Association of the Genomic Profile of Medullary Thyroid Carcinoma with Tumor Characteristics and Clinical Outcomes in an International Multicenter Study

Bin Xu,1,* Kartik Viswanathan,2,* Mahsa S. Ahadi,3–5 Sara Ahmadi,6 Bayan Alzumaili,1 Mohamed-Amine Bani,7 Eric Baudin,8 David Blake Behman,2 Marzia Capelletti,9 Nicole G. Chau,9 Federico Chiarucci,10 Angela Chou,3–5 Roderick Clifton-Bligh,3–5 Sara Coluccelli,10 Dario De Biase,11 Antonio De Leo,10 Snjezana Dogan,1 James A. Fagin,12 Talia L. Fuchs,3–5 Anthony Robert Glover,3 Julien Hadoux,3 Ludovic Lacroix,7 Livia Lamartina,8 Daniel J. Lubin,2 Catherine Luxford,3–5 Kelly Magliocco,2 Thais Maloberti,10 Abhinata S. Mohanty,1 Fedaa Najdawi,9 Aradhya Nigam,13 Alexander James Papachristos,3–5 Andrea Repaci,14 Bruce Robinson,3–5 Jean-Yves Scoazec,7 Qiuying Shi,2 Stan Sidhu,3–5 Erica Solaroli,15 Mark Sywak,3–5 R. Michael Tuttle,12 Brian Untch,13 Justine A. Barletta,9 Abir Al Ghuzlan,7 Anthony J. Gill,3–5 Ronald Ghossein,1,* Giovanni Tallini,10,* and Ian Ganly13,*

Purpose: The prognostic importance of RET and RAS mutations and their relationship to clinicopathologic parameters and outcomes in medullary thyroid carcinoma (MTC) need to be clarified.

Experimental Design: A multicenter retrospective cohort study was performed utilizing data from 290 patients with MTC. The molecular profile was determined and associations were examined with clinicopathologic data and outcomes.

Results: RET germ line mutations were detected in 40 patients (16.3%). Somatic RET and RAS mutations occurred in 135 (46.9%) and 57 (19.8%) patients, respectively. RETM918T was the most common somatic RET mutation (n = 75). RET somatic mutations were associated with male sex, larger tumor size, advanced American Joint Committee Cancer (AJCC) stage, vascular invasion, and high International Medullary Thyroid Carcinoma Grading System (IMTCGS) grade. When compared with other RET somatic mutations, RETM918T was associated with younger age, AJCC (eighth edition) IV, vascular invasion, extrathyroidal extension, and positive margins. RET somatic or germ line mutations were significantly associated with reduced distant metastasis-free survival on univariate analysis, but there were no significant independent associations on multivariable analysis, after adjusting for tumor grade and stage. There were no significant differences in outcomes between RET somatic and RET germ line mutations, or between RETM918T and other RET mutations. Other recurrent mo-
Molecular alterations included TP53 (4.2%), ARID2 (2.9%), SETD2 (2.9%), KMT2A (2.9%), and KMT2C (2.9%). Among them, TP53 mutations were associated with decreased overall survival (OS) and disease-specific survival (DSS), independently of tumor grade and AJCC stage.

**Conclusions:** RET somatic mutations were associated with high-grade, aggressive primary tumor characteristics, and decreased distant metastatic-free survival but this relationship was not significant after accounting for tumor grade and disease stage. RET<sup>3918T</sup> was associated with aggressive primary tumors but was not independently associated with clinical outcomes. TP53 mutation may represent an adverse molecular event associated with decreased OS and DSS in MTC, but its prognostic value needs to be confirmed in future studies.

**Keywords:** medullary thyroid carcinoma, grade, RET, RAS, prognosis

### Introduction

**Medullary Thyroid Carcinoma** (MTC) accounts for ~2% of all thyroid malignancies and 8% of thyroid cancer-related mortality. It may occur sporadically or in the setting of a germ line RET mutation. A large proportion of sporadic (i.e., non-germ line RET mutated) MTCs harbor somatic RET (particularly RET<sup>3918T</sup>) or RAS mutations, making them potential candidates for kinase inhibitor-targeted therapies. Some MTCs are wild type (WT) for Somatic alterations with various clinicopathologic parameters, including grading system (IMTCGS) based on mitotic count, Ki67 proliferation index, and/or tumor necrosis. Although data on RET germ line mutations were collected, the underlying molecular profile and its correlation with clinicopathologic features and outcome of MTC were not analyzed.

In this study, we investigated the molecular signatures of a large multicentric cohort of 290 patients with primarily resected MTC using the polymerase chain reaction (PCR)-based methods and 6 different next-generation sequencing (NGS) platforms. The aims were twofold: first, to examine the prognostic significance of somatic RET or RAS mutations and their associations with various clinicopathologic parameters, including IMTCGS grade; and second, to identify additional molecular alterations that could be associated with disease outcomes.

### Materials and Methods

**Study cohort**

This multicenter retrospective cohort study was approved by the Institutional Review Board (IRB) of each participating site (MSKCC 17-103). This study included 290 patients with primary MTC who underwent surgical resection between 1998 and 2021 at 6 tertiary centers (University of Bologna Medical Center [UB], Bologna, Italy; n = 64; Memorial Sloan Kettering Cancer Center [MSKCC], New York, NY; n = 54; Institut Gustave Roussy, Villejuif, France: n = 45; Brigham and Women’s Hospital [BWH], Boston, MA: n = 44; Royal North Shore Hospital, Sydney, Australia: n = 42, and Emory University Hospital Midtown [EU], Atlanta, GA: n = 41). Of these, 280 (97%) patients were included in the prior IMTCGS cohort or a subsequent validation study from EU.

All cases with a successfully sequenced primary MTC were included in this study. One center (BWH) included only patients with sporadic MTC, whereas cases from other centers also included patients with germ line RET mutations.

**Molecular platforms**

Somatic molecular alterations, including RET and RAS mutations, were detected using either PCR-based methods targeting only RET and/or RAS genes (n = 99) (Fluidigm multiplex PCR Access-Array or Agilent SureSelect XT HS platform) or by NGS using platforms in place at each of the six centers (n = 191), either commercially available or as described previously (briefly summarized in Supplementary Table S1). The number of cases tested with each NGS platform was as follows: custom-designed multigene sequencing panel with Thermo Fisher Scientific Ion GeneStudio S5 Prime System sequencer (n = 104), MSK-IMPACT (n = 54), ion Ampliseq<sup>TM</sup> cancer hotspot v. 2 (CHP2, n = 17; Thermo Fisher Scientific), OncoPanel (n = 14), Paradigm Cancer Diagnostics (PCDx) platform (n = 1; Paradigm Diagnostics), and CARIS NGS platform (n = 1; CARIS Life Sciences).

**Clinicopathologic characteristics, oncological outcomes, and statistical analyses**

Clinicopathologic review was carried out at each participating site as described previously. Oncological outcomes, including overall survival (OS), disease-specific survival (DSS), distant metastasis-free survival (DMFS), and locoregional recurrence-free survival (LRFRS), were calculated. Survival analyses were carried out using the Kaplan–Meier method and the log-rank test. Multivariable analyses examining associations between genomic profile and oncological outcomes were performed using the Cox proportional hazards models, adjusted for tumor grade and disease stage. In addition, comparisons of the clinicopathologic features among each molecular subgroup (RET germ line mutations, RET somatic mutations, RAS somatic mutations, and RET/RAS WT) were carried out using the chi-square test or Fisher’s exact test for categorical variables and two-tailed Student’s t-test for continuous variables. Statistical analyses were performed using the SPSS software 24.0 (IBM Corporation, Armonk, NY).

**Results**

**Clinicopathologic characteristics of the study cohort**

The median age of presentation was 57 years (range: 7–88 years, Table 1). The male-to-female ratio was 1:1.1.
### Table 1. Clinicopathologic Characteristics According to RET and RAS Mutation Status

<table>
<thead>
<tr>
<th></th>
<th>All cases (N = 290)</th>
<th>RET germ line mutations (n = 40)</th>
<th>RET somatic mutations (n = 133)</th>
<th>RAS somatic mutations (n = 56)</th>
<th>RET/RAS WT (n = 61)</th>
<th>p-Values (RET somatic vs. WT)</th>
<th>p-Values (RET germ line vs. WT)</th>
<th>p-Values (RET somatic vs. RET germ line)</th>
<th>p-Values (RAS vs. WT)</th>
<th>p-Values (RAS vs. WT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:female ratio</td>
<td>138:152 (1:1.1)</td>
<td>18:22 (1:1.2)</td>
<td>77:56 (1:0.7)</td>
<td>18:38 (1:2.1)</td>
<td>35:36 (1:1.4)</td>
<td>0.031</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Age, years</td>
<td>57 (7–88)</td>
<td>42 (7–66)</td>
<td>59 (22–88)</td>
<td>57 (7–79)</td>
<td>58 (25–84)</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Tumor size, cm</td>
<td>2.0 (0.1–11.0)</td>
<td>1.2 (0.1–6.0)</td>
<td>2.1 (0.4–8.0)</td>
<td>2.0 (0.3–11.0)</td>
<td>1.9 (0.3–6.0)</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>AJCC eighth edition overall stage</td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>I</td>
<td>92 (31.7)</td>
<td>13 (32.5)</td>
<td>29 (21.3)</td>
<td>24 (42.9)</td>
<td>26 (42.6)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>II</td>
<td>43 (14.8)</td>
<td>4 (10.0)</td>
<td>13 (9.6)</td>
<td>12 (21.4)</td>
<td>14 (23.0)</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
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<td>NS</td>
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<tr>
<td>III</td>
<td>41 (14.1)</td>
<td>11 (27.5)</td>
<td>17 (12.5)</td>
<td>7 (12.5)</td>
<td>6 (9.8)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IV</td>
<td>114 (39.3)</td>
<td>12 (30.0)</td>
<td>77 (56.6)</td>
<td>13 (23.2)</td>
<td>15 (24.6)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Vascular invasion</td>
<td>122 (42.1)</td>
<td>12 (30.0)</td>
<td>76 (55.9)</td>
<td>17 (30.4)</td>
<td>20 (32.8)</td>
<td>0.005</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Extrathyroidal extension</td>
<td>83 (28.8)</td>
<td>9 (22.5)</td>
<td>47 (34.6)</td>
<td>14 (25.0)</td>
<td>15 (25.4)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Positive margin</td>
<td>54 (18.6)</td>
<td>5 (12.5)</td>
<td>32 (23.5)</td>
<td>11 (19.6)</td>
<td>8 (13.1)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>First postoperative CEA, ng/mL</td>
<td>5 (0–38,335)</td>
<td>2 (0–600)</td>
<td>7 (0–26,300)</td>
<td>5 (1–539)</td>
<td>3 (0–38,335)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>First postoperative calcitonin, pg/mL</td>
<td>13 (1–970,000)</td>
<td>48 (1–7979)</td>
<td>24 (1–970,000)</td>
<td>5 (1–68,791)</td>
<td>4 (1–16,000)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Calcitonin doubling time</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Never doubled</td>
<td>105/163 (64.4)</td>
<td>23/32 (71.9)</td>
<td>40/75 (53.3)</td>
<td>19/27 (70.4)</td>
<td>23/31 (74.2)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Months if doubled</td>
<td>17 (3–139)</td>
<td>13 (3–139)</td>
<td>15 (3–84)</td>
<td>27 (8–35)</td>
<td>15 (3–99)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IMTCGS grade</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Low grade</td>
<td>218 (75.2)</td>
<td>33 (82.5)</td>
<td>88 (64.7)</td>
<td>47 (83.9)</td>
<td>50 (82.0)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>High grade</td>
<td>72 (24.8)</td>
<td>7 (17.5)</td>
<td>48 (35.3)</td>
<td>9 (16.1)</td>
<td>11 (18.0)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Necrosis</td>
<td>43 (14.8)</td>
<td>1 (25.9)</td>
<td>30 (22.1)</td>
<td>4 (71)</td>
<td>8 (131)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.002</td>
</tr>
<tr>
<td>Mitotic index, / 2 mm²</td>
<td>1 (0–29)</td>
<td>0 (0–7)</td>
<td>1 (0–18)</td>
<td>1 (0–9)</td>
<td>0 (0–7)</td>
<td>0.028</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.049</td>
</tr>
<tr>
<td>KI67, %</td>
<td>2 (0–58)</td>
<td>1 (0–15)</td>
<td>2 (0–30)</td>
<td>2 (0–58)</td>
<td>2 (0–30)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.016</td>
</tr>
<tr>
<td>DM at presentation</td>
<td>25 (8.7)</td>
<td>1 (2.5)</td>
<td>19 (14.4)</td>
<td>1 (1.8)</td>
<td>4 (6.7)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.047</td>
</tr>
<tr>
<td>Follow-up period, months</td>
<td>48 (0–285)</td>
<td>33 (0.4–285)</td>
<td>45 (0–228)</td>
<td>55 (1–268)</td>
<td>56 (0–226)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>21 (8.2)</td>
<td>4 (10.3)</td>
<td>14 (12.2)</td>
<td>1 (2.1)</td>
<td>2 (3.7)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Kinase inhibitor therapy</td>
<td>25 (8.7)</td>
<td>2 (5.0)</td>
<td>20 (15.2)</td>
<td>1 (1.8)</td>
<td>2 (3.3)</td>
<td>0.015</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as n (column %) for categorical variables, or median (range) for continuous variables.

aThe three cases with both RET germ line mutations and somatic RET or RAS mutations are classified as RET germ line mutations in this table.
AJCC, American Joint Committee Cancer; CEA, carcinoembryonic antigen; DM, distant metastasis; IMTCGS, International Medullary Thyroid Carcinoma Grading System; NS, not significant; WT, wild type.
American Joint Committee Cancer (AJCC) eighth edition stage IV, vascular invasion, extrathyroidal extension, positive margin, and IMTCGS high grade were identified in 39.3%, 42.1%, 28.8%, 18.6%, and 24.8% of the cases, respectively. Twenty-one patients (8.2%) had distant metastasis at presentation. External beam radiation therapy (RT) and kinase inhibitors were given to 8.2% and 8.7% of patients, respectively. The tyrosine kinase inhibitors (TKIs) used in the familial setting were associated with younger age at presentation (median age: 42 years in RET germ line mutation group compared with 58 years in WT; \( p < 0.001 \)). Other clinicopathologic characteristics did not differ between the two groups. Similarly, the only significant difference between the index cases (proband, first case identified to carry a germ line RET mutation) and sporadic MTCs was younger age at presentation in the index group (index cases: median age 43 years, range 9–65 years; sporadic MTC: median age: 58 years, range 22–88 years).

MTCs with RET somatic mutations were associated with aggressive tumor characteristics at presentation compared with WT MTC, including larger tumor size (median size: 2.1 cm in RET somatic mutations, 1.9 cm in WT; \( p = 0.036 \)), AJCC stage IV (56.6% in RET somatic mutations, 24.6% in WT; \( p < 0.001 \)), vascular invasion (55.9% in RET somatic mutations, 32.8% in WT; \( p = 0.005 \)), and high IMTCGS grade (35.3% in RET somatic mutations, 18.0% in WT; \( p = 0.027 \)). In addition, patients with RET somatic mutations were more commonly male (male-to-female ratio: 1:0.7 in RET somatic mutation group, 1:1.4 in WT) and more commonly treated with kinase inhibitor therapy (15.2% in RET somatic mutation group, 3.3% in WT group), which could be explained by selection criteria to receive such treatment.

Similarly, compared with MTCs with RET germ line mutations, the presence of RET somatic mutations was associated with more aggressive disease, as characterized by a larger tumor size (median 2.1 cm in RET somatic mutation group, 1.2 cm in RET germ line mutation group, \( p = 0.006 \)), AJCC stage IV (56.6% in RET somatic mutations, 30.0% in RET germ line mutations, \( p = 0.006 \)), vascular invasion (55.9% in RET somatic mutations, 30.0% in RET germ line mutations, \( p = 0.007 \)), tumor necrosis (22.1% in RET somatic mutations, 2.5% in RET germ line mutations, \( p = 0.002 \)), high mitotic count (median 0/2 mm\(^2\) in RET somatic mutations, 1/2 mm\(^2\) in RET germ line mutations, \( p = 0.049 \)), high Ki67 proliferation rate (median 1% in RET somatic mutations, 2% in RET germ line mutations, \( p = 0.016 \)), and distant metastasis at presentation (14.4% in RET somatic mutations, 2.5% in RET germ line mutations, \( p = 0.047 \)).

Clinicopathologic features of MTCs with RET somatic mutations and WT MTCs were not significantly different. RET\(^{M918T}\) somatic mutation was considered a high-risk mutation in this study. When compared with other RET somatic mutations, RET\(^{M918T}\) was more commonly associated with younger age (median age: 52 years in RET\(^{M918T}\), 63 years in other RET somatic mutations), AJCC stage IV (69.3% in RET\(^{M918T}\), 40.0% in other RET somatic mutations; \( p < 0.001 \)), vascular invasion (65.3% in RET\(^{M918T}\), 35.3% in other RET somatic mutations; \( p = 0.015 \)), extrathyroidal extension (46.7% in RET\(^{M918T}\), 20.0% in other RET somatic mutations; \( p = 0.002 \)), and positive surgical margin (30.7% in RET\(^{M918T}\), 15.0% in other RET somatic mutations, \( p = 0.042 \)). Other clinicopathologic parameters did not differ between MTCs with RET\(^{M918T}\) and those with other RET somatic mutations.

**Relationship between RET/RAS mutations and clinicopathologic parameters**

The clinicopathologic characteristics listed according to RET and RAS mutations status are shown in Table 1.

<table>
<thead>
<tr>
<th>Clinicopathologic Parameter</th>
<th>RET Somatic Mutations</th>
<th>WT MTCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Size (cm)</td>
<td>2.1 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>AJCC Stage IV</td>
<td>56.6%</td>
<td>30.0%</td>
</tr>
<tr>
<td>Vascular Invasion</td>
<td>55.9%</td>
<td>32.8%</td>
</tr>
<tr>
<td>IMTCGS Grade</td>
<td>35.3%</td>
<td>18.0%</td>
</tr>
</tbody>
</table>

**RET germ line mutations**

After excluding the BWH subgroup, which underwent preselection so that it comprised only somatic MTCs, the frequency of RET germ line mutations was 16.3% (40/246, Table 1 and Fig. 1 and Supplementary Table S2). Among the 39 cases with family history available, 19 (49%) were index cases (proband), whereas the remaining 20 (51%) had a family history.

The most frequently detected germ line mutations were C690Y (n = 14), C634R (n = 8), and G533C (n = 3). Among these familial MTCs, RET or RAS somatic mutations were detected in three cases: one case had germ line RET\(^{N791F}\) and somatic RET\(^{N918K}\), one had germ line RET\(^{V804Y}\) and somatic RET\(^{M918T}\), and the third had RET\(^{C634R}\) germ line mutation and HRAS\(^{G12V}\) somatic mutation. None of these three patients received kinase inhibitor therapy.

**RET somatic mutations and copy number alteration**

RET somatic mutations were detected in 135 cases (46.9% overall, 53.6% in sporadic MTCs, Table 1 and Fig. 1 and Supplementary Table S2). Among them, RET\(^{M918T}\) was the most common somatic mutation, being detected in 75 cases. Other common RET somatic mutations were located at codons 634 (n = 16), 630 (n = 11), 620 (n = 7), and 883 (n = 6).

RET copy number alteration was analyzed in 68 MTCs using MSK-IMPACT or OncoPanel platform. Two cases showed RET amplification (>2-fold gain), three had RET gain (1.3- to 1.9-fold gain), and two had deletion. All seven cases also harbored RET somatic mutations.

**RAS somatic mutations**

RAS somatic mutations were examined in 288 cases, and were identified in 57 MTCs (19.8%, Table 1 and Fig. 1 and Supplementary Table S2), including HRAS mutations in 37 cases (Q61R = n = 21, G13R = n = 10, Q61K = n = 3, G61L = n = 2, and G13V = n = 1), NRAS mutations in 20 cases (G12R = n = 8, G12V = n = 3, Q61R = n = 3, G61L = n = 1, G61K = n = 1, D54N = n = 1, A18D = n = 1, P34L = n = 1, and C186Msfs*16 = n = 1), and NRAS\(^{G61R}\) mutation in 1 case. One MTC harbored two RAS somatic mutations, being HRAS\(^{G13R}\) and KRAS\(^{G12V}\). RAS and RET somatic mutations were mutually exclusive.

The allele frequency was available in 91 MTCs with RET somatic mutations and 43 cases with RAS somatic mutations, and it did not differ between the 2 groups (\( p = 0.750 \)). The median allele frequency for RET and RAS somatic mutation was 35% and 36%, respectively.

In our cohort, the 61 MTCs (21.0%) without RET (somatic or germ line) or RAS mutations were grouped as RET/RAS WT.
FIG. 1. *RET* germ line and somatic mutations in MTC and their impact on clinical outcome. (A) Lollipop plot showing sites of mutations. (B) Oncoprint showing the clinicopathologic features and mutation profile of MTCs. (C–F) Kaplan–Meier curves for OS (C), (D), (E), and (F). *RET* mutations, somatic or germ line, are associated with decreased LRRFS and DMFS. OS and DSS do not differ according to *RET* or RAS mutation status. Cadherin, cadherin domain; DMFS, distant metastasis-free survival; DSS, disease-specific survival; LRRFS, locoregional recurrence-free survival; MTC, medullary thyroid carcinoma; OS, overall survival; Pkinase_Tyr, protein tyrosine kinase; TM, transmembrane.
Lastly, when comparing MTCs with RET somatic mutations and those without RET somatic or germ line mutations (RET WT, which included tumors with RAS mutations), RET somatic mutation was significantly associated with male sex, AJCC stage IV, vascular invasion, tumor necrosis, IMTCGS high grade, distant metastasis at presentation, higher frequency of cases whose postoperative calcitonin did not double, postoperative RT, and TKI therapy ($p < 0.05$, Supplementary Table S4).

**Relationship between RET and RAS mutations and oncological outcomes**

RET or RAS mutations were not significantly associated with OS or DSS (results are shown in Table 2 and Fig. 1). Compared with RET/RAS WT MTCs, those with RET somatic or RET germ line mutations were associated with poorer DMFS (RET somatic mutations: $p = 0.010$, RET germ line mutations: $p = 0.045$) in univariate analysis. The 10-year DMFS in MTCs with RET somatic mutations, in MTCs with RET germ line mutations, and in WT MTCs was 49%, 47%, and 75%, respectively. Similarly, RET somatic mutations were associated with decreased DMFS and LRRFS when compared with RET WT MTC ($p < 0.001$ and $p = 0.003$, respectively, Supplementary Table S4) in univariate analyses. Compared with other RET somatic mutations, RET$^{M918T}$ somatic mutations were not significantly associated with OS, DSS, DMFS, or LRRFS (Supplementary Table S3).

There was also a nonstatistically significant trend for RAS-mutated MTCs to be associated with improved DMFS (10-year DMFS in RAS-mutated MTCs: 88%, $p = 0.081$). The RET or RAS mutation profile was not significantly independently associated with DMFS in multivariable analyses adjusted for IMTCGS grade and AJCC stage (Supplementary Table S5, $p > 0.05$). IMTCGS grade and AJCC stage were significantly independently associated with DMFS (IMTCGS grade: hazard ratio 2.031 [confidence interval, CI 1.230–3.353], $p = 0.006$; AJCC stage IV: hazard ratio 1.478 [CI 1.221–1.789], $p < 0.001$).

**Table 2. Impact of RET and RAS Mutation on Prognosis in Medullary Thyroid Carcinoma (Log-Rank Test)**

<table>
<thead>
<tr>
<th>RET mutation</th>
<th>OS</th>
<th>DSS</th>
<th>DMFS</th>
<th>LRRFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET somatic vs. RET germ line</td>
<td>0.387</td>
<td>0.556</td>
<td>0.745</td>
<td>0.630</td>
</tr>
<tr>
<td>RET somatic vs. RET/RAS WT</td>
<td>0.748</td>
<td>0.854</td>
<td><strong>0.010</strong></td>
<td>0.118</td>
</tr>
<tr>
<td>RET germ line vs. RET/RAS WT</td>
<td>0.422</td>
<td>0.557</td>
<td><strong>0.045</strong></td>
<td>0.156</td>
</tr>
<tr>
<td>RAS mutated vs. RET/RAS WT</td>
<td>0.406</td>
<td>0.407</td>
<td>0.081</td>
<td>0.193</td>
</tr>
<tr>
<td>RET$^{M918T}$ somatic vs. other RET somatic</td>
<td>0.202</td>
<td>0.328</td>
<td>0.375</td>
<td>0.531</td>
</tr>
</tbody>
</table>

Values in the table are $p$-values. Bold values: significant $p$-values. DMFS: distant metastasis-free survival; DSS, disease-specific survival; LRRFS, locoregional recurrence-free survival; OS, overall survival.

The relationship of other gene mutations with oncological outcomes

Mutations other than RAS or RET were searched in 191 cases using 6 different NGS platforms. Their overlap with RET or RAS mutations is illustrated in Figure 1 and their features are detailed in Supplementary Table S6. In 30 of 38 cases (78%), mutations were secondary events in RET- or RAS-mutated tumors (RAS mutations: $n = 8$; RET somatic mutations: $n = 21$; RET germ line mutation: $n = 1$).

Recurrent somatic mutations or fusions included TP53 (8/191, 4.2%), PTK3CA (2/191, 1.0%), VHL (2/191, 1.0%), TERT promoter mutation (2/191, 1.0%), ATM (2/87, 2.3%), ARID2 (2/70, 2.9%), SETD2 (2/70, 2.9%), KMT2A (2/70, 2.9%), and KMT2C (2/70, 2.9%). TP53 mutations were associated with decreased OS (log-rank test, $p = 0.027$, Fig. 2), DSS ($p = 0.008$), and LRRFS ($p = 0.018$), with no statistically significant trend toward shortened DMFS ($p = 0.074$).

On multivariable survival analyses adjusted for IMTCGS grade and AJCC stage, TP53 mutations remained independently associated with reduced OS (hazard ratio 5.150 [CI 1.130–23.465], $p = 0.034$) and DSS (hazard ratio 6.078 [CI 1.279–28.887], $p = 0.023$), but not LRRFS (hazard ratio 2.952 [CI 0.892–9.764], $p = 0.076$). There was no significant association between TP53 mutations and IMTCGS grade or AJCC stage ($p = 1.000$).

Other molecular alterations were not significantly associated with clinicopathologic characteristics or survival outcomes.

**Discussion**

The key findings of this largest MTC molecular study are as follows: (1) RET somatic mutations were associated with aggressive tumor characteristics, high IMTCGS grade, and decreased DMFS in univariate analysis but not in multivariable analyses adjusted for tumor grade and disease stage; (2) MTCs with RET$^{M918T}$ somatic mutations showed more aggressive features in the primary tumor (such as AJCC stage IV, vascular invasion, extrathyroidal extension, and positive margin), but were not independently associated with survival outcomes; (3) RET and RAS mutations were not significantly associated with OS or DSS; and (4) a TP53 mutation (identified in 4.2% of MTCs) was identified as a novel potential adverse molecular event associated with reduced survival [indicating which survival outcomes].

Germ line RET mutations were detected in 16.3% of the study cohort, lower than the ~25% rate of familial cases generally reported in the literature. The relatively low frequency of germ line RET mutations may be explained by the selection bias of our study cohort toward adult patients: only four patients (1.4%) of our cohort were age ≤18 years. As the current American Thyroid Association (ATA) guideline recommended prophylactic thyroidectomy at a young age for patients with hereditary MTCs, such patients were likely not included in our cohort giving the selection bias toward adult patients.

Consistent with what has been previously reported, we found that RET germ line mutations affected codons 533, 609, 611, 618, 634, 791, 804, and 918, with codon 609 being the most prevalent. Overall, hereditary MTCs, including the index cases (proband), occurred at a younger age compared with sporadic MTCs, although it is known that the age of
MTC manifestation may differ depending on the type of RET germ line mutations. Recent studies had shown that germ line RETY791F was not associated with increased risk of MTC. It is therefore conceivable that it could represent a polymorphism. However, as the current ATA guideline regards RETY791F as pathogenic, we classified the single case with germ line RETY791F under the RET germ line mutation group.

RET somatic mutations have been identified in 45–70% of sporadic MTCs and have been shown to be associated with larger tumor size, nodal metastasis, distant metastasis, advanced overall stage, and decreased survival (overall and disease free). Similarly, we showed here that MTCs with RET somatic mutations were associated with larger tumor size, advanced AJCC stage, and decreased DMFS in univariate analysis but not in multivariable analysis, adjusted for tumor grade and AJCC stage. Together, these data imply that RET somatic mutation may be associated with more aggressive tumor characteristics and adverse outcomes, but its role in MTC may not be independent of grade and stage.

In a study of 100 sporadic MTCs, Elisei et al. identified RET somatic mutation and advanced stage as the only two factors independently correlated with persistent disease. There were several differences between their study and the current study. First, Elisei et al. included only sporadic MTCs, whereas our cohort contained both hereditary and sporadic cases. Second, RAS somatic mutation was not analyzed in the study by Elisei et al. Third, Elisei et al. examined associations between persistent disease and mutation/stage using a multivariable logistic regression model, and not a survival analysis. The fact that driver mutations are not independent from stage and other clinicopathologic factors such as grade is not unique to MTC. Indeed, the same scenario applies to follicular-derived thyroid carcinomas where the BRAFV600E mutation was not independent from clinicopathologic features such as stage in predicting mortality.

We show for the first time that RET somatic mutation is associated with high IMTCGS grade. Najdawi et al. previously reported that there was no significant association between RET somatic mutation and IMTGS grade in 44 sporadic MTCs. These 44 patients were also included in the current study. We were able to establish an association between IMTCGS high grade and RET somatic mutations in the current study for two reasons: first, we expanded the cohort size to 290 cases; and second, we excluded cases with RAS somatic mutations from the WT group.

Not all high IMTCGS grade tumors harbor RET somatic or germ line mutations. Among the 72 cases of high IMTCS grade MTCs, 55 (76.4%) had RET somatic or germ line mutations and 9 (12.5%) had RAS mutations, whereas the remaining 11 did not harbor RET or RAS mutations.

RET somatic mutation, the most prevalent RET somatic mutation in MTC, has been implied in several studies to be an adverse molecular signature. Schilling et al. analyzed the TP53 mutation in MTC. TP53 mutation is associated with decreased survival in MTC. Kaplan–Meier curves for OS, DSS, DMFS, and LRRFS. WT, wild type.
reported that \(RET^{M918T}\) was associated with decreased OS and increased risk of distant metastasis in 34 patients with sporadic MTCs. However, the authors only examined \(RET^{M918T}\) mutations in their study, and their control group theoretically included both MTCs with other \(RET\) somatic mutations and MTCs without \(RET\) mutations. Therefore, it was impossible to directly compare \(RET^{M918T}\) somatic mutations with other \(RET\) somatic mutations in this study. Romei et al.\(^\text{33}\) showed that \(RET^{M918T}\) mutations were associated with larger tumor size and were relatively infrequent in MTCs \(<1\) cm in size.

Moura et al. reported that MTCs with \(RET\) somatic mutations involving exons 15 and 16 (including 87% of M918T and 13% of A883F) had a higher prevalence of nodal metastasis, stage IV disease, and persistent disease compared with MTCs with other \(RET\) somatic mutations, in 52 sporadic MTCs.\(^\text{34}\) In contrast, Najdawi et al.\(^\text{19}\) showed that \(RET\) somatic mutations affecting exons 15 and 16 did not impact DSS and progression-free survival and did not correlate with IMTCGS grade. In the current study including 75 cases with \(RET^{M918T}\) somatic mutations and 60 cases with other \(RET\) somatic mutations, we find that \(RET^{M918T}\) somatic mutations are associated with younger age, advanced AJCC stage, and other aggressive tumor characteristics (e.g., vascular invasion, extrathyroidal extension, and positive margin) compared with MTCs with other \(RET\) somatic mutations. However, \(RET^{M918T}\) somatic mutations were not significantly associated with OS, DSS, DMFS, and LRRFS in MTC in this study.

\(RAS\) mutations are the predominant driver mutations in \(RET\)-WT sporadic MTC, being detected in 11% (range: 9–20%) of MTCs overall, 13% (range: 0–43%) of sporadic MTCs, and 61% (range: 0–81%) of \(RET\)-WT sporadic MTCs.\(^\text{7,9,10,35–38}\) Similarly, we detected \(RAS\) mutations in 19.8% of all MTCs, 22.4% of sporadic MTCs, and 44.9% of \(RE\)T-WT sporadic MTCs. Although we showed a nonsignificant trend of \(RAS\)-mutated MTCs with improved DMFS, overall \(RAS\) mutations were not associated with tumor characteristics or outcomes in MTC, confirming prior published research by Vuong et al.\(^\text{30}\)

The mutation data that we have presented suggest that additional molecular events other than \(RET\) or \(RAS\) mutations are required to explain the aggressive biological behavior seen in high-grade MTCs. In this respect, we showed that a \(TP53\) mutation may represent a novel prognostic molecular alteration in MTCs. \(TP53\) mutations are uncommon in MTCs, being reported in 9% (8/88 cases) in the literature\(^\text{38}\) and 4.2% in our cohort. In our series, \(TP53\) mutation nearly always coexisted with \(RET\) or \(RAS\) driver mutations.

The current study is the first to show that the \(TP53\) mutation is associated with decreased OS and DSS in MTC, independent of IMTCGS grade and AJCC stage. Interestingly, in the majority of our cases harboring mutations other than \(RET\) or \(RAS\), these mutations were secondary events (i.e., present in tumors already carrying \(RET\) or \(RAS\) mutations). These findings deserve further investigation to better understand the biology of MTC progression.

A limitation of the current study was the utilization of multiple molecular platforms resulting in a lack of uniformity on the molecular data. In addition, certain mutations may not be identified in the \(RET/RAS\) WT group due to the limitation of the testing platforms. This study may also be subject to selection bias as consecutive eligible cases in all institutions were not included and systematically collected data were not available to inform reporting of a participant flow diagram. Some selection bias may be reflected in the discrepancies in prevalence rates of some molecular events, relative to the published literature.

In summary, this international multicenter MTC consortium study enabled us to report detailed clinical, pathologic, and molecular characteristics of MTC. We have previously established and validated a prognostically relevant grading scheme.\(^\text{7}\) We herein present evidence that \(RET\) somatic mutations and \(TP53\) may also have some prognostic importance in MTC, although the prognostic relevance of \(RET\) somatic mutation is not independent of IMTCGS grade and AJCC stage and further studies are required to validate the prognostic values of \(TP53\) mutations. Our data represent valuable preliminary information to develop a prognostic nomogram to accurately stratify risk in individual patients with MTCs.

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Authors’ Contributions


Statistics: B.X.

Article drafting: B.X.


Author Disclosure Statement

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Supplementary Material
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References

Address correspondence to:
Ronald Ghossein, MD
Department of Pathology and Laboratory Medicine
Memorial Sloan Kettering Cancer Center
1275 York Avenue
New York, NY 10065
USA
E-mail: ghossein@mskcc.org