Medullary Thyroid Carcinoma in MEN2A: ATA Moderate- or High-Risk *RET* Mutations Do Not Predict Disease Aggressiveness

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Context: High-risk *RET* mutations (codon 634) are associated with earlier development of medullary thyroid carcinoma (MTC) and presumed increased aggressiveness compared with moderate-risk *RET* mutations.

Objective: To determine whether high-risk *RET* mutations are more aggressive.

Design: Retrospective cohort study using institutional multiple endocrine neoplasia type 2 registry.

Setting: Tertiary cancer care center.

Patients: Patients with MTC and moderate- or high-risk germline *RET* mutation.

Intervention: None (observational study).

Main Outcome Measures: Proxies for aggressiveness were overall survival (OS) and time to distant metastatic disease (DMD).

Results: A total of 127 moderate-risk and 135 high-risk patients were included (n = 262). Median age at diagnosis was 42.3 years (range, 6.4 to 86.4 years; mean, 41.6 years) for moderate-risk mutations and 23.0 years (range, 3.7 to 66.8 years; mean, 25.6 years) for high-risk mutations ($P < 0.0001$). Moderate-risk patients had more T3/T4 tumors at diagnosis ($P = 0.03$), but there was no significant difference for N or M stage and no significant difference in OS ($P = 0.40$). From multivariable analysis for OS, increasing age [hazard ratio (HR), 1.05/y; 95% confidence interval (CI), 1.03 to 1.08], T3/T4 tumor (HR, 2.73; 95% CI, 1.22 to 6.11), and M1 status at diagnosis (HR, 3.93; 95% CI, 1.61 to 9.59) were significantly associated with worse OS but high-risk mutation was not ($P = 0.40$). No significant difference was observed for development of DMD ($P = 0.33$). From multivariable analysis for DMD, only N1 status at diagnosis was significant (HR, 2.10; 95% CI, 1.03 to 4.27).

Conclusions: Patients with high- and moderate-risk *RET* mutations had similar OS and development of DMD after MTC diagnosis and therefore similarly aggressive clinical courses. High-risk connotes increased disease aggressiveness; thus, future guidelines should consider *RET* mutation classification by disease onset (early vs late) rather than by risk (high vs moderate). **(*J Clin Endocrinol Metab* 102: 2807–2813, 2017)**

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Abbreviations: ATA, American Thyroid Association; CI, confidence interval; DMD, distant metastatic disease; HR, hazard ratio; MEN2, multiple endocrine neoplasia type 2; MTC, medullary thyroid carcinoma; OS, overall survival.

The risk stratification for hereditary medullary thyroid carcinoma (MTC), which is based on the individual *RET* mutation in multiple endocrine neoplasia type 2 (MEN2), has undergone substantial modification as genotype–phenotype correlations have evolved. The American Thyroid Association (ATA) previous A, B, C, and D schema (1) was recently replaced by moderate-, high-, and highest-risk categories in its 2015 revised guidelines for the management of MTC (recommendation 1) (2). The guidelines defined a highest-risk category (*RET* M918T mutations, formerly a level D mutation) as a distinct clinical entity with its own phenotype (MEN2B), poorer clinical outcomes, and increased MTC aggressiveness as compared with MEN2A (3, 4). Within MEN2A, the guidelines defined a high-risk *RET* category (codon 634 mutations, formerly level C) associated with more aggressive disease compared with a moderate-risk category (formerly levels A and B) (5–7). The concept of a categorization based on mutations associated with similar MTC disease aggressiveness is echoed in both the National Comprehensive Cancer Network and the North American Neuroendocrine Tumor Society consensus guidelines (8, 9). The current version of the ATA guidelines states that "aggressiveness was based on the development of MTC at an early age, frequently in association with metastatic disease" (2). This observation makes intuitive sense; individuals with earlier development of disease would also develop metastatic disease earlier, leading to worse outcomes, and therefore must have more virulent mutations.

The later onset of MTC in moderate-risk patients as compared with high-risk patients is well accepted within the field. However, to our knowledge, the assumed increased aggressiveness of MTC after diagnosis of disease in high-risk patients, especially over the long term, has never been systematically analyzed (10). The purpose of this study was to investigate whether patients with ATA high-risk *RET* mutations develop more aggressive MTC than do those with ATA moderate-risk mutations after MTC diagnosis, despite high-risk mutations having earlier-onset MTC.

Methods

Patient cohort

With approval from the institutional review board of the University of Texas MD Anderson Cancer Center, a prospectively maintained, comprehensive registry of patients with MEN2A was accessed and queried. The registry contains extensive demographic, genotypic, clinical, treatment, and pathology detail. Inclusion criteria for this study were a diagnosis of MTC and a moderate- or high-risk germline *RET* mutation as defined by the current ATA guidelines (2). Patients who underwent prophylactic thyroidectomy with negative final pathologic results or who were under continued surveillance for potential future development of MTC were excluded from all analyses. Patients with highest-risk *RET* mutations or those with mutations not currently classified in a current ATA risk level were also excluded.

Primary end points

A priori, the primary end points of overall survival (OS), disease-specific survival, and the time to development of distant metastatic disease (DMD; all from the time of initial diagnosis) were selected for analysis as suitable surrogate endpoints to assess clinical aggressiveness of MTC. Development of regional metastatic disease was not selected because its detection varies depending on the thoroughness of the preoperative radiologic staging and the initial surgical approach. In the final analytic cohort, the number of disease-specific deaths that could be verified was eight in the moderate-risk group and seven in the high-risk group, so disease-specific survival was not analyzed because of the small number of events. Therefore, OS and development of DMD were selected as the final proxies for aggressiveness.

The time of origin and the time of the event were determined as follows: for both end points (OS and DMD), the time of origin was the date of diagnosis of MTC, by biopsy or surgical excision, whichever came sooner. For OS, the date of death was the event of interest. For DMD, the first development of any metastatic disease outside the locoregional lymph node chains in the neck (*e.g.*, liver, lung, bone, or brain) met criteria for the event of interest. In other words, once a patient developed one site of DMD, the patient was considered to have experienced the event, and any subsequent sites of DMD were not counted toward the final time-to-event analysis. Those who developed DMD before or at the time of initial diagnosis (M1 status at diagnosis) were excluded from the DMD analysis. We believed this to be reasonable because the absolute number was small and statistically equivalent between the moderate- and high-risk groups ($P = 0.25$).

Statistical analysis

The Fisher exact test or χ^2 test was used to evaluate the association between two categorical variables, and the Kruskal–Wallis test or Wilcoxon rank-sum test was used to evaluate the difference in continuous variables among or between patient groups. The Kaplan–Meier method was used to analyze time-to-event end points, including OS and time to development of DMD, and the log-rank test was used to evaluate the difference between patient groups. Cox proportional hazards models were fitted to evaluate the effects of covariates deemed clinically important to time-to-event end points, including age at diagnosis, TNM status at diagnosis, and ATA risk level. A variable for index vs nonindex cases was initially included in the models but was not statistically significant; thus, it was dropped during model building. All tests were two sided, and P values <0.05 were considered to indicate statistically significant differences. We used SAS software, version 9.3 (SAS Institute, Cary, NC), and *S*-Plus software, version 8.2 (TIBCO Software Inc., Palo Alto, CA), for the analyses.

Results

The final analytic cohort contained 127 moderate-risk and 135 high-risk patients (n = 262). The number and distribution of germline *RET* mutations in each patient group are listed in Table 1. The moderate-risk mutation

Table 1. Number and Types of *RET* Mutations Included in Analytic Cohort

RET Mutation	Moderate Risk (n = 127), n (%)	High Risk (n = 135), n (%)
C609R/Y	37 (29.1)	—
609 (unspecified)	1 (0.8)	—
C611F/R/S/Y	7 (5.5)	—
C618F/G/R/S/Y	32 (25.2)	—
618 (unspecified)	1 (0.8)	—
C620F/G/R/S/W	19 (15.0)	—
620 (unspecified)	1 (0.8)	—
C634F/G/R/S/W/Y	—	130 (96.3)
634 (unspecified)	—	5 (3.7)
L790F	2 (1.6)	—
R912P	2 (1.6)	—
S891A/S	10 (7.9)	—
V804M	15 (11.8)	—

group was composed mostly of codon 609, 618, and 620 mutations, whereas the high-risk group consisted entirely of 634 mutations, by definition. Demographic characteristics for both the moderate-risk and high-risk groups are displayed in Table 2. Significantly more nonwhite patients were present in the high-risk group (P = 0.0094). The median age at diagnosis was 42.3 years (range, 6.4 to 86.4 years) for patients with moderate-risk mutations and 23.0 years (range, 3.7 to 66.8 years) for those with high-risk mutations ($P < 0.0001$). Moderate-risk patients had more T3/T4 tumors at diagnosis (P = 0.034, not shown in the table), but there was no significant difference between groups for N stage or M stage at diagnosis.

Treatment received by means of surgery, radiation therapy, and/or chemotherapy did not significantly differ between groups. There was also no difference in the percentage of patients who developed DMD or who died between the moderate- and high-risk groups. The median follow-up times were 9.03 years (range, 0.003 to 54.42 years) for the censored observations, 6.49 years (range, 0.016 to 47.30 years) for the moderate-risk group, and 11.50 years (range, 0.003 to 54.42 years) for the high-risk group.

The OS by ATA risk group obtained by the Kaplan–Meier method is displayed in Fig. 1. No significant difference in OS was observed for moderate- or high-risk groups (P = 0.40 by the log-rank test). From multivariable analysis for OS (Table 3), increasing age at diagnosis [hazard ratio (HR), 1.05 per year; 95% confidence interval (CI), 1.03 to 1.08], T3/T4 tumor (HR, 2.73; 95% CI, 1.22 to 6.11), and M1 status at diagnosis (HR, 3.93; 95% CI, 1.61 to 9.59) were significantly associated with an increased risk for death from all causes; high-risk mutation was not significantly associated with worse OS (P = 0.40).

The time to development of DMD obtained by the Kaplan–Meier method is displayed in Fig. 2. No difference was observed in the time to development of DMD between moderate- and high-risk groups (P = 0.33). From multivariable analysis for DMD (Table 4), only N1 status at diagnosis was significant (HR, 2.10; 95% CI, 1.03 to 4.27). High-risk mutation status, age, and T3/T4 tumor were not significantly associated with DMD.

Discussion

We sought to determine whether MTC behavior in patients with MEN2A who have high-risk *RET* mutations is more aggressive than MTC observed in patients harboring moderate-risk mutations. When the time of diagnosis of MTC is taken as the time of origin, we found in our institutional database of 262 patients with MEN2A that OS and the occurrence of DMD were statistically equivalent in patients within the moderate- and high-risk categories. Likewise, on multivariate analysis, moderate- vs high-risk category status was not associated with DMD or OS. These results question the perception of increased clinical aggressiveness of patients categorized as high-risk. Although individuals possessing high-risk mutations presented with MTC earlier than those with moderate-risk mutations by nearly two decades, once MTC developed, the clinical course was statistically equivalent in terms of distant metastasis and survival.

In a cohort of patients with hereditary MTC, we observed that age of diagnosis, T3 or T4 tumor at diagnosis, and M1 status at diagnosis were all independent predictors of increased risk for death from all causes. The presence of distant metastasis at time of diagnosis (M1 disease) was the factor most strongly associated with death. Interestingly, node positivity at diagnosis was not a statistically significant predictor of death, which is consistent with previous findings reporting no differences in node positivity between risk groups and could be due to the relatively slow progression of nodal metastatic disease (11). Our findings are also consistent with large-database analyses, in which no differentiation is made between hereditary MTC and sporadic disease (statistically predominant proportion) (12, 13). Esfandiari *et al.* (12) found that age, larger tumor size, and distant disease were associated with an increased risk for death for MTC in the National Cancer Database (1998 to 2005). A follow-up study by Youngwirth *et al.* (13), which included National Cancer Database data through 2012, found similar results with respect to T stage at diagnosis, demonstrating that extrathyroidal extension (which defines T3/T4 along with tumor size >4 cm) was associated with decreased survival.

Table 2. Demographic Characteristics of Moderate- and High-Risk *RET* Mutations

Characteristic	Moderate Risk (n = 127)	High Risk (n = 135)	*P* Value
Female, n (%)	88 (67.7)	81 (60.0)	0.19
White ace, n (%)	105 (82.7)	93 (68.9)	0.0094
Median age (range) (y)	42.3 (6.4–86.4)	23.0 (3.7–66.8)	<0.0001
Context of diagnosis			0.0001
Index case	70 (56.0)	43 (32.1)	
Nonindex case	55 (44.0)	91 (67.9)	
Unknown/missing	2	1	
T stage at diagnosis, n (%)			0.044
T1	59 (46.5)	70 (51.9)	
T2	27 (21.3)	20 (14.8)	
T3	16 (12.6)	10 (7.4)	
T4	9 (7.1)	4 (3.0)	
TX	16 (12.6)	31 (23.0)	
N stage at diagnosis, n (%)			0.37
N0	36 (28.3)	35 (26.1)	
N1	53 (41.7)	48 (35.8)	
NX	38 (29.9)	51 (38.1)	
M stage at diagnosis, n (%)			0.25
M0	105 (82.7)	121 (89.6)	
M1	10 (7.9)	7 (5.2)	
MX	12 (9.4)	7 (5.2)	
Nonoperative treatment, n (%)	2 (1.6)	4 (3.0)	0.74
Radiation	17 (13.8)	13 (9.8)	0.31
Systemic therapy			
Standard chemotherapy	4 (6.1)	8 (11.1)	0.37
Tyrosine kinase inhibitor	8 (12.3)	7 (9.7)	0.63
Liver metastasis, n (%)	22 (17.3)	26 (19.3)	0.99
Synchronous	4 (3.1)	5 (3.7)	
Metachronous	18 (14.2)	20 (14.8)	
Unknown timing	—	1 (0.7)	
Lung metastasis, n (%)	8 (6.3)	10 (7.4)	1.00
Synchronous	1 (0.8)	1 (0.7)	
Metachronous	7 (5.5)	8 (5.9)	
Unknown timing	—	1 (0.7)	
Bone metastasis, n (%)	18 (14.2)	15 (11.1)	0.86
Synchronous	5 (3.9)	4 (3.0)	
Metachronous	13 (10.2)	10 (7.4)	
Unknown timing	—	1 (0.7)	
Other metastasis, n (%)	8 (6.3)	5 (3.7)	0.75
Synchronous	3 (2.4)	1 (0.7)	
Metachronous	4 (3.1)	3 (2.2)	
Unknown timing	1 (0.8)	1 (0.7)	
Any metastasis, n (%)	27 (21.3)	28 (20.9)	0.46[a]
Deceased, n (%)	15 (11.8)	24 (17.8)	0.18[a]
Median age at death (range) (y)	59 (27–92)	53.5 (29–85)	0.20

[a] Also analyzed as time-to-event end points.

Where our results differ from these large-database studies is our failure to find an association of node positivity and increased risk for death. One explanation for the discordant findings may be in how our reference and comparison groups were constructed; N0 and NX were included in the reference group and compared with any N1 disease in this analysis, but Esfandiari *et al.* (12) compared N0 status to NX as well as to different numbers of positive nodes (1 to 5, 6 to 10, 11 to 15, ≥16) and found differences in survival between these groups. We were not able to divide our analysis into so many different nodal groups, given the limited number of patients included who had nodal disease, so our study may be underpowered in this regard and residual confounding may remain. However, we believe it is unlikely that the difference is attributed to hereditary disease.

For time to DMD, nodal status at diagnosis was the only factor that remained significant, a finding corroborated by Machens and Dralle (14), who found that lymph node disease increased risk for lung, liver, and bony metastases. From the surgical perspective, a thorough nodal dissection (when indicated in the surgical management of hereditary MTC) can provide not only therapeutic but also prognostic benefit, based on the

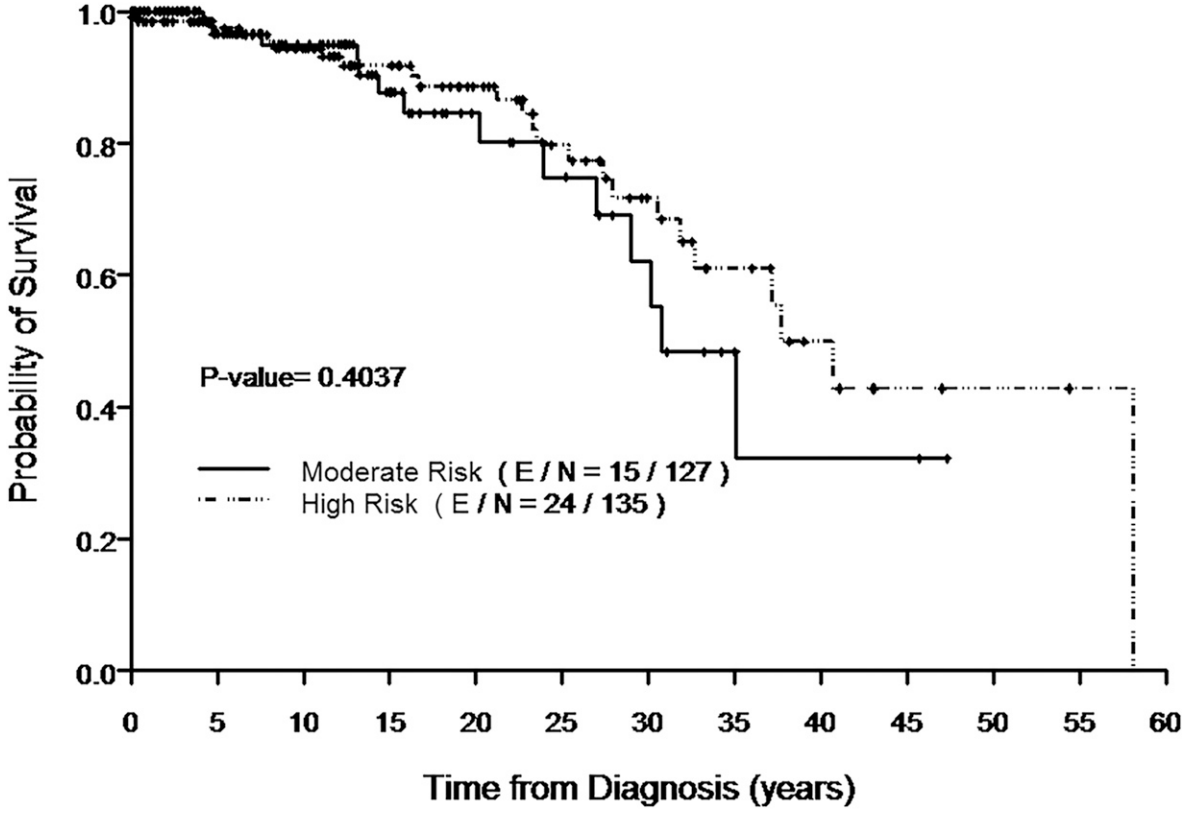

Figure 1. Kaplan–Meier curves of overall survival by ATA risk level. E, event; N, total number of patients.

results of this analysis; patients with positive nodes had a twofold higher risk for eventually developing DMD.

Our data support the widely held observation in the literature that patients who possess high-risk mutations develop MTC earlier than those with moderate-risk mutations (15, 16). An age-related penetrance among *RET* mutations is well established (7). We argue that even though patients with high-risk mutations develop MTC sooner, the clinical course of MTC after its emergence is statistically the same between the high- and moderate-risk categories. Indeed, the age at which MTC develops in an individual may not determine its long-term behavior. As the authors of this work have previously proffered, if we believe that a second or third genetic hit leads to transformation, it could be the nature of the second hit, rather than the specific *RET* mutation, that determines the long-term behavior of the tumor (10). The germline mutation may determine the age of onset of disease, and the second hit may be more responsible for the disease aggressiveness.

Table 3. Results of Cox Model Demonstrating Risk Factors for Hazard of Death

Variable	HR (95% CI)	*P* Value
Age at diagnosis (per year)	1.05 (1.03–1.08)	<0.0001
T3/T4 tumor (vs T1/T2/TX)	2.73 (1.22–6.11)	0.0145
N1 status (vs N0/NX)	1.25 (0.55–2.84)	0.60
M1 status (vs M0)	3.93 (1.61–9.59)	0.0026
MX status (vs M0)	1.79 (0.66–4.82)	0.25
High-risk mutation (vs moderate)	1.38 (0.66–2.89)	0.40

We propose a modified categorization system that emphasizes the age at onset of MTC instead of attempting to categorize risk; currently, categorization of risk connotes the clinical aggressiveness of a particular mutation, not just the chance of development of disease. We would not want an individual with a moderate-risk mutation who is diagnosed with MTC to be followed any less intensively than the same individual with a high-risk mutation because there is the perception that their disease behaves less aggressively; our data suggest this is not the case. We submit that because "high-risk" implies increased aggressiveness, future guidelines should consider *RET* mutation classification by probability of disease onset (early vs late) rather than by risk categorization (high vs moderate). Because the recommendation to genetically screen family members of individuals diagnosed with MTC has become the standard of care, we have the opportunity to perform earlier surgeries, which could result in lower-stage disease. In this setting, age at onset to prevent disease from occurring, not disease aggressiveness, will dominate the conversation.

This study had several limitations. First, some of the patient data in the analyzed institutional database were obtained through retrospective review; registrants were

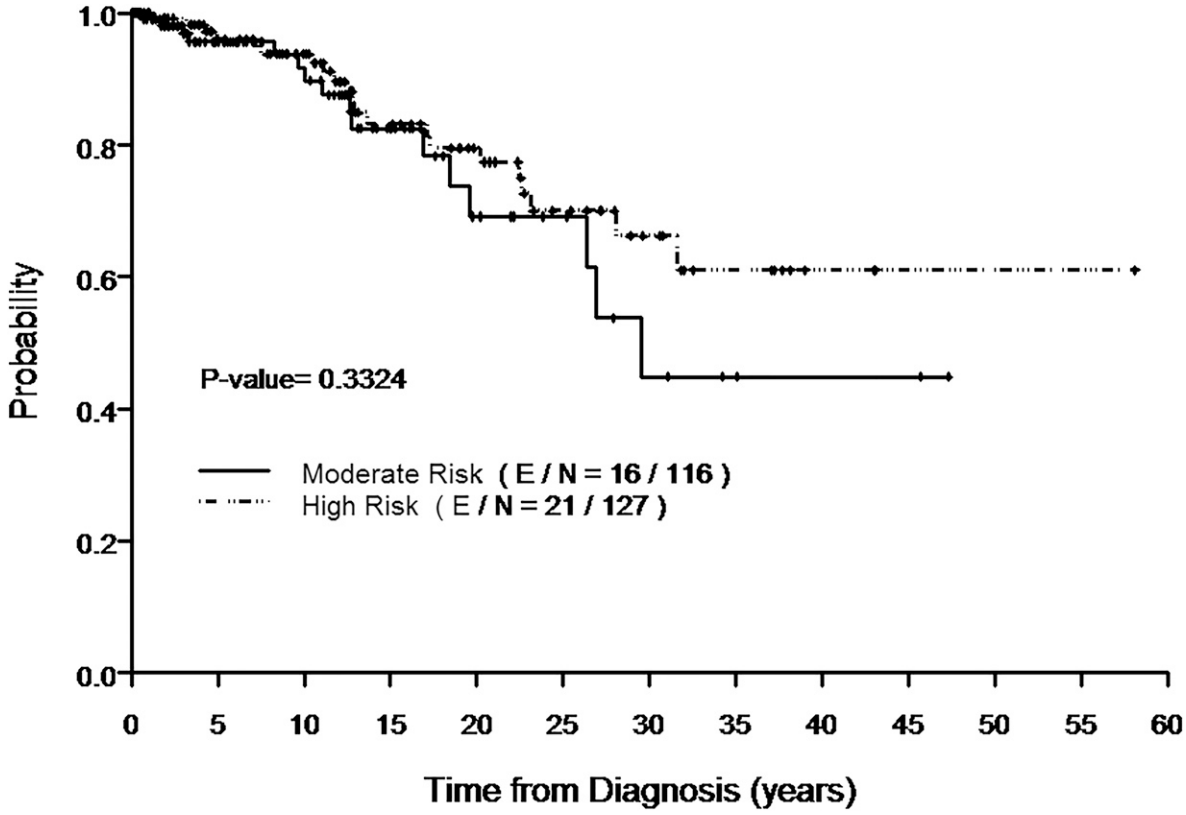

Figure 2. Kaplan–Meier curves of time to development of DMD by ATA risk level. E, event; N, total number of patients.

enrolled at various time points in the course of their disease and clinical and pathology detail had to be derived from their past medical records. Because of this, information on tumor size and nodal status at diagnosis was missing for a proportion of patients. To include patients with missing information (TX, NX, and MX), they were placed in the lower-risk group wherever possible (*e.g.*, T1/T2/TX vs T3/T4), although in actuality those with missing information generally had a survival curve that was in between that of the lower- and the higher-risk group. This likely led to some residual confounding and artificially pulled the point estimates closer to the null. Therefore, the true point estimates may be even more extreme than those reported here.

Another limitation is that the small number of events in our study prevented us from detecting small differences in survival with adequate power. Small study numbers of patients and events are a common problem in rare diseases, such as hereditary MTC, which reinforces the need to collaborate on a national and international level when possible.

The median follow-up for the moderate- and high-risk groups are quite different (6.5 years vs 11.5 years), in large part because the high-risk mutations were more commonly screened for a decade ago and were diagnosed almost two decades sooner than in the moderate-risk groups (mean age at diagnosis, 23 vs 42 years). Further long-term follow-up is essential in both of these groups, given that the malignant effects of MTC often occur over decades.

Table 4. Results of Cox Model Demonstrating Risk Factors for Hazard of Developing DMD

Variable	HR (95% CI)	*P* Value
Age at diagnosis (per year)	0.98 (0.95–1.01)	0.14
T3/T4 tumor (vs T1/T2/TX)	1.70 (0.69–4.16)	0.25
N1 status (vs N0/NX)	2.10 (1.03–4.27)	0.041
High-risk mutation (vs moderate)	0.72 (0.35–1.49)	0.38

Conclusions

We found that patients with MEN2A who had high- and moderate-risk germline *RET* mutations experienced similar OS and development of DMD after diagnosis, suggesting that MTC is similar in clinical aggressiveness after pathologic diagnosis. Because high-risk connotes increased clinical aggressiveness and worse outcomes, future guidelines should consider *RET* mutation classification by the probability of disease onset (early vs late) rather than categorizing risk.

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