Clinical significance of RET and RAS mutations in sporadic medullary thyroid carcinoma: a meta-analysis

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Abstract

There are ongoing debates with respect to the prognostic roles of molecular biomarkers in sporadic medullary thyroid carcinoma (MTC). In this study, we aimed at investigating the prognostic value of RET and RAS mutations – the two most common mutations in sporadic MTCs. A search was conducted in four electronic databases. Relevant data were extracted and pooled into odds ratios (OR), mean differences (MD) and corresponding 95% confidence intervals (CI) using the random-effect model. We used Egger’s regression test and visual of funnel plots to assess the publication bias. From 2581 studies, we included 23 studies with 964 MTCs for meta-analysis. Overall, the presence of RET mutation was associated with an elevated risk for lymph node metastasis (OR = 3.61; 95% CI = 2.33–5.60), distant metastasis (OR = 2.85; 95% CI = 1.64–4.94), advanced tumor stage (OR = 3.25; 95% CI = 2.02–5.25), tumor recurrence (OR = 3.01; 95% CI = 1.65–5.48) and patient mortality (OR = 2.43; 95% CI = 1.06–5.57). RAS mutation had no significant prognostic value in predicting tumor aggressiveness. To summarize, our results affirmed that RET mutation is a reliable molecular biomarker to identify a group of highly aggressive sporadic MTCs. It can help clinicians better assess patient prognosis and select appropriate treatment decisions.

Key Words
- RET
- RAS
- mutation
- genetic alteration
- outcome
- survival
- recurrence
- relapse
- sporadic
- hereditary
- meta-analysis
- review

Introduction

Thyroid cancer is the most common malignancy of endocrine organs in which papillary thyroid cancer accounts for the majority of cases (Kondo et al. 2006). Most of thyroid cancer subtypes are derived from follicular cells, except MTCs, which originate from parafollicular C-cell (DeLellis et al. 2004). MTC is a well-differentiated thyroid tumor that shows an intermediate prognostic outcome between papillary and anaplastic thyroid cancer (DeLellis et al. 2004). It is generally accepted that MTCs occur either sporadically or in a hereditary form. About 25% of MTCs are hereditary and are associated with multiple endocrine neoplasia type 2 (MEN2) syndromes and familial MTC, which has been incorporated into the MEN2A category in the most recent guidelines (Wells et al. 2015). Several clinicopathological parameters have been reported to be indicators of poor prognosis in MTCs including age, male
gender and distant metastasis (Raue et al. 1993, Gulben et al. 2006).

The RET oncogene was first described in 1985 by Takahashi et al. (1985). Since then, over 100 genetic alterations involving RET have been found in patients with sporadic and hereditary MTCs. RET mutation occurs in virtually all cases of MEN2 syndrome and about 50% of sporadic MTCs (DeLellis et al. 2004, Elisei et al. 2008). The second most common mutation in MTCs is RAS mutation, prevalence of which ranges from 10 to 60% of RET-negative MTCs in various studies, with HRAS being the most common genotype (Goutas et al. 2008, Moura et al. 2011, Ciampi et al. 2013, Lyra et al. 2014). RET mutation has been shown to be associated with an increased risk of tumor relapse and mortality (Elisei et al. 2008, Mian et al. 2011). However, conflicting results were reported in other series and thus raises an ongoing debate regarding the prognostic value of RET mutation in MTCs (Dvorakova et al. 2008, Grubbs et al. 2016). On the other hand, the prognostic significance of RAS mutation in MTCs is still unclear.

In this systematic review and meta-analysis, we aimed at investigating the clinical and prognostic significance of the two most common mutations in sporadic MTCs: RET and RAS mutations.

Materials and methods

Search strategy and study identification

We performed a search in four electronic databases including PubMed, Scopus, Web of Science and Virtual Health Library and included articles published from inception to October 2017. We used the following search term: ‘(RET OR RAS) AND medullary thyroid’. Additionally, a manual search by reviewing the citations within the included publications and reviews was also carried out. There was no published protocol document or registration for this meta-analysis. Our study protocol generally followed the recommendation of Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement (Moher et al. 2009).

Selection criteria and abstract screening

After searching electronic databases, we imported search results into Endnote (Thomson Reuters). Two reviewers independently screened the titles and abstracts using the predetermined selection criteria. We used the following inclusion criteria: articles investigating the clinical significance (such as gender, age, multifocality, vascular invasion, extrathyroidal extension, pathological Tumor-Node-Metastases (pTNM) classification, AJCC stage, recurrence/persistence and survival outcomes) of RET and/or RAS mutations in sporadic MTC patients. The exclusion criteria were (i) studies only providing data of hereditary MTCs or germline RET mutation, (ii) studies in which data for somatic RET mutations could not be separately extracted from germline mutations, (iii) case report or case series only reporting group of patients harboring mutations, (iv) review and (v) conference, proceeding paper, poster, thesis, book. Discrepancies between two reviewers were solved by discussion and consensus.

Full-text screening and data extraction

Two reviewers screened the full-text of potential articles and extracted data into a predefined data extraction form. Disagreements were solved again by discussion and consensus. We extracted the following data from the included studies: institution, city, country, year of publication, study design, age, gender, multifocality, vascular invasion, extrathyroidal extension, pTNM classification, AJCC stage, recurrence/persistence and patient mortality.

Data analysis

The Review Manager 5.3 software (Cochrane Collaborative, Oxford, UK) was used for statistical analysis. We estimated mean and standard deviation (s.d.) value as described previously (Hozo et al. 2005). Pooled estimates of ORs, MDs and corresponding 95% CIs were calculated using random-effect model. We did not use the fixed-effect model in this study because the random-effect model takes into account within-study heterogeneity and provide the same results as the fixed-effect model when the between-study heterogeneity is not present (Borenstein et al. 2010).

For time-to-event data including disease-free survival and overall survival, we prioritized to calculate pooled hazard ratio (HR) using the random-effect model weighted by the inverse variance method. HR and its 95% CI were directly extracted from the original articles or indirectly estimated from Kaplan–Meier curve using the methods described by Tierney et al. for studies that did not provide HR and its 95% CI (Tierney et al. 2007). If there were insufficient data to pool HR, pooled OR and its 95% CI regarding the rough number of recurrence and patient death during follow-up were used for analyses. Additionally, we also investigated the prognostic
significance of somatic RET M918T mutation, the most common genotype of RET mutation in sporadic MTCs.

Heterogeneity among the included studies was investigated using the $I^2$ statistic, which is the percentage of the total variation between studies that cannot be attributed to chance (Higgins & Thompson 2002). We classified the inconsistency across the studies as low if $0% < I^2 \leq 25\%$, moderate if $25% < I^2 \leq 50\%$ and high if $I^2 > 50\%$. If there was a presence of heterogeneity among the included studies, we used sensitivity analysis by removing one study at a time to find the single study causing the heterogeneity. If a considerable heterogeneity still existed after performing sensitivity analysis, we carried out subgroup analysis by dividing into different subgroups (e.g., mutational genotypes, follow-up duration) to further explore the origins of heterogeneity.

Publication bias was analyzed by using Egger's regression test and funnel plot which were calculated using MAVIS, version 1.1.2 – an R package. A $P$ value $\leq 0.05$ was considered a statistically significant publication bias.

Risk of bias assessment

We assessed the methodologic quality of included studies based on the Newcastle–Ottawa Scale (NOS) for the quality of cohort and case–control in our meta-analyses (Wells et al. 2000). Stars were awarded for each cohort and case–control study (maximum 9 stars) based on a developed checklist (Wells et al. 2000). Studies awarded more than six stars were considered good-quality studies; those with a NOS value of five or six stars were regarded fair quality studies and those with a NOS value below five stars were poor-quality studies.

Results


All the included studies were retrospective cohort studies. Data regarding the clinicopathological significance of RET and RAS mutations in sporadic MTCs were available in 18 and 8 studies, respectively. Patient data from the studies (Mian et al. 2011, Cavedon et al. 2017) could overlap with each other. Additionally, patients from the studies (Romei et al. 1996, Elisei et al. 2008, Ciampi et al. 2017) were recruited from the same institution (University of Pisa). Furthermore, the studies by Zedenius et al. (1994, 1995) and Frisk et al. (2000) might share the same patient origin. In cases of suspicious overlapping patient data, we included the studies with higher number of MTC patients for meta-analysis.
Table 1 Characteristics of included studies.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Mutations</th>
<th>No. of patients</th>
<th>Median FU (months)</th>
<th>Gender</th>
<th>Age</th>
<th>pT</th>
<th>LNM</th>
<th>DM</th>
<th>Stage</th>
<th>RP</th>
<th>Death</th>
<th>S</th>
<th>C</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavedon et al. 2017</td>
<td>RET</td>
<td>106</td>
<td>40</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Cho et al. 2005</td>
<td>RET</td>
<td>20</td>
<td>NA</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>RET</td>
<td>7</td>
<td>156</td>
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<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ciampi et al. 2013</td>
<td>RAS</td>
<td>108</td>
<td>79*</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
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<td>35*</td>
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<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
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<td>Dvorakova et al. 2008</td>
<td>RET</td>
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<td>24</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>4</td>
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<td>3</td>
</tr>
<tr>
<td>Elisei et al. 2008</td>
<td>RET</td>
<td>100</td>
<td>122</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Frisk et al. 2000</td>
<td>RET</td>
<td>13</td>
<td>NA</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>N</td>
<td>N</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Goutas et al. 2008</td>
<td>RAS</td>
<td>44</td>
<td>NA</td>
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<td>N</td>
<td>N</td>
<td>Y</td>
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<tr>
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<td>RET</td>
<td>62</td>
<td>118</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>4</td>
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<tr>
<td>Lyra et al. 2014</td>
<td>RAS</td>
<td>62</td>
<td>NA</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>3</td>
</tr>
<tr>
<td>Mian et al. 2011</td>
<td>RET</td>
<td>60</td>
<td>39</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<td>Y</td>
<td>Y</td>
<td>4</td>
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<tr>
<td>Moura et al. 2009</td>
<td>RET</td>
<td>51</td>
<td>93*</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Moura et al. 2011</td>
<td>RAS</td>
<td>25</td>
<td>70*</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Nascimento et al. 2016</td>
<td>RET</td>
<td>10</td>
<td>NA</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Pasquali et al. 2011</td>
<td>RET</td>
<td>50</td>
<td>NA</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>4</td>
<td>0</td>
<td>3</td>
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<tr>
<td>Romei et al. 1996</td>
<td>RET</td>
<td>18</td>
<td>33*</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Schilling et al. 2001</td>
<td>RET</td>
<td>34</td>
<td>69</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>4</td>
<td>0</td>
<td>3</td>
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<tr>
<td>Schulten et al. 2011</td>
<td>RET</td>
<td>13</td>
<td>25</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>4</td>
<td>0</td>
<td>3</td>
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<tr>
<td>Simbolo et al. 2014</td>
<td>RET</td>
<td>20</td>
<td>NA</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Tiedje et al. 2016</td>
<td>RET</td>
<td>32</td>
<td>NA</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Zedenius et al. 1994</td>
<td>RET</td>
<td>10</td>
<td>48</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
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<td>Y</td>
<td>Y</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Zedenius et al. 1995</td>
<td>RET</td>
<td>46</td>
<td>123</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Y, indicates that data for the corresponding clinicopathological parameter were provided in the study; N, indicates that data for the corresponding clinicopathological parameter were not provided in the study.

*Mean follow-up value.

C, comparability; DM, distant metastasis; FU, follow-up; LNM, lymph node metastasis; NA, not available; O, outcome; RP, recurrence/persistence; S, selection.

The number of stars awarded to each of the included studies ranged from 6 to 7 (Table 1). None of the included studies was adjusted for important confounding factors (zero stars for the comparability domain).

All results concerning the associations between RET and RAS mutations and the clinicopathological features of sporadic MTCs are presented in Table 2.

**Male gender**

Pertinent data for RET mutations from 12 studies were included for analysis (Zedenius et al. 1994, Schilling et al. 2001, Dvorakova et al. 2008, Elisei et al. 2008, Moura et al. 2009, Mian et al. 2011, Schulten et al. 2011, Simbolo et al. 2014, Chuang et al. 2016, Grubbs et al. 2016, Nascimento et al. 2016, Tiedje et al. 2016). There was a statistical difference in male proportion between two groups (Supplementary Fig. 1A, see section on supplementary data given at the end of this article). After excluding the study by Nascimento et al. (2016), the heterogeneity was removed completely and the overall effect remained significant (OR = 1.76; 95% CI = 1.17–2.64; P = 0%).

Data for RAS mutation were available in six studies (Goutas et al. 2008, Moura et al. 2011, Ciampi et al. 2013, Lyra et al. 2014, Simbolo et al. 2014, Nascimento et al. 2016). The difference in male proportion between RAS-mutated (RAS-mut) and RAS-wild-type (RAS-wt) MTCs was not statistically significant (Supplementary Fig. 2A).

**Patient age**

Eligible data for RET and RAS mutations were available to extract from 11 studies (Zedenius et al. 1994, Schilling et al. 2001, Dvorakova et al. 2008, Elisei et al. 2008,
Table 2  Associations of RET, RAS mutation and RET M918T mutation with the clinicopathological parameters of sporadic medullary thyroid carcinoma.

<table>
<thead>
<tr>
<th>Clinicopathological features</th>
<th>No. of studies</th>
<th>No. of patients</th>
<th>OR (or MD)</th>
<th>95% CI</th>
<th>P (%)</th>
<th>P-Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>12</td>
<td>442</td>
<td>1.68</td>
<td>1.07–2.63</td>
<td>13</td>
<td>0.02</td>
</tr>
<tr>
<td>Age</td>
<td>11</td>
<td>435</td>
<td>–7.19b</td>
<td>–12.47 to –1.90</td>
<td>83</td>
<td>0.008</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>4</td>
<td>98</td>
<td>1.33</td>
<td>0.57–3.12</td>
<td>0</td>
<td>0.51</td>
</tr>
<tr>
<td>pT3/T4</td>
<td>12</td>
<td>471</td>
<td>2.31</td>
<td>1.55–3.45</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>10</td>
<td>437</td>
<td>3.61</td>
<td>2.33–5.60</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>11</td>
<td>463</td>
<td>2.85</td>
<td>1.64–4.94</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Advanced AJCC stage</td>
<td>7</td>
<td>349</td>
<td>3.25</td>
<td>2.02–5.25</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Recurrence/persistence</td>
<td>9</td>
<td>474</td>
<td>3.01</td>
<td>1.65–5.48</td>
<td>41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Patient mortality</td>
<td>7</td>
<td>372</td>
<td>2.43</td>
<td>1.06–5.57</td>
<td>33</td>
<td>0.04</td>
</tr>
<tr>
<td>RAS mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>6</td>
<td>265</td>
<td>0.65</td>
<td>0.33–1.28</td>
<td>0</td>
<td>0.21</td>
</tr>
<tr>
<td>Age</td>
<td>5</td>
<td>221</td>
<td>–2.53b</td>
<td>–8.58 to 3.53</td>
<td>39</td>
<td>0.41</td>
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<tr>
<td>pT3/T4</td>
<td>4</td>
<td>153</td>
<td>0.47</td>
<td>0.18–1.24</td>
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<td>0.13</td>
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<td>7</td>
<td>342</td>
<td>0.63</td>
<td>0.33–1.23</td>
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<td>Distant metastasis</td>
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<td>144</td>
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<td>0.30–3.70</td>
<td>0</td>
<td>0.94</td>
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<tr>
<td>Advanced AJCC stage</td>
<td>4</td>
<td>275</td>
<td>0.63</td>
<td>0.33–1.23</td>
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<tr>
<td>Recurrence/persistence</td>
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<td>231</td>
<td>0.57</td>
<td>0.23–1.42</td>
<td>0</td>
<td>0.22</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>5</td>
<td>158</td>
<td>0.92</td>
<td>0.48–1.76</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>Age</td>
<td>5</td>
<td>158</td>
<td>–7.4b</td>
<td>–11.94 to –2.86</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td>pT3/T4</td>
<td>4</td>
<td>140</td>
<td>1.83</td>
<td>0.91–3.68</td>
<td>0</td>
<td>0.09</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>4</td>
<td>143</td>
<td>4.95</td>
<td>1.89–12.94</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>4</td>
<td>146</td>
<td>2.82</td>
<td>1.08–7.37</td>
<td>0</td>
<td>0.03</td>
</tr>
<tr>
<td>Recurrence/persistence</td>
<td>3</td>
<td>142</td>
<td>4.82</td>
<td>1.13–20.60</td>
<td>53</td>
<td>0.03</td>
</tr>
<tr>
<td>Patient mortality</td>
<td>3</td>
<td>106</td>
<td>1.66</td>
<td>0.71–3.86</td>
<td>0</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Boldface, statistically significant result.

aP-Values were tested for the hypothesis that the OR is 1 or the MD is 0; bmean difference value.

CI, confidence interval; MD, mean difference; OR, odds ratio.

Moura et al. 2009, Mian et al. 2011, Schulten et al. 2011, Simbolo et al. 2014, Grubbs et al. 2016, Nascimento et al. 2016, Tiedje et al. 2016 and five studies (Moura et al. 2011, Ciampi et al. 2013, Lyra et al. 2014, Simbolo et al. 2014, Nascimento et al. 2016), respectively. Patients with RET-mutated (RET-mut) tumors showed a significantly younger age compared with RET-wild type (RET-wt) tumors (MD = –7.19; 95% CI = –12.47 to –1.90; P = 0.03) (Supplementary Fig. 1B). The high heterogeneity was considerably attenuated after removing the study by Elisei et al. and the significant result was unaffected (MD = –8.74; 95% CI = –10.91 to –6.58; P = 0.05) (Elisei et al. 2008).

There was no statistically significant difference in age of MTC patients with and without RAS mutations (MD = –2.53; 95% CI = –8.58 to 3.53; P = 0.39) (Supplementary Fig. 2B). The moderate heterogeneity between the included studies completely disappeared after excluding the study by Moura et al. (2011), but the meta-analysis result was changed to a significant result (MD = –5.78; 95% CI = –10.38 to –1.18; P = 0.01).

Vascular invasion

Only data for RET mutation extracted from four studies were used for meta-analysis (Dvorakova et al. 2008, Moura et al. 2009, Schulten et al. 2011, Chuang et al. 2016). Overall, the presence of RET mutation in sporadic MTCs was not associated with a greater probability of vascular invasion (OR = 1.33; 95% CI = 0.57–3.12; P = 0%) (Supplementary Fig. 1C).

Pathological tumor (pT) factor

significantly higher proportion of pT3/T4 (OR=2.31; 95% CI=1.55–3.45; P=0%) (Supplementary Fig. 1D).

The difference in proportion of pT3/T4 tumors between RAS-mut and RAS-wt was not statistically significant (OR=0.47; 95% CI=0.18–1.24; P=0%) (Supplementary Fig. 2C).

**Lymph node metastasis**


There was no significant association between the presence of RAS mutation and risk for nodal involvement in MTCs (OR=0.63; 95% CI=0.33–1.23; P=0%) (Supplementary Fig. 2D).

**Distant metastasis at time of diagnosis**

We included 11 studies (Schilling et al. 2001, Dvorakova et al. 2008, Elisei et al. 2008, Moura et al. 2009, Mian et al. 2011, Pasquali et al. 2011, Schulten et al. 2011, Simbolo et al. 2014, Chuang et al. 2016, Grubbs et al. 2016, Tiedje et al. 2016) and three studies (Moura et al. 2011, Ciampi et al. 2013, Simbolo et al. 2014) to investigate the association of RET and RAS mutations with distant metastasis, respectively. RET-mut MTCs showed a significantly higher propensity for distant metastasis (OR=2.85; 95% CI=1.64–4.94; P=0%) (Supplementary Fig. 1F).

The presence of RAS mutation in MTCs was not correlated with an increased risk for distant metastasis (OR=1.05; 95% CI=0.30–3.70; P=0%) (Supplementary Fig. 2E).

**Advanced AJCC stage**

Seven studies (Cho et al. 2005, Elisei et al. 2008, Moura et al. 2009, Schulten et al. 2011, Chuang et al. 2016, Grubbs et al. 2016, Cavedon et al. 2017) and four studies (Goutas et al. 2008, Moura et al. 2011, Ciampi et al. 2013, Cavedon et al. 2017) were included to analyze the association of RET and RAS mutations with advanced AJCC stage, respectively. We found that RET-mut MTCs were associated with advanced tumor stage III/IV in comparison with tumors without mutation (OR=3.25; 95% CI=2.02–5.25; P=0%) (Supplementary Fig. 1G).

On the other hand, the presence of RAS mutation in MTCs was not significantly associated with advanced tumor stage (OR=0.63; 95% CI=0.33–1.23; P=0%) (Supplementary Fig. 2F).

**Tumor recurrence and patient mortality**

There was only one study reporting the hazard ratio of RET mutations on disease-free survival (HR=2.68; 95% CI=1.21–5.92) (Pasquali et al. 2011). Kaplan–Meier plot or individual patient data on disease-free survival was provided in two additional studies (Schilling et al. 2001, Dvorakova et al. 2008). Data of RET mutations on disease-specific survival or overall survival was found in only one study (Elisei et al. 2008) and two studies (Mian et al. 2011, Grubbs et al. 2016), respectively. Therefore, we pooled OR and its 95% CI based on the number of events of recurrence and death during follow-up to explore the association of these mutations with patient outcomes.

Overall, data from nine and seven studies were pooled for association of RET mutation with tumor recurrence and patient death, respectively (Zedenius et al. 1995, Frisk et al. 2000, Schilling et al. 2001, Cho et al. 2005, Dvorakova et al. 2008, Elisei et al. 2008, Moura et al. 2009, Schulten et al. 2011, Chuang et al. 2016, Grubbs et al. 2016, Cavedon et al. 2017). In sporadic MTCs, RET mutation was an indicator of poor prognosis with increased risk for both tumor recurrence (OR=3.01; 95% CI=1.65–5.48; P=41%) (Supplementary Fig. 1H) and patient death (OR=2.43; 95% CI=1.06–5.57; P=33%) (Supplementary Fig. 1I). The moderate heterogeneity in the meta-analysis on tumor recurrence considerably attenuated after excluding one study (Zedenius et al. 1995) (OR=2.60; 95% CI=1.61–4.21; P=7%). Removing the study by Dvorakova et al. helped to reduce the among-study heterogeneity in the meta-analysis on patient death (OR=2.81; 95% CI=1.34–5.90; P=15%) (Dvorakova et al. 2008). The overall estimates following the leave-one-out method both remained significant.

Eligible data for RAS mutation was only sufficient to analyze the association of this mutation with tumor recurrence and data were found in 3 studies. The presence of RAS mutation in MTCs was not an indicator for tumor relapse (OR=0.57; 95% CI=0.23–1.42; P=0%) (Supplementary Fig. 2G).
Prognostic significance of somatic RET M918T in sporadic MTCs

Data regarding prognostic implication of this genotype were found in nine studies (Zedenius et al. 1994, 1995, Frisk et al. 2000, Schilling et al. 2001, Schulten et al. 2011, Simbolo et al. 2014, Grubbs et al. 2016, Tiedje et al. 2016). After excluding potentially overlapping study population, the presence of somatic RET M918T mutations was associated with increased risks for pT3/T4, nodal involvement, distant metastasis, advanced AJCC stage and tumor recurrence in sporadic cases (Table 2).

Between-study heterogeneity assessment

Heterogeneity among the included studies was absent in most of the meta-analyses. A high degree of heterogeneity was only present in the meta-analysis on association between age and RET mutation. This high heterogeneity was mainly driven by the study by Elisei et al. (2008), which was the only study showing an older age of patients harboring RET mutation in comparison with patients without RET in the study set. The age of RET-mut patients in the remaining ten studies was younger comparing with RET-wt patients and the significant overall effect was unchanged after excluding the study by Elisei et al. (P = 5%). A moderate heterogeneity was found in the meta-analysis on age for RAS mutation. However, the overall estimate was changed from insignificant to significant after omitting the study by Moura et al. (P = 0%) (Moura et al. 2011).

A moderate heterogeneity was also found in the meta-analyses on the association between RET mutation and recurrence/mortality. After performing the leave-one-out method, the between-study heterogeneity considerably reduced, and the overall estimates were robust. The sources of heterogeneity could be explained following the sensitivity analyses so we did not carry out subgroup analysis in this study.

Publication bias

Egger’s regression test and funnel plots were performed to assess the presence of publication bias. Funnel plots showed no clear evidence of asymmetry and Egger’s regression test indicated no evidence of publication bias. Representative funnel plots are shown in Supplementary Fig. 3.

Discussion

Sporadic MTC is usually present at an advanced stage at the time of diagnosis (Wells et al. 2015, Romei et al. 2016). Therefore, it is worth to search for clinicopathological or molecular parameters to identify those aggressive cases to tailor appropriate therapeutic treatment. With the rapid development of the translational medicine over the past three decades, the understanding of MTC pathogenesis and biology has been greatly improved. For that reason, it is important to investigate the role of molecular biomarkers to predict tumor behavior and patient survival in sporadic MTCs.

Our meta-analysis results confirmed that the presence of somatic RET mutation is a strong indicator of aggressive tumors with poor survival. RET mutation is not only a risk factor for tumor aggressiveness at diagnosis (higher rate of pT3/T4, lymph node and/or distant metastasis), but this genetic marker is also associated with an increased risk for tumor recurrence and patient mortality in sporadic MTCs during follow-up.

It is generally known that older age is a classic risk factor for poor survival in thyroid cancer. In our study, it is interesting to find out that the age of sporadic MTC patients harboring RET mutation is significantly younger than those patients without RET despite the fact that patients with RET mutation are associated with shorter survival. It suggests that the aggressiveness of these patients might be caused by the considerably higher propensity for tumor extension and metastases, which trump over the positive effect of younger age. Hereditary MTCs have been reported to be associated with multifocality and bilaterality, whereas sporadic tumors were unifocal (Kihara et al. 2016). We could not assess the association of RET mutation and multifocality in sporadic MTCs due to lack of data.

Most of MTC cases occur as sporadic tumors and somatic RET mutations have been found in 40–50% of these tumors with a mutation in codon 918 of RET gene being the most common mutation (Dvorakova et al. 2008, Elisei et al. 2008). In this study, we demonstrated that the presence of somatic RET mutation is a strong predictor of highly aggressive sporadic tumors with an increased propensity for tumor extension, nodal involvement, distant metastasis, tumor relapse and mortality. Our results also pointed out that the presence of RET M918T also has prognostic significance, and it is worth screening for this genotype routinely to better assess MTC patients’ prognosis. It is of clinical interest to investigate the prognostic importance of RET codon 918 in comparison with other RET mutations. In an Italian MTC cohort, 77% of RET M918T-mut tumors had lymph node metastasis, while 43% of RET codon 634 and 27% of RET-wt tumors showed nodal involvement (Elisei et al. 2008).
It is interesting to note that more than one RET mutation can be found in the same tumor, and these RET double-mutant cases, as well as those cases with RET complex mutations, showed a worse outcome (Romei et al. 2016). In the era of targeted therapies, the use of RET mutation not only has a therapeutic implication by predicting tumor aggressiveness but can also help to predict patients who will respond to RET-targeted treatment. Several multi-kinase inhibitors such as vandetanib and carbobantinib have been approved for the treatment of patients with progressive and metastatic MTCs (Cabanillas et al. 2014).

RAS mutation can be found in many histologic subtypes of thyroid neoplasms, particular in follicular thyroid tumors. In MTC, mutations in RAS genes are the second most common mutation and mutually exclusive with RET mutation (Ciampi et al. 2013, Ji et al. 2015, Romei et al. 2016). Our results showed that RAS mutation has no significant prognostic role in MTCs. However, it is interesting to note that a significant association of younger age with RAS mutation was obtained following the leave-one-out method. Therefore, this association should be interpreted with caution and further studies are required to clarify this result.

Our study is the first meta-analysis on the prognostic value of molecular biomarkers in MTCs. It can be of clinical benefit by helping clinicians better assess patient outcome and modify appropriate therapeutic decisions. However, there are a few limitations in our study that need to be outlined. All included studies were retrospective cohort, which could raise selection bias. Additionally, as we mentioned earlier, it is of clinical importance to investigate the prognostic implication of RET M918T in comparison with other RET genotypes. Unfortunately, other genotypes of RET mutations are rare, and we could not find enough data to clarify this relationship in this meta-analysis. Future large cohort studies are needed to answer this question.

In conclusion, our study exhibits strong evidence that somatic RET mutation is a reliable molecular biomarker to identify a group of aggressive sporadic MTC tumors, while RAS mutation has no prognostic significance in this thyroid cancer entity. Screening for RET mutation in MTCs can be used to better stratify MTC patients prognostically and tailor therapeutic implication.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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