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ONCOLOGY: RESEARCH ARTICLE

Revisiting the genotype-phenotype correlation in children with medullary thyroid carcinoma: A report from the GPOH-MET registry

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Abstract

Background: Medullary thyroid carcinomas (MTC) account for 3% to 5% of all thyroid cancers. In most cases, MTC is hereditary and occurs as part of the multiple endocrine neoplasia (MEN) type 2A and 2B syndromes. There is a strong genotype-phenotype correlation associated with the respective *RET* mutations, making risk-adapted management possible.

Procedure: We report the prospectively collected data on children and adolescents of the multicenter nonrandomized German GPOH-MET registry. Children and adolescents with MTC and C-cell hyperplasia (CCH) were included.

Results: From 1997 to June 2019, a total of 57 patients with MTC and 17 with CCH were reported. In patients with MTC, median follow-up was five years (range, 0-19) and median age at diagnosis 10 years (range, 0-17). Overall survival and event-free survival (EFS) were 87% and 52%, respectively. In total 96.4% of patients were affected by MEN2 syndromes, which was in 37/42 MEN2A and 3/28 MEN2B (*M918T* mutation) inherited. EFS in MEN2A was 78%, and in MEN2B 38% ($P < 0.001$). In multivariate analyses, lymph node (LN) status and postoperatively elevated calcitonin were significant prognostic factors for EFS. Notably, modest-risk mutation carriers presented with MTC at a rather young age, without raised calcitonin, and LN metastases.

Conclusions: Identification of children carrying *de novo RET M918T* mutations by means of the characteristic phenotype is crucial to detect MTC at an early stage, which will be associated with improved survival. As calcitonin levels may be false-negative and modest-risk mutation carriers present with a variable phenotype, particular attention should be paid to these children.

KEYWORDS
children, medullary thyroid carcinoma, MEN syndrome, RET

1 | INTRODUCTION

Medullary thyroid carcinoma (MTC) accounts for 3% to 5% of all thyroid cancers in children and adults with an incidence in children of 0.03 per 100.000 population per year.[1,2] In most cases, MTC is hereditary.[2] Hereditary MTC occurs as part of the multiple endocrine neoplasia (MEN) type 2A and 2B syndrome.[3] MEN type 2 can additionally present with pheochromocytoma (PHEO) and primary hyperparathyroidism (PHPT) (referred to as MEN2A) as well as mucosal neurinomas, intestinal ganglioneuromatosis, and among

Abbreviations: (M)MTC, (metastatic) medullary thyroid carcinoma; ATA, American Thyroid Association; CCH, C-cell hyperplasia; CI, confidence interval; CPS, cancer predisposition syndrome; CR, complete remission; EFS, event-free survival; H, high risk; HST, highest risk; MEN, multiple endocrine neoplasia; MET, malignant endocrine tumor; MOD, modest risk; OS, overall survival; PET, positron emission tomography; PHEO, pheochromocytoma; PHPT, primary hyperparathyroidism; RLN, recurrent laryngeal nerve; SD, standard deviation; TE, thyroidectomy.

others a marfanoid habitus (referred to as MEN2B).[3–7] Almost all MTCs diagnosed in childhood result from autosomal dominant inherited or *de novo* gain-of-function pathogenic germline variants in the *RET* proto-oncogene.[3,8,9]

In contrast to most cancer predisposition syndromes (CPS) in children, there is a striking genotype-phenotype correlation and, thus, predictability for the development of the three pathognomonic tumors in MEN2.[4,10–13] Typically, MEN2 initially manifests with the development of MTC. However, the age of onset of MTC varies by genotype, from early childhood (i.e., *p.M918T*, MEN2B) to adolescent and early adulthood and even middle age in MEN2A.[6,10,12,13] The development of MTC is preceded by calcitonin-secreting parafollicular C-cell hyperplasia (CCH) and the progression to MTC is age related.[14] The latter correlates with the transforming capacity of the respective *RET* mutations.[15]

Guidelines for the management of patients with hereditary MTC/MEN2 have been published by the American Thyroid Association (ATA) in 2001 and have since then been modified thrice. The pathogenic codon-specific variants are classified in "highest" (HST), "high" (H), and "modest" (MOD) due to the clinical aggressiveness of the MTC and to the incidence of PHEO and PHPT.[10] Management of mutation carriers including the recommended timing of prophylactic thyroidectomy (TE) depends on the specific *RET* mutation. This takes into account the probability of developing MTC at an early age and of metastatic disease. Prophylactic TE is recommended in the first year of life in carriers with highest risk mutation, at or before five years of age based on serum calcitonin levels in the high-risk category, and in modest-risk mutation carriers TE is recommended when the serum calcitonin levels start to rise.[10]

Hence, pathogenic *RET* mutations afford the unique opportunity for early treatment before metastatic and thus incurable disease has been occurred. MEN2 syndromes represent an impressive paradigm of CPS manifesting in childhood, in which individualization of care and prophylactic therapy based on the patient's mutation is possible. As illustrated by the need for a repeated update of ATA guidelines within a short time period, ongoing collection of data and outcomes is necessary to provide the most accurate, variant-specific risk estimates particularly in carriers of moderate-risk alleles.

The clinical reality of the clinical situation in MEN2B syndrome contrasts with current recommendations. The *M918T* mutation of *RET* for instance, conferring the highest MTC risk level, most frequently occurs *de novo*.[16,17] Thus, in case the characteristic phenotype is overlooked, patients are diagnosed with MEN2B when MTC is already present.

We herein report the data on all children and adolescents with MTC and CCH reported to the German GPOH-MET study center since 1997. We review the genotype-phenotype correlation of these patients regarding the ATA guidelines and comment on individual patient's characteristics deviating from the expected phenotype.

1.1 | Patients and methods

Data collection was prospectively conducted in the GPOH-MET 97 study and the GPOH-MET registry. The GPOH-MET 97 study was a prospective multicenter interdisciplinary nonrandomized trial collecting data on children and adolescents with malignant endocrine tumors (MET), including MTC and CCH. Since January 2013, the GPOH-MET 97 study has continued as the GPOH-MET registry. Children and adolescents aged 0 to 18 years were analyzed.

Patients were diagnosed according to the TNM classification system by local pathology assessment. Noteworthy, the 8th edition of the American Joint Committee on Cancer (AJCC)/TNM cancer staging system was introduced on January 1, 2018. One important modification was that the microscopic extrathyroidal tumor invasion is no longer considered as a criterion for the classification as a T3 tumor.[18] However, in our cohort, only one patient was diagnosed after this reference date and was not affected by this modification. Consistently, the reported data were classified according to the previous edition of the classification system.

The GPOH-MET 97 study protocol and the recommendations of the GPOH-MET registry document were conducted according to the Declaration of Helsinki and approved by the ethical committees of the University of Luebeck (97-125) and Otto von Guericke University Magdeburg (174/12), Germany. Written informed consent was obtained from either the patients themselves, if they were aged 15 years or older, and the child's parents or legal guardians, respectively.

Due to missing data in some patients, the exact number of cases used for the frequency analyses (including only those patients in whom data were available) varied and is given in the tables. Overall survival (OS) and event-free survival (EFS) were calculated using the Kaplan-Meier method; groups were compared using the log-rank test. For estimation of OS, death of any cause was used as an event, for EFS, non-remission, persisting/increased calcitonin levels, development of new metastases, second malignancies, and death of any cause were used. Survivors were censored at the last date of last known follow-up. Cox proportional hazards model was used to evaluate the impact of prognostic factors on EFS in a univariate and multivariate manner including the following factors potentially associated with outcome: indication for thyroidectomy, pN, pM, postoperative calcitonin levels, and type of MEN2 syndrome. All *P* values below 0.05 were considered significant. Statistical analyses were performed by SPSS version 26. Data will be made available upon reasonable request.

2 | RESULTS

2.1 | Children with medullary thyroid carcinoma

From 1997 to June 2019, a total of 57 children and adolescents with MTC were registered within the GPOH-MET databases with a mean follow-up of 5.8 years (SD ±4.8). Detailed patient characteristics are given in Table 1. OS and EFS of children diagnosed with MTC were 87% and 52%, respectively.

Preoperative calcitonin levels were above 100 ng/l in nine (20.0%) of the patients (thereof $pN_1 = 5$, $pM_1 = 1$), 14 (30.4%) presented with calcitonin levels above 1000 ng/l ($pN_1 = 13$, $pM_1 = 9$). Details of preoperative calcitonin levels, CEA levels, and radiological diagnostics are given in Table 2.

TABLE 1 Demographic and clinical characteristics of children and adolescents with MTC ($n = 57$)

	Children with MTC	MEN2A	MEN2B
No	57[a]	27	27
Gender			
Female	25 (43.9%)	12 (44.4%)	14 (51.9%)
Male	32 (56.1%)	15 (55.6%)	13 (48.1%)
Age at diagnosis			
Mean (SD)	9.9 (5.0)	9.6 (4.7)	9.2 (5.3)
Median (range in years)	10 (0-17)	9 (1-17)	10 (0-17)
Time of follow-up			
Mean (SD)	5.8 (4.8)	5.4 (4.6)	5.3 (4.8)
Median (range in years)	5 (0-19)	5 (0-17)	5 (0-19)
TNM classification			
pT1	33 (57.9%)	21 (77.8%)	12 (44.4%)
pT2	4 (7.0%)	1 (3.7%)	1 (3.7%)
pT3	4 (7.0%)	1 (3.7%)	3 (11.1%)
pT4	11 (19.3%)	0	10 (37.0%)
Unknown	5 (8.8%)	4 (14.8%)	1 (3.7%)
pN0	28 (49.1%)	20 (74.1%)	8 (29.6%)
pN1	26 (45.6%)	4 (14.8%)	19 (70.4%)
Unknown	3 (5.3%)	3 (11.1%)	0
pM0	42 (73.7%)	24 (%)	16 (59.3%)
pM1	13 (22.8%)[b]	1 (3.7%)	11 (40.7%)
Unknown	2 (3.5%)	2 (7.4%)	0
Remission at last follow-up			
Complete remission	28 (49.1%)	20 (74.1%)	8 (29.6%)
Partial remission	2 (3.5%)	0	2 (7.4%)
Stable disease	4 (7.0%)	1 (3.7%)	3 (11.1%)
Progressive disease	10 (17.5%)	0	8 (29.6%)
Hypercalcitoninemia	4 (7.0%)	2 (7.4%)	1 (3.7%)
Death	6 (10.5%)	1 (3.7%)	5 (18.5%)
Unknown	3 (5.3%)	3 (11.1%)	0
Status at last follow-up			
Alive	47 (82.5%)	23 (85.2%)	22 (81.5%)
Deceased	8 (14.1%)	1 (3.7%)	5 (18.5%)
Unknown	3 (5.3%)	3 (11.1%)	0

[a]Somatic *RET* mutation $n = 1$; no somatic and no germline *RET* mutation $n = 2$.
[b]Lung $n = 13$, liver $n = 5$, bone $n = 2$, cerebral $n = 2$.
n/a, not applicable; SD, standard deviation; TNM, tumor node metastasis.

In 25 (43.9%) patients, prophylactic TE was performed, and 21 (36.8%) patients underwent therapeutic TE. Details of surgical treatment are given in Table 3.

Postoperative complications included unilateral recurrent laryngeal nerve (RLN) paralysis in four (25%) patients with a preoperatively functioning RLN. Due to extensive tumor invasion including preoperative RLN paralysis, unilateral RLN resection was performed in five patients and bilateral resection in two patients (requiring tracheostomy).

Permanent hypoparathyroidism was reported in 12 (26.7%) patients. Hypoparathyroidism occurred significantly more frequently in patients undergoing therapeutic TE compared with prophylactic TE ($P = 0.016$).

Postoperative calcitonin levels were not detectable in 12 (26.1%) patients, normalized in 15 (32.6%), and remained elevated in 19 (41.3%) patients.

Only a minority of patients ($n = 12$; 21.1%)—all with advanced local and/or metastatic disease—received additional therapy. Details of therapeutic modalities are given in Table 3. Seven (12.3%) patients underwent combinatorial treatment approaches.

At last follow-up, of 13 children and adolescents with metastatic disease at diagnosis, five (38.5%) had subsequently died due to their disease, one (7.7%) due to acute airway obstruction, and one (7.7%) due to an accident (both patients were extensively metastasized). Five patients did not achieve a complete remission (CR), subsequently developed new metastases or relapsed, respectively. Of the 13 patients with lymph node metastases at initial presentation, only three (23.1%) achieved a complete or partial remission, whereas 9 (69.2%) developed elevated calcitonin levels and metastases, respectively.

With regard to calcitonin levels, only one (7.1%) patient with calcitonin levels above 1000 pg/mL achieved a CR, five (35.7%) had died at last follow-up.

2.2 | Children with C-cell hyperplasia

In addition to patients with MTC, 17 children and adolescents with CCH were reported to the GPOH-MET registry. The mean age at diagnosis of CCH was 6.8 years (SD 3.9), and the mean follow-up was 1.6 years (SD 2.7).

Pathologic calcitonin levels (mean 13.3 pg/mL, SD 7.0) were reported in 77.8% ($n = 7$) of the patients. CCH most frequently occurred multifocal (66.7%). Apart from prophylactic TE, no additional treatment was given in these children. Neither paralysis of the RLN nor hypoparathyroidism was reported as postoperative complications following prophylactic TE for CCH. Until last follow-up, no child developed disease progression/relapse or PHEO.

2.3 | Children affected by MEN2 syndrome

MEN2 syndrome was reported in 70 (94.6%) children and adolescents; of these, 42 (60.0%) were diagnosed with MEN2A and 28 (40.0%) with MEN2B syndrome. MEN2A syndrome was inherited in 37 children (unknown in $n = 3$) and MEN2B syndrome in three children (unknown in $n = 4$).

The majority of children ($n = 24$, 66.7%) with MEN2A syndrome carried the pathogenic *RET* codon 634 mutation in the cadherin-like domain of exon 11 followed by pathogenic mutations in the tyrosine

TABLE 2 Overview of diagnostics and preoperative, postoperative, and follow-up levels of biomarkers in children and adolescents with MTC

	Children with MTC		MEN2A		MEN2B	
	($n = 57$)[a]		($n = 27$)		($n = 27$)	
Diagnostics						
Ultrasound of the neck						
Normal	17 (29.8%)		12 (44.4%)		4 (14.8%)	
Uncertain	4 (7.0%)		1 (3.7%)		17 (63.0%)	
Pathologic	25 (43.9%)		6 (22.2%)		3 (11.1%)	
Not done	11 (19.3%)		8 (29.6%)		3 (11.1%)	
Magnetic resonance imaging						
No tumor detectable	6 (10.5%)		1 (3.7%)		6 (22.2%)	
Tumor verified	19 (33.3%)		1 (3.7%)		14 (51.9%)	
Not done	32 (56.1%)		25 (92.6%)		7 (25.9%)	
PET imaging						
Negative	1 (1.8%)		1 (3.7%)		0	
Uncertain	1 (1.8%)		0		0	
Positive	6 (10.5%)		0		6 (22.2%)	
Not done	49 (86.0%)		26 (96.3%)		21 (77.8%)	
Biochemical markers	***Preoperative***	***Postoperative***	***Preoperative***	***Postoperative***	***Preoperative***	***Postoperative***
Calcitonin (ng/l; normal < 5 female, < 8.4 male)						
Not detectable	n/a	12 (21.1%)	n/a	9 (33.3%)	n/a	3 (11.1%)
Normal	5 (8.8%)	15 (26.3%)	5 (18.5%)	11 (40.7%)	0	4 (14.8%)
Pathologic	46 (80.7%)	19 (33.3%)	18 (66.7%)	1 (3.7%)	25 (92.6%)	15 (55.6%)
Unknown	6 (10.5%)	11 (19.3%)	4 (14.8%)	6 (22.2%)	2 (7.4%)	5 (18.5%)
Mean (range)	5015 (1-105 416)	6733 (0-106 250)	684 (1-13 000)	4 (0-41)	8369 (45-105 416)	12 765 (0-106 250)
CEA (ng/ml; normal < 2.5)						
Not detectable	n/a	1 (1.8%)	n/a	1 (3.7%)	n/a	0
Normal	12 (21.1%)	8 (14.0%)	8 (29.6%)	3 (11.1%)	4 (14.8%)	5 (18.5%)
Pathologic	15 (26.3%)	8 (14.0%)	1 (3.7%)	1 (3.7%)	12 (44.4%)	6 (22.2%)
Unknown	30 (52.6%)	40 (70.2%)	18 (66.7%)	22 (81.5%)	11 (40.7%)	16 (59.3%)
Mean (range)	65.3 (0.1-360.0)	42.7 (0.4-233.3)	1.6 (0.1-6.1)	8.8 (0.4-40.9)	101.7 (2.0-360.0)	63.3 (0.6-233.3)

n/a, not applicable; PET, positron emission tomography.
[a]No MEN syndrome in three patients.

kinase domain of exon 13 in codons 790 and 791 ($n = 6$; 16.7%). In six children with MEN2A syndrome, *RET* variant data were not available. For mutation patterns and clinical characteristics of children diagnosed with MEN2 syndrome, please refer to Figure 1 and Tables 1 to 3.

2.4 | Revisiting the ATA criteria in children affected by MEN2 syndrome

Referring to the ATA guideline in the current version (8th edition), 12 (19.4%) children were assigned to the "modest," 24 (38.7%) to the "high," and 26 (41.9%) to the "highest" risk group. Details of age, preoperative calcitonin levels, and events in patients in each risk group are given in Figure 1.

According to the ATA guideline, in the modest-risk group, TE is recommended above five years of age if basal calcitonin levels are within normal limits. Noteworthy, in this group, one child (carrying a mutation in codon 630) was diagnosed with MTC as early as one year of age. In only two (40.0%) of five children who presented with MTC due to pathogenic mutations in codons 611, 630, and 790, calcitonin levels were elevated according to age and gender (missing information in one patient). This highlights that calcitonin levels in pediatric *RET* carriers may be an insufficient biomarker. In line with this finding, in two adolescents affected by the pathogenic high-risk *C634* mutation, calcitonin levels at diagnosis of MTC (at 12 and 14 years of age) were within the normal range.

The ATA recommendations also take into account aggressiveness of the disease as reflected by the potential to develop metastatic

TABLE 3 Overview on treatment in children and adolescents with MTC

	Children with MTC ($n = 57$)[a]	MEN2A ($n = 27$)	MEN2B ($n = 27$)
Treatment			
Type of first surgery			
Prophylactic TE	25 (43.9%)[b]	21 (77.8%)	4 (14.8%)
Therapeutic TE	21 (36.8%)	2 (7.4%)	18 (66.7%)
Total TE	18	2	15
Subtotal thyroidectomy	2	0	2
Hemithyroidectomy	1	0	1
Lymphadenectomy only	3 (5.3%)	0	2 (7.4%)
Biopsy only	2 (3.5%)	1 (3.7%) (liver)	1 (3.7%) (lung)
Unknown	6 (10.5%)	3 (11.1%)	2 (7.4%)
*Second **surgery***	7 (12.3%)	0	7 (25.9%)
Radiotherapy			
No	53 (93.0%)	26 (96.3%)	24 (88.9%)
Yes	3 (5.3%)	0	3 (11.1%)
Unknown	1 (1.8%)	1 (3.7%)	0
Chemotherapy[c]			
No	50 (87.7%)	25 (92.6%)	22 (81.5%)
Yes	6 (10.5%)[d]	1 (3.7%)	5 (18.5%)
Unknown	1 (1.8%)	1 (3.7%)	0
Octreotide therapy			
No	52 (91.2%)	23 (85.2%)	26 (96.3%)
Yes	1 (1.8%)	1 (3.7%)	0
Unknown	4 (7.0%)	3 (11.1%)	1 (3.7%)
Radioiodine therapy			
No	52 (91.2%)	23 (85.2%)	26 (96.3%)
Yes	2 (3.5%)	1 (3.7%)	1 (3.7%)
Unknown	3 (5.3%)	3 (11.1%)	0
Compassionate use			
No	45 (78.9%)	23 (85.2%)	19 (70.4%)
Yes	9 (15.8%)[e]	1 (3.7%)	8 (29.6%)
Unknown	3 (5.3%)	3 (11.1%)	0

CCH, C-cell hyperplasia; LND, lymph node dissection, MTC, medullary thyroid carcinoma; n/a, not applicable; TE, thyroidectomy.
[a]No MEN syndrome in three patients.
[b]Due to increased calcitonin levels $n = 19$; on families wish $n = 6$.
[c]Consisting of either vincristine, carboplatin, and etoposide or vindesine, ifosfamide, dacarbazine, and adriamycin.
[d]Resulting in stable disease and progressive disease in three patients each.
[e]Interferon alpha $n = 2$, imatinib $n = 1$, vandetanib $n = 4$, sorafenib $n = 1$, and sunitinib $n = 1$.

disease. Advanced local (pT3/4) and metastatic disease (pM1) were present only in children affected by mutations in codons 634 and 918. In line with this, children carrying the *M918T* mutation were frequently affected by advanced local disease ($n = 13$; 50.0%), lymph node ($n = 18$; 69.2%), and distant metastases ($n = 10$; 38.5%). However, looking closely at the modest-risk group, in which MTC is assumed to be less aggressive, it is noteworthy that one child (aged 11 years; codon 790) presented with lymph node metastases (pN1) at diagnosis of MTC (missing information on calcitonin levels) and one child (codon 611) developed lymph node metastases three years after the diagnosis of MTC.

Except for one child carrying a codon 634 mutation (in whom distant metastases were already present at initial diagnosis of MTC), children dying due to MTC were only affected by the *RET* codon *M918T* mutation. Of the 17 children who subsequently developed any disease-associated event other than death, 13 children were carriers of the *M918T* mutation and one child of a codon 634 mutation.

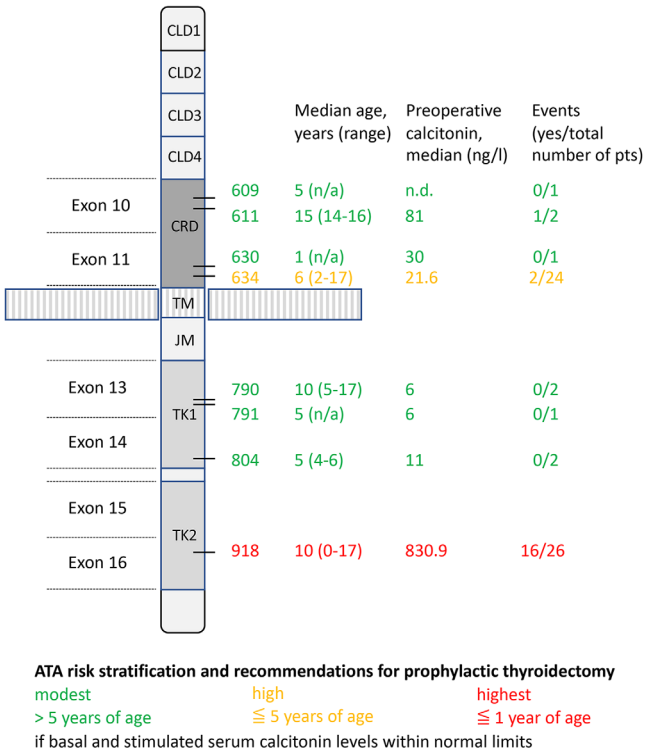

FIGURE 1 *RET* mutations, ATA risk stratification, and clinical presentation in 59 patients affected by MTC and CCH. ATA, American Thyroid Association; CCH, C-Cell hyperplasia; CLD, cadherin-like domain; CRD, cysteine-rich domain; green, modest risk; JM, juxtamembrane domain; pts, patients; red, highest risk; TK, tyrosine kinase domain; TM, transmembrane domain; yellow, high risk

The ATA guideline takes the lifetime risk to develop MTC, PHEO, and PHTP into account. In line with this, thus far, two children carrying the *RET* codon *M918T* mutation developed PHEO, whereas in children carrying "H" and "MOD" mutations, no other malignancy was reported so far.

2.5 | Prognostic factors for outcome

Differences in OS and EFS were observed between indication for thyroidectomy (prophylactic vs therapeutic), lymph node, and distant metastases status at diagnosis, and postoperative calcitonin levels (Figure 2A-D). Patients with MEN2B syndrome did worse when compared with those with MEN2A syndrome (10-year EFS 38% vs 78%, $P < 0.001$).

In multivariate analysis of EFS (including indication for thyroidectomy, pN status, pM status, postoperative calcitonin status, and type of MEN syndrome), the presence of lymph node metastases (HR = 10.92, 95% confidence interval [CI], 1.89-62.99, $P = 0.008$) and postoperative elevated calcitonin (HR = 7.34, 95% CI, 1.18-45.84; $P = 0.033$) were identified as significant adverse prognostic factors. The type of MEN2 syndrome had no significant influence on outcome (HR = 3.10, $P = 0.401$).

3 | DISCUSSION

This is the first report on children and adolescents diagnosed with MTC and CCH reported to the German GPOH-MET registry. In line with previous reports on patients with MTC,[1,2,4,6,8,9,11,19–22] children and adolescents presenting with localized disease at diagnosis of MTC had an excellent outcome, whereas children with metastatic disease did worse.

The standard treatment for MTC is the surgical removal of all thyroid and tumor tissue, respectively.[23,24] On the other hand, surgical cure is virtually impossible, when 10 or more lymph nodes are involved or serum calcitonin levels exceed 1000 ng/l.[25,26] In our study, 45% of patients presented with lymph node metastases and 23% with distant metastases at diagnosis. In line with known outcome predictors in MTC,[27,28] lymph node metastases and elevated calcitonin postoperatively (as a sign of persistent disease) were independently associated with poorer outcome on EFS in multivariable analyses.

MTC does not respond to radiotherapy and standard cytotoxic chemotherapy.[29,30] Therapeutic options other than surgery are scarce for patients with advanced disease. The multikinase inhibitors vandetanib and cabozantinib (including the inhibition of RET) have been approved for the treatment of inoperable (progressive) MTC.[31,32] In phase I/II trials of vandetanib in children, partial response rates of 47% to 58% have been reported.[33,34] Vandetanib was administered in a compassionate need intention to four of our patients with progressive disease. One patient subsequently died due to MTC, and the remaining patients are in progressive disease.

Pediatric (hereditary) MTC is deemed to be a paramount example of a striking genotype-phenotype correlation, including an age-related progression to MTC depending on the respective *RET* sequence variant. Corresponding to the ATA risk estimation, children carrying variants in codon 634 and particularly in codon 918 presented with more advanced and aggressive disease and had a poorer outcome than children carrying modest-risk variants. Of note, the type of MEN syndrome had no impact on EFS in multivariate analysis.

Our data clearly indicate that particular attention is required for carriers of modest-risk variants. The phenotype may be more variable, and recommendations are primarily based on few data confronting treating physicians with greater inaccuracy. This is impressively underlined by five children from our cohort carrying modest-risk variants, in whom MTC was diagnosed as early as one year of age, was accompanied by lymph node metastases, or was associated with calcitonin levels within the normal range.

Typically, MTC cells secrete calcitonin. For that reason, serum calcitonin is generally used as a biomarker for detection, staging, postoperative management, and prognostication in MTC patients.[35] However, in some cases, MTC cells do not secrete calcitonin and, thus, cannot be detected and followed by monitoring of this biomarker.[36] This is indeed crucial when monitoring carriers of pathogenic modest-risk *RET* mutations, in whom the ATA guidelines thus far

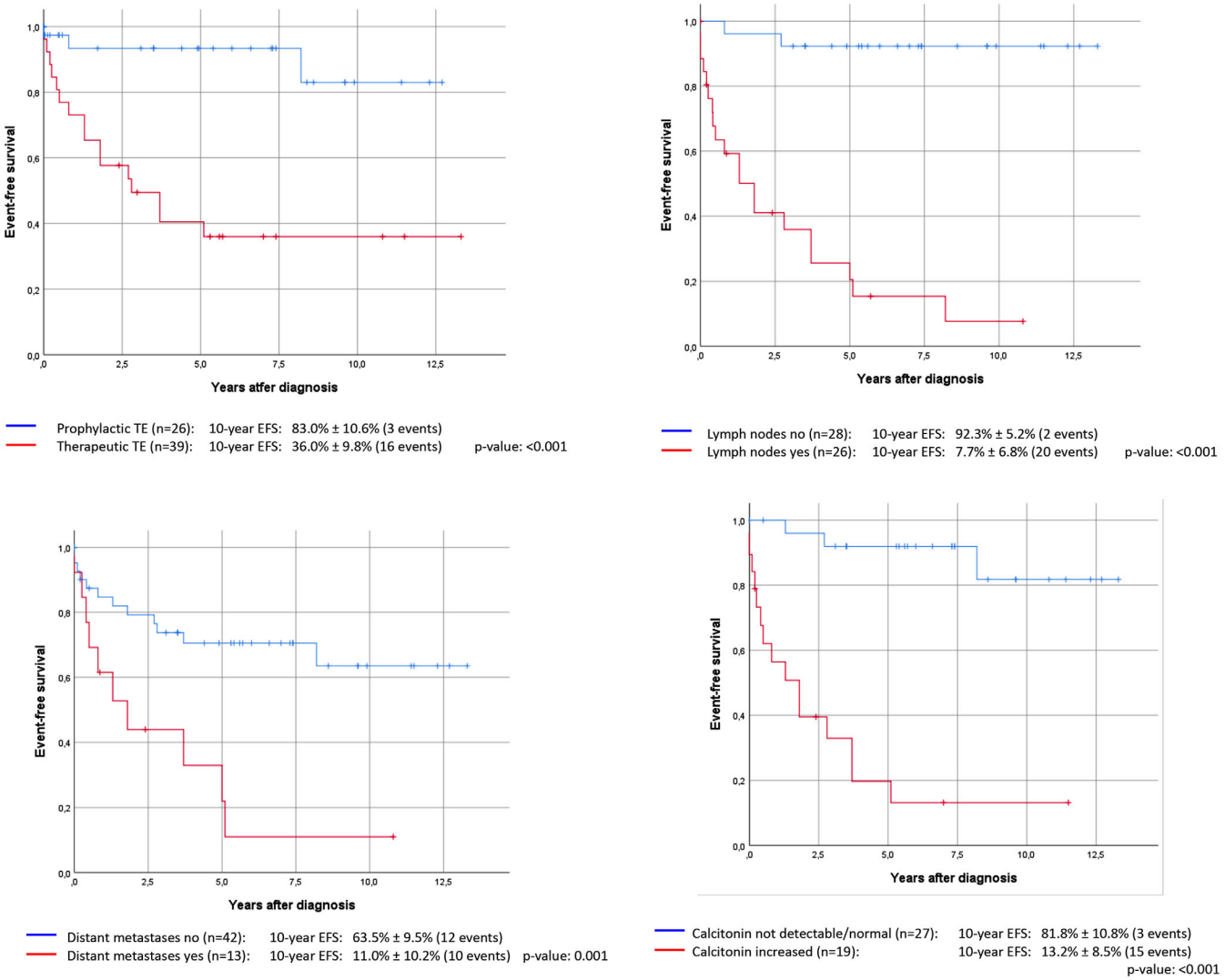

FIGURE 2 Probability of EFS depending on the indication for thyroidectomy **(A)**, the presence of lymph node metastases **(B)** and distant metastases **(C)** at diagnosis, and postoperative calcitonin levels **(D)**

recommend prophylactic TE based on calcitonin levels. According to our calcitonin data, this recommendation may be misleading at least in modest-risk mutation carriers. We suggest it may require revision based on larger patient numbers and include prophylactic TE in childhood.

Another feature of MEN2 syndrome is that predictive genetic testing is recommended as early as six months of age in families carrying the *M918T* variant.[37,38] By identifying carriers and performing prophylactic TE before the development of MTC, incidence and mortality of MTC in families affected by MEN2 syndrome have significantly been reduced.[39] In line with previous reports,[16,17] in 85.7% of children and adolescents affected by MEN2B syndrome, the aggressive *RET M918T* mutation occurred *de novo*, however. In these patients, prophylactic or early TE could not be performed; instead, MTC was diagnosed at an advanced and even metastatic stage. For these patients, novel therapeutic approaches are urgently needed.

4 | CONCLUSION

In children and adolescents enrolled in the GPOH-MET registry, lymph node status and postoperative elevated calcitonin were identified as significant prognostic factors for EFS. To avoid the occurrence of advanced MTC and to improve survival, the identification of children carrying *de novo RET M918T* mutations by means of the characteristic phenotype is crucial.

Notably, modest-risk mutation carriers presented with a variable phenotype including false-negative calcitonin levels and, thus, need particular attention.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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