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# 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

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## 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

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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

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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

We found 2581 articles for title and abstract screening and 58 of them were selected for full-text reading. After reading full-texts, we further excluded 35 studies and the remaining 23 studies, comprising 964 sporadic MTCs were included for final analyses (Fig. 1) (Zedenius *et al.* 1994, 1995, Romei *et al.* 1996, Frisk *et al.* 2000, Schilling *et al.* 2001, Cho *et al.* 2005, Dvorakova *et al.* 2008, Elisei *et al.* 2008, Goutas *et al.* 2008, Moura *et al.* 2009, 2011, Mian *et al.* 2011, Pasquali *et al.* 2011, Schulten *et al.* 2011, Ciampi *et al.* 2013, 2017, Lyra *et al.* 2014, Simbolo *et al.* 2014, Chuang *et al.* 2016, Grubbs *et al.* 2016, Nascimento *et al.* 2016, Tiedje *et al.* 2016, Cavedon *et al.* 2017). The characteristics of all included studies are shown in Table 1.

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.

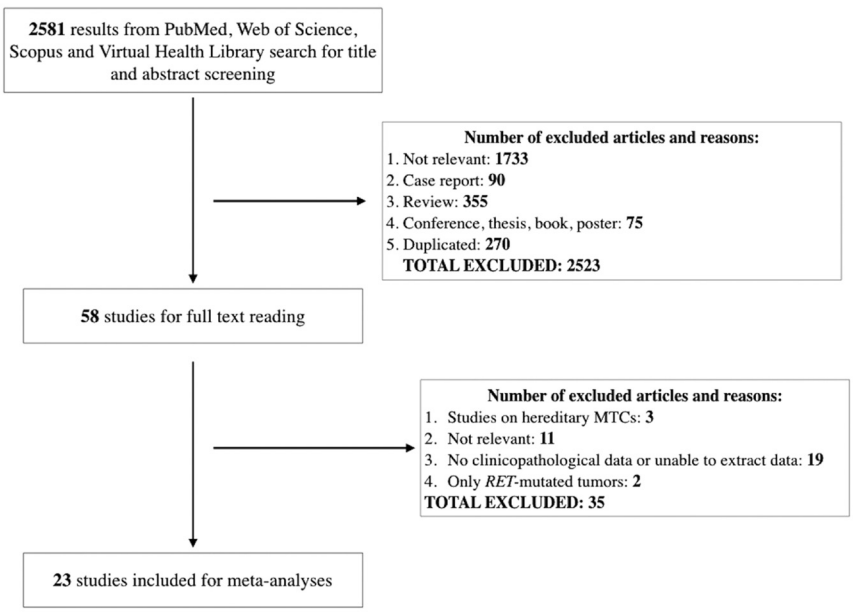


**Figure 1**
Flowchart of study selection.

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**Table 1** Characteristics of included studies.

| Reference | Mutations | No. of patients | Median FU (months) | Gender | Age | pT | LNM | DM | Stage | RP | Death | NOS domains S | C | O |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cavedon *et al.* 2017 | RET<br>RAS | 106 | 40 | N | N | N | Y | N | Y | Y | Y | 4 | 0 | 3 |
| Cho *et al.* 2005 | RET | 20 | NA | N | N | N | N | N | Y | N | N | 3 | 0 | 3 |
| Chuang *et al.* 2016 | RET | 7 | 156 | Y | N | Y | Y | Y | Y | Y | Y | 4 | 0 | 3 |
| Ciampi *et al.* 2013 | RAS | 108 | 79* | Y | Y | Y | Y | Y | Y | Y | N | 4 | 0 | 3 |
| Ciampi *et al.* 2017 | RAS | 19 | 35* | N | Y | Y | Y | Y | Y | N | N | 3 | 0 | 3 |
| Dvorakova *et al.* 2008 | RET | 48 | 24 | Y | Y | Y | Y | Y | Y | Y | Y | 4 | 0 | 3 |
| Elisei *et al.* 2008 | RET | 100 | 122 | Y | Y | Y | Y | Y | Y | Y | Y | 4 | 0 | 3 |
| Frisk *et al.* 2000 | RET | 13 | NA | Y | Y | N | N | N | N | N | Y | 4 | 0 | 3 |
| Goutas *et al.* 2008 | RAS | 44 | NA | Y | N | N | Y | N | Y | N | N | 3 | 0 | 3 |
| Grubbs *et al.* 2016 | RET | 62 | 118 | Y | Y | Y | Y | Y | Y | N | Y | 4 | 0 | 3 |
| Lyra *et al.* 2014 | RAS | 62 | NA | Y | Y | N | Y | N | N | N | N | 3 | 0 | 3 |
| Mian *et al.* 2011 | RET | 60 | 39 | Y | Y | Y | Y | Y | Y | Y | Y | 4 | 0 | 3 |
| Moura *et al.* 2009 | RET | 51 | 93* | Y | Y | Y | Y | Y | Y | Y | N | 4 | 0 | 3 |
| Moura *et al.* 2011 | RAS | 25 | 70* | Y | Y | Y | Y | Y | Y | Y | N | 4 | 0 | 3 |
| Nascimento *et al.* 2016 | RET<br>RAS | 10<br>6 | NA | Y | Y | Y | Y | N | N | N | N | 3 | 0 | 3 |
| Pasquali *et al.* 2011 | RET | 50 | NA | N | N | N | N | Y | N | N | N | 4 | 0 | 3 |
| Romei *et al.* 1996 | RET | 18 | 33* | Y | Y | Y | Y | N | N | Y | Y | 4 | 0 | 3 |
| Schilling *et al.* 2001 | RET | 34 | 69 | Y | Y | Y | Y | Y | N | Y | Y | 4 | 0 | 3 |
| Schulten *et al.* 2011 | RET | 13 | 25 | Y | Y | Y | Y | Y | Y | N | Y | 4 | 0 | 3 |
| Simbolo *et al.* 2014 | RET<br>RAS | 20 | NA | Y | Y | Y | Y | Y | N | N | N | 4 | 0 | 3 |
| Tiedje *et al.* 2016 | RET | 32 | NA | Y | Y | Y | Y | Y | N | N | N | 3 | 0 | 3 |
| Zedenius *et al.* 1994 | RET | 10 | 48 | Y | Y | N | N | N | N | Y | Y | 4 | 0 | 3 |
| Zedenius *et al.* 1995 | RET | 46 | 123 | N | N | N | N | N | N | Y | N | 4 | 0 | 3 |

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; $I^2$ = 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,

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**Table 2** Associations of *RET*, *RAS* mutation and *RET* M918T mutation with the clinicopathological parameters of sporadic medullary thyroid carcinoma.

| Clinicopathological features | No. of studies | No. of patients | OR (or MD) | 95% CI | $I^2$ (%) | *P*-Value[a] |
|---|---|---|---|---|---|---|
| *RET* mutation | | | | | | |
| Male gender | 12 | 442 | 1.68 | 1.07–2.63 | 13 | **0.02** |
| Age | 11 | 435 | −7.19[b] | −12.47 to −1.90 | 83 | **0.008** |
| Vascular invasion | 4 | 98 | 1.33 | 0.57–3.12 | 0 | 0.51 |
| pT3/T4 | 12 | 471 | 2.31 | 1.55–3.45 | 0 | **<0.001** |
| Lymph node metastasis | 10 | 437 | 3.61 | 2.33–5.60 | 0 | **<0.001** |
| Distant metastasis | 11 | 463 | 2.85 | 1.64–4.94 | 0 | **<0.001** |
| Advanced AJCC stage | 7 | 349 | 3.25 | 2.02–5.25 | 0 | **<0.001** |
| Recurrence/persistence | 9 | 474 | 3.01 | 1.65–5.48 | 41 | **<0.001** |
| Patient mortality | 7 | 372 | 2.43 | 1.06–5.57 | 33 | **0.04** |
| *RAS* mutation | | | | | | |
| Male gender | 6 | 265 | 0.65 | 0.33–1.28 | 0 | 0.21 |
| Age | 5 | 221 | −2.53[b] | −8.58 to 3.53 | 39 | 0.41 |
| pT3/T4 | 4 | 153 | 0.47 | 0.18–1.24 | 0 | 0.13 |
| Lymph node metastasis | 7 | 342 | 0.63 | 0.33–1.23 | 0 | 0.18 |
| Distant metastasis | 3 | 144 | 1.05 | 0.30–3.70 | 0 | 0.94 |
| Advanced AJCC stage | 4 | 275 | 0.63 | 0.33–1.23 | 0 | 0.18 |
| Recurrence/persistence | 3 | 231 | 0.57 | 0.23–1.42 | 0 | 0.22 |
| *RET* M918T mutation | | | | | | |
| Male gender | 5 | 158 | 0.92 | 0.48–1.76 | 0 | 0.8 |
| Age | 5 | 158 | −7.4[b] | −11.94 to −2.86 | 0 | **0.001** |
| pT3/T4 | 4 | 140 | 1.83 | 0.91–3.68 | 0 | **0.09** |
| Lymph node metastasis | 4 | 143 | 4.95 | 1.89–12.94 | 0 | **0.001** |
| Distant metastasis | 4 | 146 | 2.82 | 1.08–7.37 | 0 | **0.03** |
| Recurrence/persistence | 3 | 142 | 4.82 | 1.13–20.60 | 53 | **0.03** |
| Patient mortality | 3 | 106 | 1.66 | 0.71–3.86 | 0 | 0.24 |

Boldface, statistically significant result.
[a]*P*-Values were tested for the hypothesis that the OR is 1 or the MD is 0; [b]mean 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; $I^2$ = 83%) (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; $I^2$ = 5%) (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; $I^2$ = 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; $I^2$ = 0%).

## 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; $I^2$ = 0%) (Supplementary Fig. 1C).

## Pathological tumor (pT) factor

We found adequate data regarding the significance of pT factor for *RET* and *RAS* mutations in 12 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, Nascimento *et al*. 2016, Tiedje *et al*. 2016) and four studies (Moura *et al*. 2011, Ciampi *et al*. 2013, Simbolo *et al*. 2014, Nascimento *et al*. 2016), respectively. The presence of *RET* mutation was associated with a

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significantly higher proportion of pT3/T4 (OR=2.31; 95% CI=1.55–3.45; $I^2$=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; $I^2$=0%) (Supplementary Fig. 2C).

### Lymph node metastasis

In total, ten studies (Schilling *et al.* 2001, Dvorakova *et al.* 2008, Elisei *et al.* 2008, Moura *et al.* 2009, Schulten *et al.* 2011, Simbolo *et al.* 2014, Chuang *et al.* 2016, Grubbs *et al.* 2016, Nascimento *et al.* 2016, Cavedon *et al.* 2017) and seven 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, Cavedon *et al.* 2017) investigated the association of *RET* and *RAS* mutations with risk for nodal involvement, respectively. *RET* mutation showed a significantly increased risk for lymph node metastasis (OR=3.61; 95% CI=2.33–5.60; $I^2$=0%) (Supplementary Fig. 1E).

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; $I^2$=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; $I^2$=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; $I^2$=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; $I^2$=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; $I^2$=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; $I^2$=41%) (Supplementary Fig. 1H) and patient death (OR=2.43; 95% CI=1.06–5.57; $I^2$=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; $I^2$=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; $I^2$=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; $I^2$=0%) (Supplementary Fig. 2G).

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### 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.* ($I^2$=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.* ($I^2$=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).

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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 carbozantinib 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.

**Supplementary data**

This is linked to the online version of the paper at https://doi.org/10.1530/ERC-18-0056.

**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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