenotype	Age of MTC Onset
R844Q	14	A	FMTC	na
R866W	15	A	2A, FMTC	44
S891A	15	A	2A, FMTC	13
R912P	16	A	2A, FMTC	14