A meta-analysis of prognostic roles of molecular markers in papillary thyroid carcinoma

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Abstract

The prognostic role of molecular markers in papillary thyroid carcinoma (PTC) is a matter of ongoing debate. The aim of our study is to investigate the impact of *RAS*, *BRAF*, *TERT* promoter mutations and *RET/PTC* rearrangements on the prognosis of PTC patients. We performed a search in four electronic databases: PubMed, Scopus, Web of Science and Virtual Health Library (VHL). Data of hazard ratio (HR) and its 95% confidence interval (CI) for disease-specific survival (DSS) and disease-free survival (DFS) were directly obtained from original papers or indirectly estimated from Kaplan–Meier curve (KMC). Pooled HRs were calculated using random-effect model weighted by inverse variance method. Publication bias was assessed by using Egger's regression test and visual inspection of funnel plots. From 2630 studies, we finally included 35 studies with 17,732 patients for meta-analyses. *TERT* promoter mutation was significantly associated with unfavorable DSS (HR = 7.64; 95% CI = 4.00–14.61) and DFS (HR = 2.98; 95% CI = 2.27–3.92). *BRAF* mutations significantly increased the risk for recurrence (HR = 1.63; 95% CI = 1.27–2.10) but not for cancer mortality (HR = 1.41; 95% CI = 0.90–2.23). In subgroup analyses, *BRAF* mutation only showed its prognostic value in short-/medium-term follow-up. Data regarding *RAS* mutations and *RET/PTC* fusions were insufficient for meta-analyses. *TERT* promoter mutation can be used as an independent and reliable marker for risk stratification and predicting patient's outcomes. The use of *BRAF* mutation to assess patient prognosis should be carefully considered.

Key Words
- BRAF
- TERT promoter
- RAS
- RET/PTC
- mutation
- genetic alteration
- rearrangement
- outcome
- survival
- recurrence
- relapse
- disease-free survival
- disease-specific survival

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Introduction

PTC is the most common histologic type of thyroid cancer, and its incidence has been increasing over the years (1). PTCs rarely behave as aggressive tumors clinically, with a cancer-specific mortality rate less than 5% (2). However, there is a small proportion of cases with aggressive features at presentation that develop early distant metastasis or relapse and are associated with adverse outcomes. Various clinicopathological factors have been investigated as prognostic factors in PTCs, and some of them have been reported to associate with poor outcomes such as old age, large tumor size or distant metastasis (2, 3).

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Recent progress in molecular analyses has improved our understanding of tumorigenesis and pathogenesis in PTC. Several genetic alterations have been described in PTC (4). Among them, *BRAF* mutation, especially *BRAF V600E*, is the most common mutation in PTC; however, its prognostic role in PTC is still debated (5, 6, 7). Another recently described genetic marker, *TERT* promoter mutations, has shown promise in predicting patient's outcomes (8, 9). The prognostic implications of *RAS* mutations and *RET/PTC* rearrangements in PTC are still controversial.

In the present study, we performed a comprehensive systematic review and meta-analysis of observational studies to examine the prognostic impact of molecular markers on tumor recurrence and cancer-related mortality in PTC.

Materials and methods

Literature search

Four electronic databases, including PubMed, Web of Science, Scopus and VHL were searched for relevant articles from inception to September 2016. We used the following search term: (BRAF OR TERT OR RAS OR RET/PTC) AND (papillary thyroid) AND (carcinoma OR cancer). We also searched for potential studies by reviewing the citations within the included studies and reviews. Our study protocol strictly followed the recommendation of Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement (10).

Selection criteria and abstract screening

We imported all search results from each electronic database into Endnote (Thompson Reuters, PA, USA) and deleted duplicates. Titles and abstracts of included studies were independently screened by two reviewers. Studies were included if they reported the association between at least one of the following molecular markers (*BRAF*, *TERT* promoter, *RAS* mutations or *RET/PTC* rearrangements) and PTC patient outcomes (tumor recurrence or cancer-related mortality). We excluded studies if they were (i) studies on other thyroid cancer subtype other than PTC, (ii) case reports, (iii) reviews, (iv) posters, conference papers, theses or books, and (v) duplicated articles. Discordant results between two reviewers were solved by discussion and consensus.

Full-text screening and data extraction

Full-text of all relevant studies were consecutively downloaded and screened independently by two reviewers. Available data were extracted into a predefined extraction form. The following data were extracted from full-text papers: authors, institution, city, country, publication year, surgical period, study design, number of patients, mutational detection method, follow-up periods, data of HR and its 95% CI on DFS and DSS and adjusted variables if available. Data of HR and its 95% CI were directly obtained from full-text papers or indirectly estimated from KMC using the methods by Tierney and coworkers (11). Any disagreements between two reviewers, if present, were resolved again by discussion and consensus. In cases of insufficient data in the original papers or unpublished data, we tried to obtain potential further data by contacting the authors via email. Studies in which data of HR and KMC on DFS or DSS were not provided in original paper or via email were further excluded from the final analyses.

Quality assessment and risk of bias analysis

We used the Newcastle–Ottawa Scale (NOS) to evaluate the quality of included studies in our meta-analyses (12). Two reviewers independently awarded stars for cohort or case–control studies (maximum nine stars) based on a developed checklist (12). In the second domain of outcome category, we awarded one star if the study had a median time of follow-up longer than five years, which was considered long enough for tumor recurrence and mortality to occur. In the last domain of outcome category, studies with the follow-up rate ≥80% or description of those lost suggesting no difference from those followed were awarded one star. Studies awarded at least six stars were considered moderate-to-high-quality studies and those with a NOS value of less than six were regarded low-quality studies.

Meta-analysis

Review Manager 5.3 (Cochrane Collaborative, Oxford, UK) was used for statistical analysis. Pooled HR for DSS and DFS was calculated using the random-model effect weighted by inverse variance method. An HR >1 indicated a compromised prognosis in PTC patients with mutations. If the authors provided various data of HR in the same study, we selected the most powerful one for primary outcome analysis (adjusted HR was superior to

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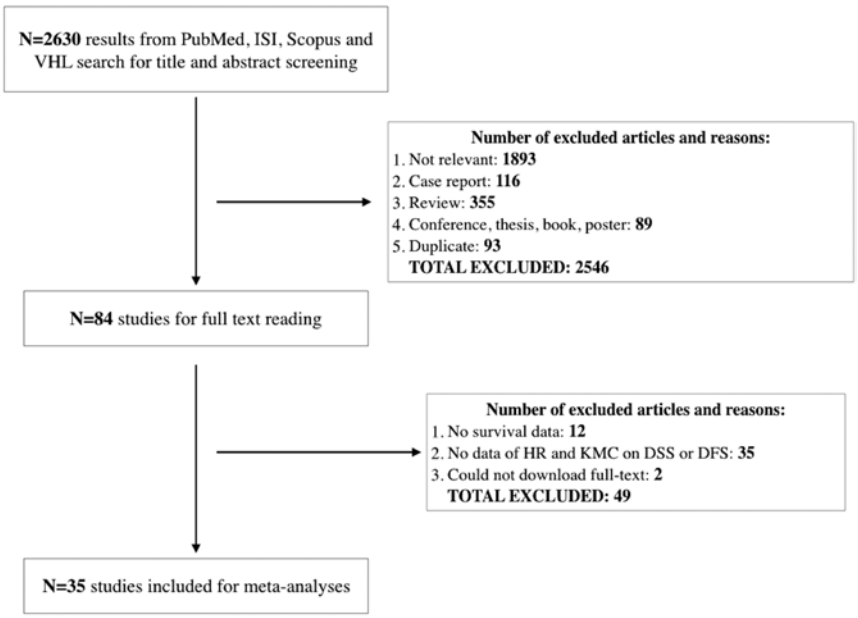

Figure 1
Study flowchart. DFS, disease-free survival; DSS, disease-specific survival; HR, hazard ratio; KMC, Kaplan Meier curve; VHL, Virtual Health Library.

unadjusted HR and unadjusted HR was superior to HR estimated from KMC).

Among-study heterogeneity was assessed by the I^2 statistic, which shows the total variation across studies that is not a result of chance (13). An I^2 statistic <25% indicates a low amount of heterogeneity and >50% indicates a high amount of heterogeneity (14). We examined the sources of heterogeneity by using (i) subgroup analyses and (ii) sensitivity analysis. Egger's regression test and funnel plot were carried out to further assess the presence of publication bias and calculated by Meta-Essentials: Workbook for meta-analysis (15). A P value less than 0.05 was considered statistically significant publication bias.

Results

We found total 6444 articles after initial search and 2630 articles after deleting duplicates. Three additional studies were found by reading citations within included studies. After title and abstract screening step, 84 potential studies were identified to read full-text. By reading full-texts, we further excluded 49 articles that did not meet the inclusion criteria. Finally, 35 articles with a total of 17,732 PTC patients were included for final analysis (Fig. 1).

We contacted all corresponding authors of the included studies via email requesting unreported HR and its 95% CI for effects of mutations on DSS and DFS. We received responses from authors of six studies in Korea, Italy, Poland and Turkey to provide their unpublished data (16, 17, 18, 19, 20, 21).

Study characteristics

Characteristics of included studies were described in Supplementary Table 1 (see section on supplementary data given at the end of this article). We found data of HR for effects of *RAS*, *TERT* promoter and *BRAF* mutations on DSS in one, six and eight studies, respectively. For DFS, survival data are available for *RAS*, *TERT* promoter and *BRAF* mutations in two, six and 26 studies, respectively. No survival data were found for effect of *RET/PTC* rearrangements on DSS or DFS. In cases of duplicated study population from same institutions, we selected data of higher statistical power as described previously or studies with higher number of cases. Because of insufficient data, we did not perform meta-analyses for effects of *RAS* mutations and *RET/PTC* rearrangements in this study.

Impact of *TERT* promoter and *BRAF* mutations on DSS

We found eligible data to pool HR for *TERT* promoter mutations in six studies and for *BRAF* mutations in eight studies, including 1396 and 6659 patients with PTC, respectively. The effect estimate for *TERT* mutations demonstrated that upon comparing patients without mutations, PTCs harboring mutations showed a significantly poor DSS (HR=6.81; 95% CI= 3.63–12.80) (Fig. 2A). Among-study heterogeneity was not present (I^2=0%).

The pooled result for *BRAF* mutations showed an insignificant association of patients possessing mutations

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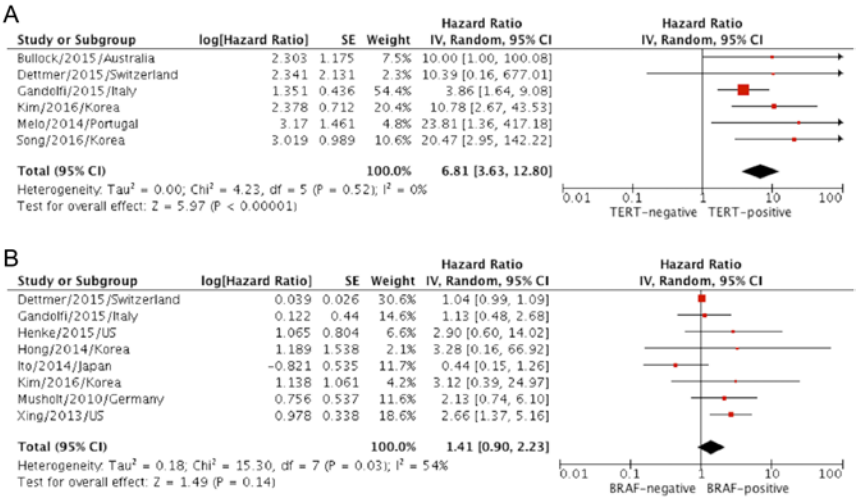

Figure 2
Forest plots of effects of *TERT* promoter (A) and *BRAF* mutations (B) on DSS.

with compromised DSS (HR=1.41; 95% CI=0.90–2.23) (Fig. 2B). A high amount of heterogeneity between included studies was found (I^2=54%). Excluding the study by Xing and coworkers (22) considerably decreased the heterogeneity among studies, but the overall effect remained insignificant (HR=1.14; 95% CI=0.80–1.63; I^2=22%).

Impact of *TERT* promoter and *BRAF* mutations on DFS

Six and 26 studies, including 1589 and 13,213 patients with PTC contained relevant data to pool HR for *TERT* promoter and *BRAF* mutations, respectively. PTCs from the studies by Alzahrani and coworkers (23), Fraser and coworkers (24), Czarniecka and coworkers (17), Xing and coworkers (8, 25), Kim and coworkers (26), Fernandez and coworkers (27), and Lee and coworkers (28) possibly overlapped with the multicenter study by Xing and coworkers (5) and the study by Kim and coworkers (29) in the meta-analysis of HR for *BRAF* mutations and were, therefore, excluded from the pooled estimate of HR for *BRAF* mutations.

The overall estimates showed a significant impact for both *TERT* promoter and *BRAF* mutations on DFS (HR=3.08; 95% CI=2.40–3.96 and HR=1.63; 95% CI=1.27–2.10, respectively) (Fig. 3). No heterogeneity among studies was found in the meta-analysis of *TERT* promoter mutations (I^2=0%). A high amount of heterogeneity was

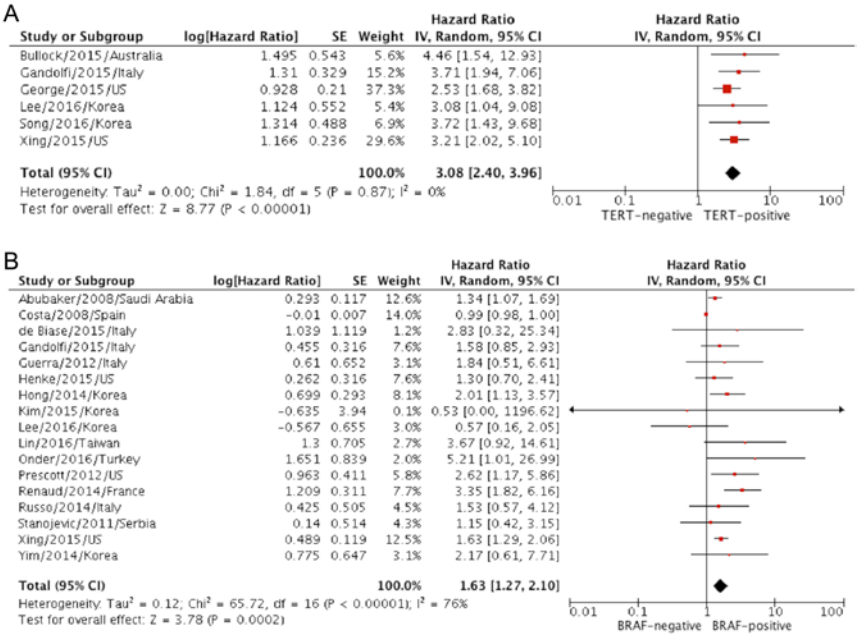

Figure 3
Forest plots of effects of *TERT* promoter (A) and *BRAF* mutations (B) on DFS.

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found among included studies for pooled estimate of *BRAF* mutations (I^2=76%). Excluding the study by Costa and coworkers (30), resulted in a significant decrease of the heterogeneity among included studies (I^2=17%), and the overall effect remained statistically significant (HR=1.69; 95% CI=1.41–2.02).

Subgroup analyses

Subgroup analyses were conducted according to HR calculation method (unadjusted and adjusted), study origin (Caucasian and Asian), follow-up duration (short/medium term and long term) and detection method (direct sequencing and other methods). We classified studies with median value (or mean value in case of no median value) of follow-up duration more than five years as long-term duration and studies with median value of five or less than five years as short-/medium-term follow-up duration.

TERT promoter mutations were significantly associated with unfavorable DFS and DSS in all subgroup analyses. We also found that *BRAF* mutations were significantly associated with poor DFS and DSS in subgroup of short-/medium-term follow-up but not in long-term follow-up. After subgroup analyses, the source of heterogeneity in the effects of *BRAF* mutations on DFS and DSS might be attributed to the follow-up duration (Table 1). All results of subgroup analyses are presented in Table 1.

Impact of coexisting *TERT* promoter and *BRAF* mutations on DSS

We carried out a meta-analysis to further compare the effects of coexisting *TERT* and *BRAF* mutations with effects of *TERT* mutation only and *BRAF* mutation only. In 35 included studies, six studies reported the prevalence of each genetic subgroup when combining *BRAF* and *TERT* promoter mutations (Supplementary Table 2). PTCs wild type for both mutations and PTCs harboring *BRAF* mutation only were the most prevalent subgroups (48.4% and 41.2%, respectively). PTCs with concomitant *BRAF* and *TERT* comprised 6.2% of cases and the subgroup of PTCs with only *TERT* promoter mutation was the least common genotype (4.2%). We could directly or

Table 1 Results of subgroup analyses for DSS and DFS.

	DSS					DFS				
Subgroup	No. of studies	No. of patients	HR	95% CI	I^2 (%)	No. of studies	No. of patients	HR	95% CI	I^2 (%)
HR calculation method										
TERT promoter mutations										
Unadjusted HR	6	1396	14.30[a]	5.31–38.53	58	6	1589	3.22[a]	2.52–4.13	0
Adjusted HR	4	1177	13.60[a]	5.22–35.44	0	3	1146	3.27[a]	2.22–4.82	0
BRAF mutations										
Unadjusted HR	8	6659	1.72	0.91–3.26	78	15	10,327	1.68[a]	1.30–2.17	80
Adjusted HR	2	4450	2.69[a]	1.34–5.39	0	4	5972	1.65[a]	1.28–2.12	7
Study origin										
TERT promoter mutations										
Caucasian	4	556	5.03[a]	2.35–10.73	0	4	950	3.04[a]	2.32–3.97	0
Asian	2	841	13.42[a]	4.32–41.65	0	2	639	3.42[a]	1.67–7.01	0
BRAF mutations										
Caucasian	5	2860	1.58	0.94–2.64	64	14	7234	1.94[a]	1.33–2.82	80
Asian	3	3799	1.14	0.25–5.08	46	7	6591	1.31	0.93–1.84	71
Follow-up duration										
TERT promoter mutations										
Short-term (≤5 years)	1	432	20.47[a]	2.95–142.22	NA	3	1146	3.27[a]	2.22–4.82	0
Long-term (>5 years)	5	965	5.98[a]	3.07–11.65	0	3	443	2.95[a]	2.12–4.11	0
BRAF mutations										
Short/medium-term (≤5 years)	3	4730	2.52[a]	1.45–4.37	0	9	7746	2.35[a]	1.81–3.07	0
Long-term (>5 years)	5	1929	1.05	0.71–1.54	25	11	1922	1.09	0.96–1.23	68
Detection method										
BRAF mutations										
Direct sequencing	4	3122	1.33	0.55–3.22	67	14	6183	1.7	1.22–2.35[a]	57
Other methods	4	3537	1.32	0.81–2.17	24	7	4283	1.68	1.04–2.71[a]	82

[a]Statistically significant.

CI, confidence interval; DFS, disease-free survival; DSS, disease-specific survival; HR, hazard ratio; NA, not available.

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indirectly obtain HR data from two studies (31, 32) and received unpublished data via email from one additional study (19). The group with concomitant *TERT* and *BRAF* mutations exhibited a worse but statistically insignificant effect on DSS as compared with the group harboring *TERT* mutations only (HR = 1.69; 95% CI = 0.42–6.90; I^2 = 57%). On the other hand, a significant effect was found when comparing the dual mutations group and the group of *BRAF* mutations only (HR = 30.08; 95% CI = 3.14–287.98; I^2 = 88%).

Risk of bias assessment and quality of studies

The NOS tool was used to assess the quality of included studies. The majority of included studies were retrospective studies. The number of stars awarded to each study ranged from four to seven stars. Details of given stars within each domain of NOS were described in Supplementary Table 3.

Publication bias

Funnel plot observation did not show strong evidence of publication bias among the set of studies. In addition, Egger's regression test of all effects did not suggest any evidence of publication bias (Supplementary Figs 1, 2, 3 and 4).

Discussion

A number of clinicopathological factors have been assessed as prognostic factors in PTC, and several potential factors have been identified such as old age, large tumor size, the presence of nodal and distant metastasis or extrathyroidal extension (33, 34, 35). The understanding of pathogenesis and genetic profiles in thyroid cancer has been much improved in recent years with the rapid growth of translational medicine. The majority of thyroid cancer cases are driven by the activation of RAS–mitogen-activated protein kinase (MAPK) signaling pathway via *BRAF* or *RAS* point mutations (36) or chromosomal fusions (*RET/PTC* or *TRK*) (37, 38). Therefore, it is very essential to investigate the usefulness of genetic events as trustworthy prognostic markers for risk stratification and patient management.

The *BRAF* mutations are the most common genetic events in PTCs, and the *BRAF V600E* is the most common mutation in *BRAF* mutation family (4). In the past two decades, the significance of *BRAF V600E* in tumor aggressiveness and its usefulness as prognostic marker have been extensively studied. The *BRAF V600E* has been reported to associate with aggressive behaviors in PTC patients (39, 40, 41). However, there were remarkable inconsistencies regarding its prognostic role among various studies (7, 18, 22, 42). It is most likely that the heterogeneities in patient selections, follow-up periods and statistical analyses are the major factors responsible for these discrepancies. Hence, meta-analysis, the most powerful statistical method of pooling results from multiple studies, is required to solve the controversial result. In our meta-analyses, we identify a significant effect of *BRAF* mutations on patient DFS but not on patient DSS. This significant effect, however, should be interpreted with caution as there is a considerable amount of heterogeneity among included studies. Although the meta-analysis for multivariate HR on DFS remains significant, the result is dominated by studies with short-/medium-term follow-up (5, 21). Interestingly, we only find a significant association of *BRAF* mutations with unfavorable DFS and DSS in subgroup of studies with short-/medium-term follow-up, and this significant result completely disappears in long-term follow-up subgroup (Table 1). Thus, *BRAF* mutation should only be used very cautiously as a prognostic marker in PTC patients, given that it only differentiates outcomes in short-/medium-term follow-up and does not show good predictive value in long-term prognosis. However, it is important to note that the majority of PTC recurrence occurs in the first five years of follow-up (43), and the use of *BRAF* mutations as a prognostic factor in PTC, therefore, can be considered.

TERT promoter mutations, the more recently discovered mutations in thyroid cancer, have been found to correlate with aggressive clinicopathological features and poor outcomes in PTCs (8, 19, 44). Our pooled analyses show a promising value of these mutations as a prognostic marker in PTCs. *TERT* promoter mutations are significantly associated with worse patient DFS and DSS in primary and all subgroup analyses. Although the number of included studies with survival data of *TERT* promoter mutations is relatively small, there are no inconsistencies among the included studies. In addition, subgroup analyses of multivariate HR on DFS and DSS both demonstrate an independent and significant association of *TERT* promoter mutations with poor survival outcomes. *TERT* promoter mutations are not prevalent in PTCs but are more frequently detected in poorly differentiated and anaplastic thyroid carcinoma (45, 46). On the other hand, Landa and coworkers reported that *TERT* promoter mutations are subclonal in a small subset of PTCs but are

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clonal in poorly differentiated and anaplastic cancer (46). Furthermore, the survival of anaplastic thyroid carcinoma patients harboring *TERT* promoter mutations was significantly worse in comparison with patients without these mutations (46), thus supporting the association of *TERT* promoter mutations with aggressive clinical course and poor outcome in thyroid cancer.

The presence and roles of *RAS* mutations and *RET/PTC* rearrangements in thyroid cancer have been established since a long time. *RAS* mutations have been shown to be associated with aggressive tumor phenotypes, distant metastasis and poor prognosis in thyroid cancer (47). However, their association with clinical course in PTC is controversial. We could find data for *RAS* mutation impact on DFS and DSS in only two studies (30, 48). In the study by Hara and coworkers, the authors reported that the presence of *RAS* mutations was a significant and independent predictor of both death and recurrence of PTC (48). However, these results should be carefully scrutinized as the authors selected a high number of cases with distant metastasis in which the majority of *RAS* mutations were detected (48). Additionally, *TERT* promoter mutations were found to be associated with distant metastasis in PTCs (19, 49, 50), and these mutations could be coexisting in those cases harboring *RAS* mutations and thus have enhanced the significant effect on patient's outcomes. In addition, Song and coworkers (50), Muzza and coworkers (51) and Shen and coworkers (52) reported that concomitant *RAS* and *TERT* promoter mutations enhanced the risk prediction in differentiated thyroid cancer. These findings support a hypothesis that differentiated thyroid cancer will most likely lack aggressiveness when harboring *RAS* mutations alone but will be associated with adverse outcome when harboring concomitant *RAS* and *TERT* promoter mutations (53). *RET/PTC* rearrangements may seem to play an unimportant prognostic role in PTCs as there were no published data on this topic, but further investigation may be needed to affirm this view.

At an earlier period, genetic alterations in thyroid cancer were thought to be mutually exclusive (54). With the improvements of detection methods and discoveries of novel genetic events, concomitant mutations have been reported in a number of cases and studies (8, 50, 55, 56). Concomitant mutations in thyroid cancer were proposed to enhance tumor aggressiveness and worsen patients' survival, especially coexisting *TERT* promoter and *BRAF* mutations in PTC (31, 56, 57). *TERT* promoter mutations are not common events in PTCs, but they are prevalent in aggressive tumors (58). Interestingly, these mutations have been found to frequently occur in coexistence with *BRAF* mutations in PTCs (8, 32, 50). Our pooled results show that PTCs with concomitant *BRAF* and *TERT* mutations showed a significantly worse DSS as compared to PTCs with *BRAF* mutations only. We find an insignificant result in the comparison of PTCs with concomitant *BRAF* and *TERT* mutations and PTCs with *TERT* mutations only, and it further supports the fact that *TERT* promoter mutations have an independent prognostic value, irrespective of *BRAF* status. There are several published papers (8, 19, 31, 32, 50, 59, 60) reporting the impact of *BRAF* and *TERT* interaction on clinical significance, but we could only find available HR data to analyze the interaction between *BRAF* and *TERT* on patient prognosis in only three studies including one provided via email request (19, 31, 32). Therefore, additional studies are needed to confirm the results from the large cohort study in the United States in which the authors demonstrated PTCs with coexisting *BRAF* and *TERT* mutations to have the highest risk for mortality (31).

This is the first meta-analysis to investigate the prognostic impact of various genetic events on PTC, and we included a high number of studies, 35 studies with nearly 18,000 PTC patients from 15 countries for meta-analyses. We also conducted detailed subgroup analyses on different statistical and clinicopathological features to systematically evaluate the prognostic effect of genetic alterations in PTC and to identify the potential source of heterogeneity, the follow-up duration. Our subgroup analyses further emphasize the prognostic values of *TERT* promoter mutations and prompt reconsideration of the usefulness of *BRAF* mutations to predict patient prognosis. However, our present study might have limitations that need to be addressed. First, most of included studies are retrospective studies so selection biases are unavoidable such as treatment models or mutational detection methods. Secondly, several data of HRs were estimated from KMC and the results, therefore, sometimes lack precision (11). To minimize this bias, we have made all efforts to contact the authors to provide unreported HR and 95% CI, and we received responses from authors of six studies. PTCs harboring *BRAF* V600E have been reported to represent a diverse group of tumors, consisting at least four molecular subtypes, with variable degrees of thyroid differentiation and these tumors, therefore, should not be considered a homogenous group in clinical studies (36). However, the majority of our included studies only provided unadjusted hazard ratio of *BRAF* mutation on patient outcomes and did not take into account other genetic alterations. Additional studies should include

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other genetic events designed to capture the extent of genetic diversity in PTCs.

In conclusion, our present study indicates strong evidence that *TERT* promoter mutation is an independent and reliable molecular marker to predict recurrence and mortality in PTCs. *TERT* should be used for risk stratification in PTC patients, especially in high-risk patients, in preference to other molecular markers. The use of *BRAF* mutation to assess patient prognosis should be considered carefully as it can only be shown to have prognostic value in short/medium-term follow-up.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EC-17-0010.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References

1 Davies L & Welch HG. Current thyroid cancer trends in the United States. *JAMA Otolaryngology: Head and Neck Surgery* 2014 **140** 317–322. (doi:10.1001/jamaoto.2014.1)

2 Orosco RK, Hussain T, Brumund KT, Oh DK, Chang DC & Bouvet M. Analysis of age and disease status as predictors of thyroid cancer-specific mortality using the Surveillance, Epidemiology, and End Results database. *Thyroid* 2015 **25** 125–132. (doi:10.1089/thy.2014.0116)

3 Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, *et al*. 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid* 2016 **26** 1–133. (doi:10.1089/thy.2015.0020)

4 Nikiforov YE & Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. *Nature Reviews Endocrinology* 2011 **7** 569–580. (doi:10.1038/nrendo.2011.142)

5 Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, Elisei R, Bendlová B, Yip L, Mian C, *et al*. Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. *Journal of Clinical Oncology* 2015 **33** 42–50. (doi:10.1200/JCO.2014.56.8253)

6 Ito Y, Yoshida H, Kihara M, Kobayashi K, Miya A & Miyauchi A. BRAFV600E mutation analysis in papillary thyroid carcinoma: Is it useful for all patients? *World Journal of Surgery* 2014 **38** 679–687. (doi:10.1007/s00268-013-2223-2)

7 Henke LE, Pfeifer JD, Ma C, Perkins SM, DeWees T, El-Mofty S, Moley JF, Nussenbaum B, Haughey BH, Baranski TJ, *et al*. BRAF mutation is not predictive of long-term outcome in papillary thyroid carcinoma. *Cancer Medicine* 2015 **4** 791–799. (doi:10.1002/cam4.417)

8 Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, Pai S & Bishop J. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. *Journal of Clinical Oncology* 2014 **32** 2718–2726. (doi:10.1200/JCO.2014.55.5094)

9 Melo M, Da Rocha AG, Vinagre J, Batista R, Peixoto J, Tavares C, Celestino R, Almeida A, Salgado C, Eloy C, *et al*. TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E754–E765. (doi:10.1210/jc.2013-3734)

10 Moher D, Liberati A, Tetzlaff J, Altman DG & PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Medicine* 2009 **6** e1000097. (doi:10.1371/journal.pmed.1000097)

11 Tierney JF, Stewart LA, Ghersi D, Burdett S & Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 2007 **8** 16. (doi:10.1186/1745-6215-8-16)

12 Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *European Journal of Epidemiology* 2010 **25** 603–605. (doi:10.1007/s10654-010-9491-z)

13 Higgins JP & Thompson SG. Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine* 2002 **21** 1539–1558. (doi:10.1002/sim.1186)

14 Ioannidis JP, Patsopoulos NA & Evangelou E. Uncertainty in heterogeneity estimates in meta-analyses. *BMJ* 2007 **335** 914–916. (doi:10.1136/bmj.39343.408449.80)

15 Van Rhee HJ, Suurmond R & Hak T. *User Manual for Meta-Essentials: Workbooks for Meta-Analysis (Version 1.0)*. Rotterdam, The Netherlands: Erasmus Research Institute of Management, 2015. (available at: http://www.erim.eur.nl/research-support/meta-essentials)

16 de Biase D, Gandolfi G, Ragazzi M, Eszlinger M, Sancisi V, Gugnoni M, Visani M, Pession A, Casadei G, Durante C, *et al*. TERT promoter mutations in papillary thyroid microcarcinomas. *Thyroid* 2015 **25** 1013–1019. (doi:10.1089/thy.2015.0101)

17 Czarniecka A, Kowal M, Rusinek D, Krajewska J, Jarzab M, Stobiecka E, Chmielik E, Zembala-Nozynska E, Poltorak S, Sacher A, *et al*. The risk of relapse in papillary thyroid cancer (PTC) in the context of BRAFV600E mutation status and other prognostic factors. *PLoS ONE* 2015 **10** e0132821. (doi:10.1371/journal.pone.0132821)

18 Russo M, Malandrino P, Nicolosi ML, Manusia M, Marturano I, Trovato MA, Pellegriti G, Frasca F & Vigneri R. The BRAFV600E mutation influences the short- and medium-term outcomes of classic papillary thyroid cancer, but is not an independent predictor of unfavorable outcome. *Thyroid* 2014 **24** 1267–1274. (doi:10.1089/thy.2013.0675)

19 Gandolfi G, Ragazzi M, Frasoldati A, Piana S, Ciarrocchi A & Sancisi V. TERT promoter mutations are associated with distant metastases in papillary thyroid carcinoma. *European Journal of Endocrinology* 2015 **172** 403–413. (doi:10.1530/EJE-14-0837)

20 Onder S, Ozturk Sari S, Yegen G, Sormaz IC, Yilmaz I, Poyrazoglu S, Sanlı Y, Giles Senyurek Y, Kapran Y & Mete O. Classic architecture with multicentricity and local recurrence, and absence of TERT promoter mutations are correlates of BRAF V600E harboring pediatric papillary thyroid carcinomas. *Endocrine Pathology* 2016 **27** 153–161. (doi:10.1007/s12022-016-9420-0)

21 Hong AR, Lim JA, Kim TH, Choi HS, Yoo WS, Min HS, Won JK, Lee KE, Jung KC, Park DJ, *et al*. The frequency and clinical

http://www.endocrineconnections.org
DOI: 10.1530/EC-17-0010

implications of the BRAFv600E mutation in papillary thyroid cancer patients in Korea over the past two decades. *Endocrinology and Metabolism* 2014 **29** 505–513. (doi:10.3803/EnM.2014.29.4.505)
22 Xing MM, Alzahrani AS, Carson KA, Viola D, Elisei R, Bendlova B, Yip L, Mian C, Vianello F, Tuttle RM, *et al*. Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. *JAMA* 2013 **309** 1493–1501. (doi:10.1001/jama.2013.3190)
23 Alzahrani AS & Xing M. Impact of lymph node metastases identified on central neck dissection (CND) on the recurrence of papillary thyroid cancer: Potential role of BRAFV600E mutation in defining CND. *Endocrine-Related Cancer* 2013 **20** 13–22. (doi:10.1530/ERC-12-0309)
24 Fraser S, Go C, Aniss A, Sidhu S, Delbridge L, Learoyd D, Clifton-Bligh R, Tacon L, Tsang V, Robinson B, *et al*. BRAF(V600E) mutation is associated with decreased disease-free survival in papillary thyroid cancer. *World Journal of Surgery* 2016 **40** 1618–1624. (doi:10.1007/s00268-016-3534-x)
25 Xing M, Clark D, Guan H, Ji M, Dackiw A, Carson KA, Kim M, Tufaro A, Ladenson P, Zeiger M, *et al*. BRAF mutation testing of thyroid fine-needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer. *Journal of Clinical Oncology* 2009 **27** 2977–2982. (doi:10.1200/JCO.2008.20.1426)
26 Kim TY, Kim WB, Rhee YS, Song JY, Kim JM, Gong G, Lee S, Kim SY, Kim SC, Hong SJ, *et al*. The BRAF mutation is useful for prediction of clinical recurrence in low-risk patients with conventional papillary thyroid carcinoma. *Clinical Endocrinology* 2006 **65** 364–368. (doi:10.1111/j.1365-2265.2006.02605.x)
27 Fernandez IJ, Piccin O, Sciascia S, Cavicchi O, Repaci A, Vicennati V & Fiorentino M. Clinical significance of braf mutation in thyroid papillary cancer. *Otolaryngology-Head and Neck Surgery* 2013 **148** 919–925. (doi:10.1177/0194599813481942)
28 Lee JI, Jang HW, Kim SW, Kim JW, Oh YL & Chung JH. BRAFV600E mutation in fine-needle aspiration aspirates: association with poorer prognostic factors in larger papillary thyroid carcinomas. *Head and Neck* 2013 **35** 548–553. (doi:10.1002/hed.22997)
29 Kim SK, Woo JW, Lee JH, Park I, Choe JH, Kim JH & Kim JS. Role of BRAF V600E mutation as an indicator of the extent of thyroidectomy and lymph node dissection in conventional papillary thyroid carcinoma. *Surgery* 2015 **158** 1500–1511. (doi:10.1016/j.surg.2015.05.016)
30 Costa AM, Herrero A, Fresno MF, Heymann J, Alvarez JA, Cameselle-Teijeiro J & García-Rostán G. BRAF mutation associated with other genetic events identifies a subset of aggressive papillary thyroid carcinoma. *Clinical Endocrinology* 2008 **68** 618–634. (doi:10.1111/j.1365-2265.2007.03077.x)
31 Liu R, Bishop J, Zhu G, Zhang T, Ladenson PW & Xing M. Mortality risk stratification by combining BRAF V600E and TERT promoter mutations in papillary thyroid cancer: genetic duet of BRAF and TERT promoter mutations in thyroid cancer mortality. *JAMA Oncology* 2016 **3** 202–208. (doi:10.1001/jamaoncol.2016.3288)
32 Kim TH, Kim YE, Ahn S, Kim JY, Ki CS, Oh YL, Kim K, Yun JW, Park WY, Choe JH, *et al*. TERT promoter mutations and long-term survival in patients with thyroid cancer. *Endocrine-Related Cancer* 2016 **23** 813–823. (doi:10.1530/ERC-16-0219)
33 Akslen LA, Myking AO, Salvesen H & Varhaug JE. Prognostic importance of various clinicopathological features in papillary thyroid carcinoma. *European Journal of Cancer* 1992 **29a** 44–51. (doi:10.1016/0959-8049(93)90574-y)
34 Thoresen SO, Akslen LA, Glattre E, Haldorsen T, Lund EV & Schoultz M. Survival and prognostic factors in differentiated thyroid cancer – a multivariate analysis of 1055 cases. *British Journal of Cancer* 1989 **59** 231–235. (doi:10.1038/bjc.1989.47)
35 Tubiana M, Schlumberger M, Rougier P, Laplanche A, Benhamou E, Gardet P, Caillou B, Travagli JP & Parmentier C. Long-term results and prognostic factors in patients with differentiated thyroid carcinoma. *Cancer* 1985 **55** 794–804. (doi:10.1002/1097-0142(19850215)55:4<794::AID-CNCR2820550418>3.0.CO;2-Z)
36 Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. *Cell* 2014 **159** 676–690. (doi:10.1016/j.cell.2014.09.050)
37 Nikiforov YE. RET/PTC rearrangement in thyroid tumors. *Endocrine Pathology* 2002 **13** 3–16. (doi:10.1385/EP:13:1:03)
38 Russell JP, Powell DJ, Cunnane M, Greco A, Portella G, Santoro M, Fusco A & Rothstein JL. The TRK-T1 fusion protein induces neoplastic transformation of thyroid epithelium. *Oncogene* 2000 **19** 5729–5735. (doi:10.1038/sj.onc.1203922)
39 Li C, Lee KC, Schneider EB & Zeiger MA. BRAF V600E mutation and its association with clinicopathological features of papillary thyroid cancer: a meta-analysis. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 4559–4570. (doi:10.1210/jc.2012-2104)
40 Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, Carson KA, Vasko V, Larin A, Tallini G, *et al*. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6373–6379. (doi:10.1210/jc.2005-0987)
41 Lupi C, Giannini R, Ugolini C, Proietti A, Berti P, Minuto M, Materazzi G, Elisei R, Santoro M, Miccoli P, *et al*. Association of BRAF V600E mutation with poor clinicopathological outcomes in 500 consecutive cases of papillary thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 4085–4090. (doi:10.1210/jc.2007-1179)
42 Yim JH, Kim WG, Jeon MJ, Han JM, Kim TY, Yoon JH, Hong SJ, Song DE, Gong G, Shong YK, *et al*. Association between expression of X-linked inhibitor of apoptosis protein and the clinical outcome in a BRAFV600E-prevalent papillary thyroid cancer population. *Thyroid* 2014 **24** 689–694. (doi:10.1089/thy.2012.0585)
43 Durante C, Montesano T, Torlontano M, Attard M, Monzani F, Tumino S, Costante G, Meringolo D, Bruno R, Trulli F, *et al*. Papillary thyroid cancer: time course of recurrences during postsurgery surveillance. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 636–642. (doi:10.1210/jc.2012-3401)
44 Alzahrani AS, Alsaadi R, Murugan AK & Bin Sadiq B. TERT promoter mutations in Thyroid Cancer. *Hormones and Cancer* 2016 **7** 165–177. (doi:10.1007/s12672-016-0256-3)
45 Landa I, Ganly I, Chan TA, Mitsutake N, Matsuse M, Ibrahimpasic T, Ghossein RA & Fagin JA. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** E1562–E1566. (doi:10.1210/jc.2013-2383)
46 Landa I, Ibrahimpasic T, Boucai L, Sinha R, Knauf JA, Shah RH, Dogan S, Ricarte-Filho JC, Krishnamoorthy GP, Xu B, *et al*. Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. *Journal of Clinical Investigation* 2016 **126** 1052–1066. (doi:10.1172/JCI85271)
47 Garcia-Rostan G, Zhao HY, Camp RL, Pollan M, Herrero A, Pardo J, Ran W, Carcangiu ML, Costa J & Tallini G. ras mutations are associated with aggressive tumor phenotypes and poor prognosis in thyroid cancer. *Journal of Clinical Oncology* 2003 **21** 3226–3235. (doi:10.1200/JCO.2003.10.130)
48 Hara H, Fulton N, Yashiro T, Ito K, DeGroot LJ & Kaplan EL. N-ras mutation: an independent prognostic factor for aggressiveness of papillary thyroid carcinoma. *Surgery* 1994 **116** 1010–1016.
49 Bae JS, Kim Y, Jeon S, Kim SH, Kim TJ, Lee S, Kim M-H, Lim DJ, Lee YS & Jung CK. Clinical utility of TERT promoter mutations and ALK rearrangement in thyroid cancer patients with a high prevalence of the BRAF V600E mutation. *Diagnostic Pathology* 2016 **11** 21. (doi:10.1186/s13000-016-0458-6)
50 Song YS, Lim JA, Choi H, Won J-K, Moon JH, Cho SW, Lee KE, Park YJ, Yi KH, Park DJ, *et al*. Prognostic effects of TERT promoter mutations are enhanced by coexistence with BRAF or RAS mutations and strengthen the risk prediction by the ATA or TNM staging system in differentiated thyroid cancer patients. *Cancer* 2016 **122** 1370–1379. (doi:10.1002/cncr.29934)

http://www.endocrineconnections.org
DOI: 10.1530/EC-17-0010

51 Muzza M, Colombo C, Rossi S, Tosi D, Cirello V, Perrino M, De Leo S, Magnani E, Pignatti E, Vigo B, *et al*. Telomerase in differentiated thyroid cancer: promoter mutations, expression and localization. *Molecular and Cellular Endocrinology* 2015 **399** 288–295. (doi:10.1016/j.mce.2014.10.019)
52 Shen X, Liu R & Xing M. A six-genotype genetic prognostic model for papillary thyroid cancer. *Endocrine-Related Cancer* 2017 **24** 41–52. (doi:10.1530/ERC-16-0402)
53 Xing M. Clinical utility of RAS mutations in thyroid cancer: a blurred picture now emerging clearer. *BMC Medicine* 2016 **14** 12. (doi:10.1186/s12916-016-0559-9)
54 Xing M. BRAF mutation in thyroid cancer. *Endocrine-Related Cancer* 2005 **12** 245–262. (doi:10.1677/erc.1.0978)
55 Guerra A, Zeppa P, Bifulco M & Vitale M. Concomitant BRAFV600E mutation and RET/PTC rearrangement is a frequent occurrence in papillary thyroid carcinoma. *Thyroid* 2014 **24** 254–259. (doi:10.1089/thy.2013.0235)
56 Zou M, Baitei EY, Alzahrani AS, Binhumaid FS, Alkhafaji D, Al-Rijjal RA, Meyer BF & Shi Y. Concomitant RAS, RET/PTC, or BRAF mutations in advanced stage of papillary thyroid carcinoma. *Thyroid* 2014 **24** 1256–1266. (doi:10.1089/thy.2013.0610)
57 Liu X, Qu S, Liu R, Sheng C, Shi X, Zhu G, Murugan AK, Guan H, Yu H, Wang Y, *et al*. TERT promoter mutations and their association with BRAF V600E mutation and aggressive clinicopathological characteristics of thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E1130–E1136. (doi:10.1210/jc.2013-4048)
58 Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, Sun H, El-Naggar A & Xing M. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. *Endocrine-Related Cancer* 2013 **20** 603–610. (doi:10.1530/ERC-13-0210)
59 Lee SE, Hwang TS, Choi YL, Han HS, Kim WS, Jang MH, Kim SK & Yang JH. Prognostic significance of TERT promoter mutations in papillary thyroid carcinomas in a BRAF(V600E) mutation-prevalent population. *Thyroid* 2016 **26** 901–910. (doi:10.1089/thy.2015.0488)
60 Dettmer MS, Schmitt A, Steinert H, Capper D, Moch H, Komminoth P & Perren A. Tall cell papillary thyroid carcinoma: new diagnostic criteria and mutations in BRAF and TERT. *Endocrine-Related Cancer* 2015 **22** 419–429. (doi:10.1530/ERC-15-0057)

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