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A Six-genotype Genetic Prognostic Model for Papillary Thyroid Cancer

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Abstract

A unique prognostic role of the genetic duet of *BRAF* V600E and *TERT* promoter mutation in papillary thyroid cancer (PTC) has been recently established, but the role of *RAS* mutation in this genetic interplay remains to be established. Using The Cancer Genome Atlas (TCGA) data of patients with PTC from 19 medical centers, we investigated interactions among the three mutations in clinical outcomes of PTC. We found that *BRAF* and *RAS* mutations were mutually exclusive but both were associated with *TERT* promoter mutations, with the genetic duet of *BRAF*/*RAS* and *TERT* mutations occurring in 34/388 (8.76%) patients. *BRAF*/*RAS* or *TERT* mutation each alone had no or minimal effect while coexisting *BRAF*/*RAS* and *TERT* mutations had a robust synergistic effect on poor clinicopatholgoic outcomes of PTC, including disease recurrence and patient mortality. For example, PTC recurrence rate was 52% with coexisting *BRAF* V600E/*RAS* and *TERT* promoter mutations versus 6.9% with no mutation, corresponding to a HR of 8.17 (95% CI 3.09–21.58), which remained significant at 14.71 (95% CI 2.79–77.61) after adjustment for clinicopathologic factors and institution. *BRAF/RAS* mutation or *TERT* mutation alone minimally affected Kaplan-Meier patient survival curves while the genetic duet was associated with a sharp curve decline. Thus, by confirming and expanding previous findings in single-institution studies, this multicenter data analysis establishes a six-genotype genetic prognostic model for poor outcomes of PTC with a risk order of genetic duet of *BRAF* V600E/*RAS* mutation and *TERT* mutation >>>> *BRAF* V600E = *TERT* mutation alone >*RAS* mutation alone = wild-type genes.

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Declaration of interest:

M Xing receives royalties as co-holder of a licensed USA patent related to *BRAF* mutation in thyroid cancer. Other authors declare no conflict of interest.

Author contributions

M X conceived, designed and supervised the study. X S collected the data. X S, R L and M X performed the analysis and interpreted the results. X S and M X wrote the manuscript. X S, R L and M X revised and approved the manuscript.

Keywords

thyroid cancer; *TERT* promoter mutation; *BRAF* V600E mutation; *RAS* mutation; prognostic molecular markers

INTRODUCTION

Thyroid cancer is a common endocrine malignancy, consisting mostly of papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC), with the former accounting for nearly 90% of all thyroid malignancies (Siegel *et al.* 2015; Howlader *et al.* 2016). PTC can be further classified into several variant types, including mainly conventional PTC (CPTC), follicular-variant PTC (FVPTC), and tall-cell PTC (TCPTC), among which CPTC was the most common. *BRAF* V600E and *RAS* mutations have been well established as the main genetic drivers of thyroid cancer, particularly PTC (Garcia-Rostan *et al.* 2003; Xing 2013). Following the initial report of two mutually exclusive *TERT* promoter mutations—chr5:1,295,228C>T and chr5:1,295,250 C>T (termed C228T and C250T, respectively) in melanoma in 2013 (Horn *et al.* 2013; Huang *et al.* 2013), we reported their occurrence also in thyroid cancer (Liu *et al.* 2013), which has been widely confirmed (Alzahrani *et al.* 2016; Liu & Xing 2016). This represents an exciting recent development in understanding the genetic mechanisms of thyroid cancer. Studies from us (Liu *et al.* 2014; Shi *et al.* 2015) and others (Alzahrani *et al.* 2016; Liu & Xing 2016) have consistently shown a strong association of *TERT* promoter mutations with aggressive clinicopathologic outcomes of thyroid cancer, suggesting a prognostic role of *TERT* promoter mutations in this cancer.

In our initial study on *TERT* promoter mutations in thyroid cancer, we made an interesting observation of the association between *BRAF* V600E and *TERT* promoter mutations (Liu *et al.* 2013). Our subsequent studies on extended cohorts of patients with different ethnic backgrounds again showed this phenomena (Liu *et al.* 2014; Shi *et al.* 2015), which has now been widely confirmed (Liu & Xing 2016). *BRAF* V600E, the most common oncogene in PTC (Xing 2005), has been shown to be associated with a poor prognosis of PTC (Xing 2007; Xing *et al.* 2013; 2015). We found the unique genetic duet of coexisting *BRAF* V600E and *TERT* promoter mutations to be even more robustly associated with aggressive clinicopathologic outcomes of PTC, including tumor recurrence, and patient mortality (Xing *et al.* 2014a, b; Liu *et al.* 2016). The genetic duet of *RAS* and *TERT* promoter mutations was shown to be marginally associated with aggressiveness of FTC in relatively small cohorts (Muzza *et al.* 2015; Sohn *et al.* 2016; Song *et al.* 2016). The role of this genetic duet in PTC has not been established.

These previous studies on the coexisting *BRAF* V600E and *TERT* promoter mutations were virtually all single institution-based. The genetic duet of *BRAF* V600E and *TERT* promoter mutations was studied in PTC virtually always with *RAS* mutations mixed in the study cohort. It is important and ideal to use multicenter studies to validate and establish an exclusive role of the genetic duet of *BRAF* V600E and *TERT* promoter mutations and to also investigate the role of the genetic duet of *RAS* and *TERT* promoter mutations in PTC. To this end, we conducted the present study using the unique multicenter cohort of PTC

patients in The Cancer Genome Atlas (TCGA) database (Cancer Genome Atlas Research Network 2014) to comprehensively investigate the interactions among *BRAF* V600E, *RAS* and *TERT* promoter mutations in affecting the clinical outcomes of PTC.

MATERIALS AND METHODS

Mutation and clinical information of the PTC patients in the TCGA database

Whole-exon mutation data were obtained from the TCGA Genome Data Analysis Center (GDAC) firehose website (http://firebrowse.org/). Information on *BRAF* V600E and *RAS* (including *NRAS*, *KRAS*, *HRAS*) mutations was extracted from the data. For *RAS* mutations, only missense mutations were included. *TERT* promoter mutation information was extracted from the TCGA thyroid cancer mark paper (Cancer Genome Atlas Research Network 2014), which included the *TERT* promoter mutation information from the Sanger sequencing and whole genome sequencing. A total of 388 patients with available information on both exon mutation and *TERT* promoter mutation were included for analyses in the present study. Clinical data for these patients were extracted directly from the TCGA Data Portal (https://tcga-data.nci.nih.gov/tcga/). The information on new tumor event, disease status, and patient mortality was updated to the latest follow-up data (07-30-2015, v4.0).

Definition of disease recurrence and patient mortality

PTC recurrence was defined here as recurrent or persistent disease as defined previously (Xing *et al.* 2014a), which was identified based on the clinical information of new thyroid cancer tumor related events and the tumor status during the follow-up time for each patient. Patients with available information for both tumor status and new tumor event status during the follow-up time were included. Patients with any of the three types of new tumor events, including locoregional recurrent tumor, distant recurrent tumor and biochemical recurrent tumor, were identified as having disease recurrence. The recurrence time was defined as the time from the initial treatment of the original tumor to the discovery of the tumor recurrence/persistence. If a patient has multiple new thyroid tumor events, the recurrence time for this patient was defined as the recurrence time for the first new tumor event. If the tumor status was “with tumor” at any follow-up times even if it represented a persistent disease but not new tumor event, it was also treated as disease “recurrence” in this study and the earliest follow-up time for the persistent tumor status was used as the recurrence time. Patients who had no new thyroid tumor event and were in ‘tumor free’ status were treated as having no disease recurrence. As a result, a final 306 cases out of the initially selected 388 patients were used for PTC recurrence analysis. For patient mortality, the follow-up time was defined as the time period from the treatment of the initial thyroid cancer to the time of the death of the patient as defined previously (Xing 2013).

Statistical analyses

Comparisons of categorical variables were performed with either Pearson’s chi-squared test or, for cases with small number, Fisher’s exact test. Wilcoxon-Mann-Whitney test was used for continuous variables. Survival curves were plotted with the Kaplan-Meier method with log-rank statistical analyses. Cox proportional hazards regression was used to assess the

hazard ratio (HR) of the risk of recurrence and mortality. Statistical significance was defined as two-sided P values < 0.05.

RESULTS

BRAF V600E, *RAS* and *TERT* promoter mutations and their relationship in PTC in the TCGA database

From the TCGA thyroid cancer database (Cancer Genome Atlas Research Network 2014), we identified 388 PTC patients from 19 sources/medical centers (Table S1) with available information both on exon mutation and *TERT* promoter mutation and analyzed the genetic status for *BRAF* V600E, *RAS*, and *TERT* promoter mutations. *BRAF* V600E was found in 226/388 (58.2%) cases, including 183/271 (67.5%) CPTC, 13/83 (15.7%) FVPTC, and 28/30 (93.3%) TCPTC. *RAS* mutations were found in 49/388 (12.6%) cases, including 21/271 (7.7%) CPTC, 28/83 (33.7%) FVPTC and 0/30 (0%) TCPTC. *TERT* promoter mutations were found in 26/271 (9.6%) CPTC, 5/83 (6.0%) FVPTC and 7/30 (23.3%) TCPTC, with an overall prevalence of 39/388 (10.1%) in all PTC. The 39 cases of *TERT* promoter mutations included 30 C228T (76.9%), 8 C250T (20.5%), and 1 C228A (2.6%). The relationship among *BRAF* V600E, *RAS*, and *TERT* promoter mutations is illustrated in Fig 1. A significant association of *TERT* promoter mutations with the *BRAF* V600E/*RAS* mutation was observed (P=0.016). Because *BARF* V600E and *RAS* mutations were mutually exclusive (P<2.2e-16), we analyzed the relationship between *TERT* and *BRAF* V600E or *RAS* mutations in the subsets of *RAS* mutation- or *BRAF* V600E mutation-negative patients, respectively. In the *RAS* mutation-negative patients, *BRAF* V600E mutation was still significantly associated with *TERT* promoter mutation (P=0.019). In the relatively small number of *BRAF* V600E mutation-negative patients, *RAS* mutations were marginally associated with *TERT* promoter mutation (P=0.085). Overall, the majority (34/39; 87.2%) of the patients with *TERT* promoter mutations had coexisting either *BRAF* V600E or *RAS* mutations, and 34/388 (8.76%) of all PTC harbored *BRAF* V600E/*RAS* and coexisting *TERT* promoter mutations.

Impacts of *BRAF* V600E or *TERT* promoter mutation alone or in their coexistence on clinicopathologic outcomes of PTC in the TCGA data

When dividing the TCGA PTC cohort into two groups—*TERT* promoter mutation-positive versus –negative groups, a strong association of *TERT* promoter mutation with multiple poor clinicopathologic outcomes was seen (Table S2). Similar effects of *TERT* promoter mutations on clinicopathologic outcomes were seen when the analysis was performed only on CPTC (Table S3). On Kaplan-Meier analyses by dividing the cohort into *TERT* promoter mutation-negative and –positive groups, *TERT* promoter mutation was associated with a significant decline in the patient survival curve (Fig S1A) and recurrence-free survival curve (Fig S1B). Similar results were obtained on the effect of *TERT* promoter mutations when only CPTC was analyzed (Figs S1C and D).

To examine the effects of individual and coexisting genetic events of *BRAF* V600E and *TERT* promoter mutations, we divided the patients into four genotypes (Table 1). In this analysis, to exclude the influence of *RAS* mutations, we focused the analyses on the 339

RAS mutation-negative PTC patients. In comparison with the group negative for either mutation, *BRAF* V600E alone was significantly associated with extrathyroidal invasion, late disease stage III&IV, high tumor stages T3&T4 and lymph node metastasis (P<0.001, P=0.050, 0.034 and 0.040, respectively) and *TERT* promoter mutation alone did not show a significant association with any clinicopathologic outcome. In contrast, coexistence of *BRAF* V600E and *TERT* promoter mutation was robustly associated with virtually all the high-risk multiple clinicopathologic characteristics with more profound significance, including distant metastasis, disease recurrence and patient mortality (P=0.047, 0.002 and 0.010, respectively). Similar robust synergistic effects of coexisting *BRAF* V600E and *TERT* promoter mutations were observed when only CPTC in the TCGA data was analyzed (Table S4).

A recent study demonstrated a differential aggressiveness risk for the three major PTC variants in the order of TCPTC > CPTC ≫ FVPTC (Shi *et al.* 2016). We observed here a similar distribution pattern of coexisting *BRAF* V600E and *TERT* promoter mutations in the three PTC variants, with a prevalence being 7/30 (23.3%), 20/270 (7.4%), and 1/83 (1.2%) in TCPTC, CPTC, and FVPTC (Table S5), respectively, consistent with an aggressive role of the genetic duet.

Table 2 summarizes the hazard ratios (HRs) of the impacts of *BRAF* V600E and *TERT* promoter mutations on PTC recurrence in *RAS* mutation-negative patients. The HR for recurrence was not significant for *BRAF* or *TERT* promoter mutation alone but robustly significant for coexisting *BRAF* V600E and *TERT* promoter mutations. Specifically, patients harboring the genetic duet had the highest recurrence rate at 7/19 (36.8%), corresponding to 106.94 recurrences per 1,000 person-years (95% CI, 50.98 to 224.31), versus only 6/87 (6.9%), corresponding to 22.15 recurrences per 1,000 person-years (95% CI, 9.94 to 49.25), in patients harboring neither mutation, with a HR of 4.75 (95% CI, 1.58 to 14.29; P= 0.006). This HR remained significant at 6.59 (95% CI, 1.55 to 27.94; P= 0.011) after adjustment for patient age and sex and marginally (P=0.102) missed the significance after additional adjustment for tumor behaviors, but remained significant (P=0.044) after further additional adjustment for institution (Table 2). Similar HR results for tumor recurrence was observed in the analysis of only CPTC (Table S6).

We next performed Kaplan-Meier analyses of patient survival and recurrence-free survival by genotype in *RAS* mutation-negative patients. Comparison of the four genotype groups globally revealed a significant difference in survival and recurrence-free survival (P<0.0001 and P=0.0097, Fig 2A and 2B). As shown in Fig 2A, in paired group comparison, compared with the group negative for either mutation, *BRAF* V600E mutation was not significantly associated with survival decline (P=0.19), but coexisting *BRAF* V600E and *TERT* promoter mutations were significantly associated with a decline in the survival curve (P=0.045). *TERT* promoter mutation alone was associated with a survival decline but the number of cases was limited. For disease recurrence-free survival (Fig 2B), *BRAF* V600E or *TERT* promoter mutation alone had a modest effect (P=0.21 and 0.08, respectively), but their coexistence was robustly associated with a sharp decline in the recurrence-free survival curve (P=0.002). These results were consistent with the synergistic effects of *BRAF* V600E and *TERT* promoter mutations on other clinicopathologic outcomes of PTC (Table 1). Similar results

were obtained when Kaplan-Meier analyses were performed only on CPTC (Figs S2A, S2B).

Impacts of *RAS* or *TERT* promoter mutation alone or their coexistence on clinicopathologic outcomes of PTC in the TCGA data

We analyzed the impacts of *RAS* or *TERT* promoter mutation alone or in coexistence on clinicopathologic outcomes in the TCGA data (Table 3). To exclude the influence of *BRAF* V600E, this analysis was focused on the 162 *BRAF* V600E mutation-negative PTC patients. Interestingly, in comparison with the group negative for either mutation, *RAS* mutation alone showed no adverse effect on any of the clinicopathological characteristics; in fact, *RAS* mutation was even inversely associated with lymph node metastasis (P=0.043). In contrast, the coexistence of *RAS* and *TERT* promoter mutations was strongly associated with older patient age, male sex, late disease stages III&IV, distant metastasis, and recurrence compared with the group negative for either mutation (P=0.024, 0.044, 0.004, <0.001 and <0.001, respectively) (Table 3). Mortality could not be analyzed due to small number of deaths. In the analysis on CPTC, we similarly observed synergistic effects of the two mutations on poor clinicopathologic outcomes (Table S7).

On the HR analysis for disease recurrence (Table 2), all 6 patients harboring both *RAS* and *TERT* promoter mutations had disease recurrence (100%, 1,173 recurrences per 1,000 person-years; 95% CI, 526.99 to 2610.97) versus only 6/87 (6.9%, 22.15 recurrences per 1,000 person-years; 95% CI, 9.94 to 49.25) in patients harboring neither mutation, corresponding to a HR of 39.04 (95% CI, 10.56 to 144.3; P<0.001). The HR remained significant at 106.76 (95% CI, 15.30–744.49, P<0.001) after adjustment for patient age and sex and at 89.88 (95% CI, 8.89–909.14, P<0.001) after additional adjustment for clinicopathologic risk factors and still significant at 138.0 (95% CI, 5.75–3313, P=0.002) after further additional adjustment for institution. Similar HR results for recurrence were obtained when only CPTC was analyzed (Table S6).

We next performed Kaplan-Meier analyses of the impacts of *RAS* and *TERT* promoter mutations on patient survival and disease recurrence-free survival. With the limited number of deaths, no difference was seen in patient survival among the different genotypes (Fig 2C). This was also the case when only CPTC was analyzed (Fig S2C). In contrast, although *RAS* or *TERT* promoter mutation each alone was associated with only a modest decline in recurrence-free survival curve, coexistence of the two mutations was robustly associated with a sharp decline in the recurrence-free survival curve (Fig 2D). These results were similarly observed when only CPTC was analyzed (Fig S2D).

Impacts of *BRAF* V600E/*RAS* or *TERT* promoter mutation alone or in coexistence on clinicopathologic outcomes of PTC in the TCGA data

Since *BRAF* V600E and *RAS* mutations were mutually exclusive but were both associated with *TERT* promoter mutations in PTC, we pooled the two mutations to collectively examine their relationship with *TERT* promoter mutations in affecting the clinicopathologic outcomes in the 388 PTC patients from the TCGA database. As shown in Table 4, in comparison with the group negative for any mutation, *BRAF* V600E/*RAS* mutation alone

was only associated with extrathyroidal invasion (P=0.005) and *TERT* promoter mutation alone was not significantly associated with any poor clinicopathologic characteristics. In contrast, the coexistence of *BRAF* V600E/*RAS* and *TERT* promoter mutation was strongly associated with older patient age, extrathyroidal invasion, advanced disease stages III/IV, high tumor stages T3&T4, distant metastasis, disease recurrence (P<0.001 for all), and patient mortality (P=0.019). Similar results were obtained when only CPTC patients were analyzed (Table S8).

We analyzed the HR of these genotypes for PTC recurrence (Table 2). Specifically, 13/25 patients harboring both *BRAF* V600E/*RAS* and *TERT* promoter mutations had disease recurrence (52%, 184.2 recurrences per 1,000 person-years; 95% CI, 106.96 to 317.28) versus only 6/87 (6.9%, 22.15 recurrences per 1,000 person-years; 95% CI, 9.94 to 49.25) patients harboring no mutation, corresponding to a HR of 8.17 (95% CI, 3.09 to 21.58; P<0.001). This HR remained significant at 12.44 (95% CI, 3.33–46.56, P<0.001) after adjustment for patient age and sex and still robustly significant at 13.16 (95% CI, 2.39–72.42, P=0.003) after additional adjustment for classical clinicopathologic risk factors and at 14.71 (95% CI, 2.79–77.61, P=0.002) after further additional adjustment for institution. A similar robust HR of recurrence for coexisting *BRAF* V600E/*RAS* and *TERT* promoter mutations was observed when only CPTC was analyzed (Tables S6).

The HR for patient mortality was not significant for *BRAF* V600E or *TERT* promoter mutation alone but was significant for the genetic duet of *BRAF* V600E and *TERT* promoter mutations (P = 0.038; Table S9), consistent with a synergistic effect of the two mutations. Due to the low mortality of PTC and relatively small cohorts, HRs for mortality was not or only marginally significant for other genetic duet conditions. Similar results were obtained when only CPTC was analyzed (Table S10). However, on Kaplan-Meier analyses, like the genetic duet of *BRAF* V600E and *TERT* promoter mutations (Figs 2A and 2B), the genetic duet of *BRAF* V600E/*RAS* and *TERT* promoter mutations robustly affected the patient survival and disease recurrence-free survival curves. Specifically, as shown in Fig 2E, while *BRAF* V600E/*RAS* had no significant impact and *TERT* promoter mutation had limited cases, coexistence of *BRAF* V600E/*RAS* and *TERT* promoter mutations was significantly associated with a sharp decline in the patient survival curve. *BRAF* V600E/*RAS* or *TERT* promoter mutation alone had only a modest effect on disease recurrence-free survival, while coexistence of *BRAF* V600E/*RAS* and *TERT* promoter mutations was associated with a sharp decline in the disease-free survival curve (Fig 2F). Similar results were obtained when only CPTC was analyzed (Figs S2E and S2F).

As for the genetic duet of *BRAF* V600E and *TERT* promoter mutations, a similar distribution pattern of the genetic duet of *BRAF* V600E/*RAS* and *TERT* promoter mutations was seen in the PTC subtypes, with a prevalence being 7/30 (23.3%), 23/270 (8.5%), and 4/83 (4.8%) in TCPTC, CPTC, and FVPTC (Table S5), respectively, corresponding to the aggressiveness order of TCPTC > CPTC ≫ FVPTC reported recently (Shi *et al.* 2016). These results were again consistent with an aggressive role of the genetic duet of *BRAF* V600E/*RAS* and *TERT* promoter mutations in PTC.

DISCUSSION

Since the initial report of *TERT* promoter mutations in thyroid cancer three years ago (Liu *et al*. 2013), many studies have been devoted to investigating their role in the pathogenesis and clinicopathologic outcomes of thyroid cancer (Liu & Xing 2016). An interesting aspect in this regard is the association of *TERT* promoter mutation with *BRAF* V600E, which, after its initial report (Liu *et al*. 2013), has been widely confirmed (Liu & Xing 2016). We hypothesized that the coexisting event of these two major oncogenic mutations likely played a special role in thyroid cancer pathogenesis and conferred a subset of PTC unique clinicopathologic properties (Liu *et al*. 2013). Indeed, this was proven to be true in our subsequent studies which demonstrated a robust synergistic role of coexisting *BRAF* V600E and *TERT* promoter mutations in the development of poor clinicopatholoigc outcomes of PTC, including sharply increased tumor recurrence and patient mortality (Xing *et al*. 2014a, b). As a result, we proposed that the genetic duet of *BRAF* V600E and *TERT* promoter mutations constitutes a unique genetic background that strongly promotes the aggressiveness of thyroid cancer and predicts the worst clinical outcomes of PTC, which was lauded by other investigators (Ngeow & Eng 2014). These findings on the genetic duet of *BRAF* V600E and *TERT* promoter mutations were confirmed by other studies (Bullock *et al*. 2016; Jin *et al*. 2016; Kim *et al*. 2016; Song *et al*. 2016). Our recent study on an extended cohort of 1,051 PTC patients again demonstrated a robust role of coexisting *BRAF* V600E and *TERT* promoter mutations in the aggressiveness of PTC and a strong prognostic value of this genetic duet for the mortality of PTC patients (Liu *et al*. 2016). Nevertheless, the potential role of *RAS* mutations, which are second most common after *BRAF* V600E mutation in PTC, in this genetic interplay in affecting the aggressiveness of PTC has not been established.

Our recent meta analysis on *TERT* promoter mutations in thyroid cancer demonstrated a significant association between *RAS* and *TERT* promoter mutations in FTC and poorly differentiated and anaplastic thyroid cancers (Liu & Xing 2016). This was confirmed in two recent studies (Landa *et al*. 2016; Sohn *et al*. 2016). Marginal association of coexisting *RAS* and *TERT* promoter mutations with poor tumor behaviors was seen in limited FTC cohorts (Muzza *et al*. 2015; Sohn *et al*. 2016). A recent study reported an association between this genetic duet and poor clinical outcomes in a cohort of patients mixed with FTC and PTC, in which the genetic duet occurred mostly in the FTC patients (Song *et al*. 2016). Also, the potential influence of *BRAF* V600E was not dissected in this analysis. Thus, the specific role of coexisting *RAS* and *TERT* promoter mutations in the pathogenesis and clinical outcomes of PTC remains undefined. Importantly, because *BRAF* V600E and *RAS* mutations are mutually exclusive (Xing 2013) and the previous studies on the role of the genetic duet of *BRAF* V600E and *TERT* promoter mutations in PTC did not separate the potential influence of *RAS* mutations, it is not clear whether the *RAS* mutation status could in fact affect the conclusions on the genetic duet of *BRAF* and *TERT* promoter mutations in the previous studies.

We performed the present study using the unique PTC cohort in the TCGA database to address the issues discussed above. The TCGA thyroid cancer database provided an ideal cohort of PTC for the present study as it consisted of patients from a large number of

medical institutions in the North America with comprehensive genetic, pathological and clinical information. The present study was therefore multicenter in nature. The first goal of this study was to confirm the previous findings in single-institution studies and validate the role of *TERT* promoter mutations and the genetic duet of *BRAF* V600E and *TERT* promoter mutations in PTC. This was successfully achieved. Specifically, we demonstrated that *BRAF* V600E and *TERT* promoter mutations each alone had no or only a modest effect but the genetic duet of the two had a robust effect on virtually all the poor clinicopatholgoic outcomes of PTC. Thus, the findings in previous studies on *BRAF* V600E and *TERT* promoter mutations were essentially all reproduced in this multicenter data analysis, confirming and validating the recent conclusions on the genetic duet of *BRAF* V600E and *TERT* promoter mutations in PTC. A similar pattern of the effects was seen for *RAS* and *TERT* promoter mutations. Specifically, RAS mutation alone had no effect while the genetic duet of the two had a strong synergistic effect on the poor clinicopathologic outcomes of PTC. This was the case whether the whole PTC cohort or only CPTC was analyzed. This is the first demonstration of a robust synergistic role of coexisting *RAS* and *TERT* promoter mutations in the aggressiveness of PTC.

In addition to the multicenter nature, another unique strength that distinguishes the present study from previous studies is the separation of *BRAF* V600E and *RAS* mutations from each other in the analysis of their synergistic effects with *TERT* promoter mutations. This provided definitive evidence supporting that coexistence of either *BRAF* V600E or *RAS* mutation with *TERT* promoter mutation is a robust genetic mechanism that drives the worst aggressiveness of PTC. The underlying molecular mechanism was proposed to involve the activation of the MAP kinase pathway which promotes the *TERT* expression by upregulating ETS transcription factors acting at the mutation sites in the *TERT* promoter (Liu *et al.* 2013; Liu & Xing 2016). This is consistent with the finding that coexisting *BRAF* V600E and *TERT* promoter mutations were associated with increased expression of *TERT* (Vinagre *et al.* 2013). It is not clear how the genetic duet occurs. Since either *BRAF*/*RAS* mutation or *TERT* promoter mutation can occur individually and each alone has limited aggressive role, one possibility is that the occurrence of each of the two individual mutations is a random event independent of each other; but once the two individually random mutations happen to occur in the same thyroid cancer cell, the genetic duet confers the cell superior survival (and aggressiveness) ability and consequently, from an evolutionary perspective, such a cell can be preferentially selected naturally, resulting in the observed common concurrence of the two mutations in aggressive thyroid cancers.

A weakness of the present study is the relatively small cohort of 388 patients, which was smaller than many of the previous single-institution studies. Separation of *BRAF* V600E from *RAS* mutations made some of the subgroup analyses even smaller. This may explain the marginal significance of multivariate analyses in some subgroups. This was particularly an issue when HRs for the relatively low mortality were analyzed. Nevertheless, this study was able to essentially reproduce all the previous findings on *TERT* promoter mutations and demonstrate a robust role of the genetic duet of *TERT* promoter and *BRAF* V600E or *RAS* mutations in PTC, establishing a strong prognostic power for these genetic events in the aggressiveness of PTC. In fact, when the *BRAF* V600E and *RAS* mutation patients were pooled to analyze their synergistic effects with *TERT* promoter mutations, even more robust

effects of the genetic duet of *BRAF* V600E/*RAS* mutations on poor clinicopathologic outcomes were observed (Table 4) and the effect on PTC recurrence remained significant even upon adjustment for all the conventional high-risk clinicopathologic factors (Table 2).

In summary, this study using the unique multicenter PTC cohort in the TCGA database confirmed the recent findings in single-institution studies on the role of *BRAF* V600E and *TERT* promoter mutations in the aggressiveness of PTC. The role of the genetic duet of *BRAF* V600E and *TERT* promoter mutations was particularly firmly established by focused analyses on them with exclusion of *RAS* mutations. Importantly, this study for the first time established a similar role of the genetic duet of *RAS* and *TERT* promoter mutations in PTC. The overall occurrence of coexisting *BRAF* V600E and *TERT* promoter mutations in a large series of PTC was 145/1,892 (7.7%) (Liu *et al*. 2016) and the overall coexisting *BRAF* V600E/*RAS* and *TERT* promoter mutations in the present study was 34/388 (8.76%), which numerically correspond well to the conventionally known about 5–10% of PTC patients that inherently have a particularly aggressive disease course and are the source of virtually all PTC-related mortality (Haugen *et al*. 2016). We recently proposed a four-genotype risk stratification system for PTC with a risk order of the genetic duet >>>> *BRAF* V600E alone = *TERT* promoter mutation alone > the wild-type for both genes (Liu *et al*. 2016). Given the findings in the present study, this genetic prognostication system may now be modified into a six-genotype prognostic system by incorporating also *RAS* mutation into it with a risk order of genetic duet of *BRAF* V600E/*RAS* mutation and *TERT* promoter mutations >>>> *BRAF* V600E = *TERT* mutation alone > *RAS* mutation alone = wild-type genes in papillary thyroid cancer. This simple but powerful genetic molecular prognostic system may help pinpoint the small subgroup of PTC patients with the highest aggressiveness risk for personalized precision treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Alzahrani AS, Alsaadi R, Murugan AK, Sadiq BB. TERT Promoter Mutations in Thyroid Cancer. Hormones & Cancer. 2016; 7:165–177. DOI: 10.1007/s12672-016-0256-3 [PubMed: 26902827]

Bullock M, Ren Y, O'Neill C, Gill A, Aniss A, Sywak M, Sidhu S, Delbridge L, Learoyd D, de Vathaire F, et al. TERT promoter mutations are a major indicator of recurrence and death due to papillary thyroid carcinomas. Clinical Endocrinology. 2016; 85:283–290. DOI: 10.1111/cen.12999 [PubMed: 26667986]

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 [PubMed: 25417114]

Garcia-Rostan G, Zhao H, Camp RL, Pollan M, Herrero A, Pardo J, Wu R, 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 [PubMed: 12947056]

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 [PubMed: 26462967]

Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, Kadel S, Moll I, Nagore E, Hemminki K, et al. TERT promoter mutations in familial and sporadic melanoma. Science. 2013; 339:959–961. DOI: 10.1126/science.1230062 [PubMed: 23348503]

Howlader, N.; Noone, A.; Krapcho, M.; Miller, D.; Bishop, K.; Altekruse, S.; Kosary, C.; Yu, M.; Ruhl, J.; Tatalovich, Z., et al. SEER Cancer Statistics Review, 1975–2013. National Cancer Institute; Bethesda, MD: 2016. http://seer.cancer.gov/csr/1975_2013/, based on November 2015 SEER data submission, posted to the SEER web site

Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013; 339:957–959. DOI: 10.1126/science.1229259 [PubMed: 23348506]

Jin L, Chen E, Dong S, Cai Y, Zhang X, Zhou Y, Zeng R, Yang F, Pan C, Liu Y, et al. BRAF and TERT promoter mutations in the aggressiveness of papillary thyroid carcinoma: a study of 653 patients. Oncotarget. 2016; 7:18346–18355. DOI: 10.18632/oncotarget.7811 [PubMed: 26943032]

Kim TH, Kim Y-E, Ahn S, Kim J-Y, Ki C-S, Oh YL, Kim K, Yun JW, Park W-Y, Choe J-H, 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 [PubMed: 27528624]

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 [PubMed: 26878173]

Liu R, Xing M. TERT promoter mutations in thyroid cancer. Endocrine-Related Cancer. 2016; 23:R143–R155. DOI: 10.1530/ERC-15-0533 [PubMed: 26733501]

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; Published online September 01, 2016. doi: 10.1001/jamaoncol.2016.3288

Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, Sun H, El-Naggar AK, Xing M. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocrine-Related Cancer. 2013; 20:603–610. DOI: 10.1530/ERC-13-0210 [PubMed: 23766237]

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. The Journal of Clinical Endocrinology & Metabolism. 2014; 99:E1130–E1136. DOI: 10.1210/jc.2013-4048 [PubMed: 24617711]

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 [PubMed: 25448848]

Ngeow J, Eng C. TERT and BRAF in Thyroid Cancer: Teaming Up for Trouble. Journal of Clinical Oncology. 2014; 32:2683–2684. DOI: 10.1200/JCO.2014.56.5614 [PubMed: 25024083]

Shi X, Liu R, Basolo F, Giannini R, Shen X, Teng D, Guan H, Shan Z, Teng W, Musholt TJ, et al. Differential Clinicopathological Risk and Prognosis of Major Papillary Thyroid Cancer Variants. The Journal of Clinical Endocrinology & Metabolism. 2016; 101:264–274. DOI: 10.1210/jc.2015-2917 [PubMed: 26529630]

Shi X, Liu R, Qu S, Zhu G, Bishop J, Liu X, Sun H, Shan Z, Wang E, Luo Y, et al. Association of TERT promoter mutation 1,295,228 C>T with BRAF V600E mutation, older patient age, and

distant metastasis in anaplastic thyroid cancer. The Journal of Clinical Endocrinology & Metabolism. 2015; 100:E632–E637. DOI: 10.1210/jc.2014-3606 [PubMed: 25584719]

Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: A Cancer Journal for Clinicians. 2015; 65:5–29. DOI: 10.3322/caac.21254 [PubMed: 25559415]

Sohn SY, Park W-Y, Shin H-T, Bae JS, Ki C-S, Oh YL, Kim SW, Chung JH. Highly concordant key genetic alterations in primary tumors and matched distant metastases in differentiated thyroid cancer. Thyroid. 2016; 26:672–682. DOI: 10.1089/thy.2015.0527 [PubMed: 26971368]

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 [PubMed: 26969876]

Vinagre J, Almeida A, Pópulo H, Batista R, Lyra J, Pinto V, Coelho R, Celestino R, Prazeres H, Lima L, et al. Frequency of TERT promoter mutations in human cancers. Nature Communications. 2013; 4:2185.doi: 10.1038/ncomms3185

Xing M. BRAF mutation in thyroid cancer. Endocrine-Related Cancer. 2005; 12:245–262. DOI: 10.1677/erc.1.0978 [PubMed: 15947100]

Xing M. BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocrine Reviews. 2007; 28:742–762. DOI: 10.1210/er.2007-0007 [PubMed: 17940185]

Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nature Reviews Cancer. 2013; 13:184–199. DOI: 10.1038/nrc3431 [PubMed: 23429735]

Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, Elisei R, Bendlova 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 [PubMed: 25332244]

Xing M, 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 [PubMed: 23571588]

Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, Pai S, Bishop S. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. Journal of Clinical Oncology. 2014a; 32:2718–2726. DOI: 10.1200/JCO.2014.55.5094 [PubMed: 25024077]

Xing M, Liu R, Bishop S. TERT promoter and BRAF mutations cooperatively promote papillary thyroid cancer-related mortality. Thyroid. 2014b; 24(S1):A-131–A-139. DOI: 10.1089/thy.2014.2410.abstracts.sc [PubMed: 25340533]

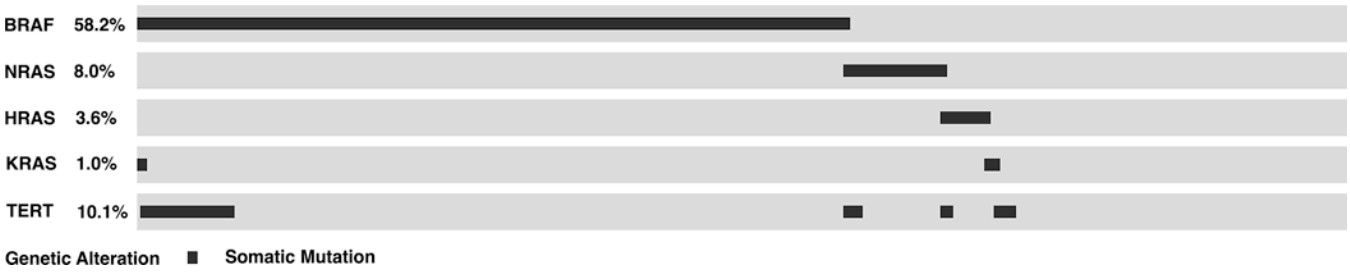

Figure 1. Gene-sample matrix of mutations on *BRAF* V600E, *RAS* and *TERT* promoter mutations

The matrix illustrates the genetic status of the 388 cases of papillary thyroid cancer (PTC) included in the present study from the TCGA database. Cases positive for the indicated gene mutations are marked with dark color. Several relationships among the different mutations are evident, including the mutual exclusiveness between *BRAF* V600E and *RAS* mutations, the association between *BRAF* V600E and *TERT* promoter mutations, and the association between *RAS* and *TERT* promoter mutations in PTC. Occurrence of *TERT* promoter mutation alone is uncommon. Thirty-four of the 39 (87.2%) patients positive for *TERT* promoter mutations have coexisting either *BRAF* V600E or *RAS* mutations and 34/388 (8.76%) of all PTC harbor the genetic duet of coexisting *BRAF* V600E/*RAS* and *TERT* promoter mutations.

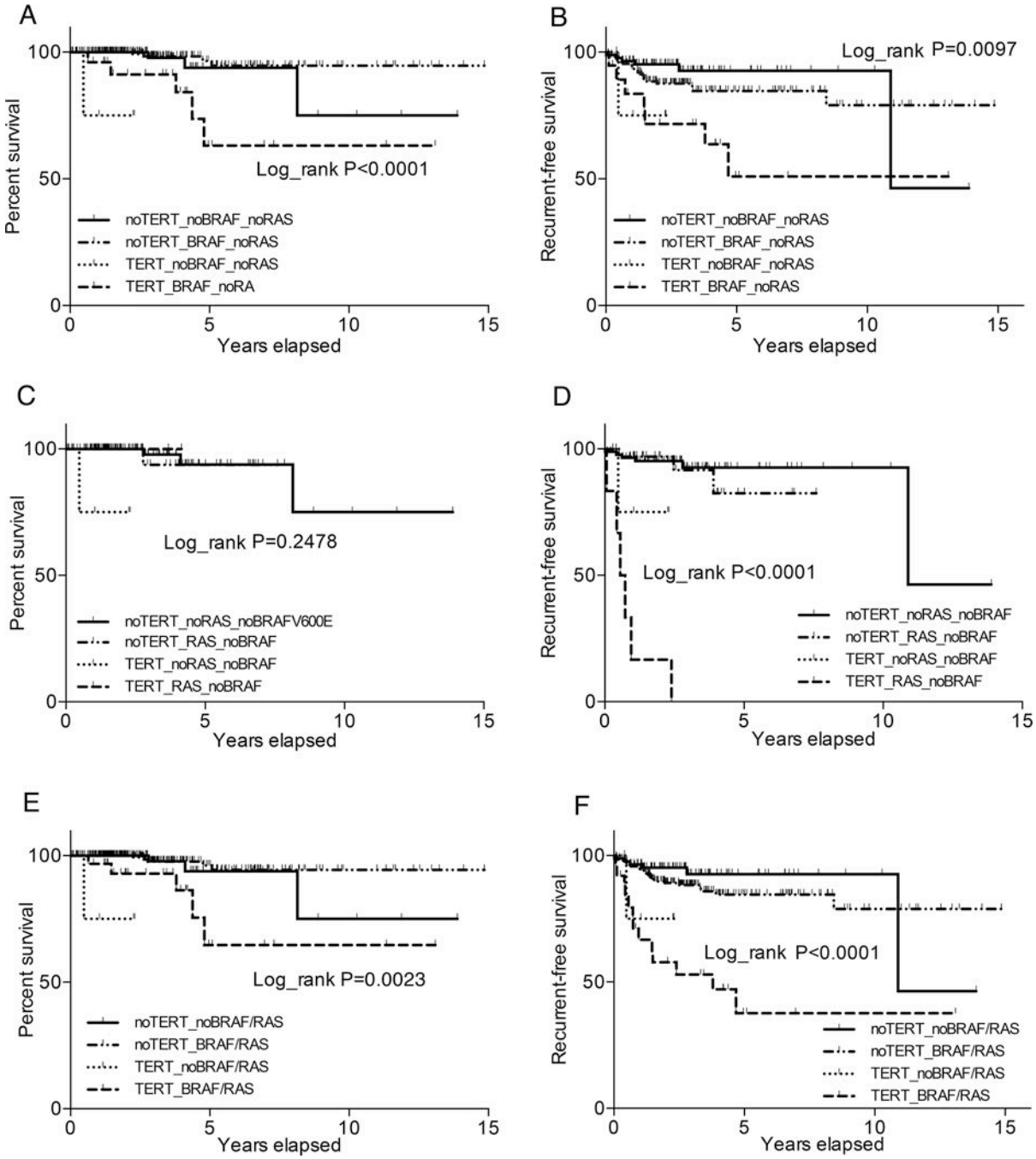

Figure 2. Kaplan-Meier analyses of the impacts of *BRAF* V600E, *RAS* or *TERT* promoter mutations or their coexistence on patient survival and disease recurrence-free survival of patients with papillary thyroid cancer (PTC) in the TCGA database

A. Impacts of *BRAF* V600E or *TERT* promoter mutations or their coexistence on patient survival. **B**. Impacts of *BRAF* V600E or *TERT* promoter mutations or their coexistence on PTC recurrence-free survival. The analyses in **A** and **B** were performed with exclusion of the cases positive for *RAS* mutations. **C**. Impacts of *RAS* or *TERT* promoter mutations or their coexistence on patient survival. **D**. Impacts of *RAS* or *TERT* promoter mutations or their coexistence on PTC recurrence-free survival. The analyses in **C** and **D** were performed with

exclusion of the cases positive for *BRAF* V600E mutation. **E**. Impacts of *BRAF*/*RAS* or *TERT* promoter mutations or their coexistence on patient survival. **F**. Impacts of *BRAF*/*RAS* or *TERT* promoter mutations or their coexistence on PTC recurrence-free survival. The analyses in **E** and **F** were performed on the whole cohort of PTC patients without genetic-based exclusion. The Log-rank P value in each panel represents the comparison among the four groups globally.

Table 1

Impacts of *BRAF* V600E or *TERT* promoter mutation alone or in their coexistence on clinicopathologic outcomes of PTC

Characteristic	No Mutation	*BRAF* V600E only		*TERT* Mutation Only		*BRAF+TERT* Mutation	
	No. (Percent)	No. (Percent)	P	No. (Percent)	P	No. (Percent)	P
Sample size	109	197		5		28	
Age at diagnosis, Median (IQR), y	46 (33–59)	46 (34–54)	.52	49 (37–68)	.52	66 (59–73)	<.001
Sex, male	27/109 (24.8)	53/197 (26.9)	.79	0/5 (0)	.34	9/28 (32.1)	.47
Multifocality	53/103 (51.5)	89/194 (45.9)	.43	0/5 (0)	.06	12/28 (42.9)	.52
Tumor size, Median (IQR), cm	2.9 (1.9–4)	2.5 (1.5–3.6)	.28	4 (3.0–5.0)	.68	3 (2.4–4.2)	.21
Extrathyroidal invasion	17/104 (16.3)	69/193 (35.8)	<.001	1/5 (20)	1	17/28 (60.7)	<.001
AJCC tumor stage							
stage I	66/108 (61.1)	115/196 (58.7)		3/5 (60)		5/28 (17.9)	
stage II	18/108 (16.7)	15/196 (7.7%)	.031	1/5 (20)	.31	2/28 (7.1)	<.001
stage III	19/108 (17.6)	47/196 (24)		0/5 (0)		10/28 (35.7)	
stage IV	5/108 (4.6)	19/196 (9.7)		1/5 (20)		11/28 (39.3)	
stage III + stage IV	24/108 (22.2)	66/196 (33.7)	.050	1/5 (20)	1	21/28 (75)	<.001
AJCC_T							
T1	31/107 (29.0)	61/197 (31)		1/5 (20)		4/28 (14.3)	
T2	46/107 (43.0)	56/197 (28.4)	.022	2/5 (40)	.06	6/28 (21.4)	<.001
T3	30/107 (28.0)	74/197 (37.6)		1/5 (20)		11/28 (39.3)	
T4	0/107 (0)	6/197 (3)		1/5 (20)		7/28 (25)	
T3+T4	30/107 (28.0)	80/197 (40.6)	.034	2/5 (40)	.62	18/28 (64.3)	<.001
AJCC_N, N1	38/92 (41.3)	99/179 (52.4)	.040	3/5 (60)	.64	17/27 (63.0)	.052
AJCC_M, M1	0/54 (0)	3/120 (2.5)	.55	0/4 (0)	1	2/19 (10.5)	.07
Distant metastasis	1/50 (2)	8/103 (7.8)	.27	0/4 (0)	1	3/17 (17.6)	.047
Tumor recurrence	6/87 (6.9)	22/154 (14.3)	.13	1/5 (20)	.33	7/19 (36.8)	.002

Characteristic	No Mutation	*BRAF* V600E only		*TERT* Mutation Only		*BRAF+TERT* Mutation	
	No. (Percent)	No. (Percent)	P	No. (Percent)	P	No. (Percent)	P
Mortality rate	3/108 (2.8)	4/189 (2.1)	.71	1/5 (20)	.17	5/28 (17.9)	.010
Total follow-up, Median (IQR), month	30 (17–46)	37 (21–65)	.056	13 (6–28)	.15	43 (16–55)	.13

This table summarizes the analyses in 339 cases of patients with PTC negative for *RAS* mutation. IQR, interquartile range; AJCC, American Joint Committee on Cancer. AJCC_M refers to distant metastasis status at the diagnosis of thyroid cancer; M1, presence of metastasis. The group “Distant metastasis” below the group “AJCC_M” in the table refers to any distant metastasis, including distant metastasis at the diagnosis and distant metastasis discovered during the follow-up time, i.e., metastatic recurrence.

Table 2

Hazard ratios of *BRAF* V600E/*RAS* or *TERT* promoter mutations each alone or their coexistence for the recurrence of PTC

	Mutations	Recurrence	Recurrence per 1,000 Person-Years (95% CI)	Unadjusted Hazard Ratios (95% CI)	Adjustment 1 Hazard Ratios (95% CI)	Adjustment 2 Hazard Ratios (95% CI)	Adjustment 3 Hazard Ratios (95% CI)
BRAF	No mutation	6/87 (6.9)	22.15 (9.94–49.25)	1			
	*BRAF*V600E mutation only	22/154 (14.3)	35.5 (23.14–54.44)	1.88 (0.76–4.63)	1.84 (0.74–4.56)	1.36 (0.5–3.7)	1.34 (0.43–4.17)
	TERT mutation only	1/5 (20)	154.27 (21.73–1095.16)	5.82 (0.64–52.64)	6.24 (0.65–60.10)	0.90 (0.05–15.55)	25.30 (0.52–1241)
	*BRAF*V600E+ *TERT* mutations	7/19 (36.8)	106.94 (50.98–224.31)	4.75 (1.58–14.29)	6.59 (1.55–27.94)	5.46 (0.71–41.97)	71.36 (1.11–4560)
RAS	No mutation	6/87 (6.9)	22.15 (9.94–49.25)	1			
	RAS mutation only	3/34 (8.8)	31.62 (10.20–98.03)	1.51 (0.36–6.34)	1.44 (0.34–6.06)	0.65 (0.11–3.77)	0.35 (0.04–2.82)
	TERT mutation only	1/5 (20)	154.27 (21.73–1095.16)	5.82 (0.64–52.64)	6.24 (0.65–60.10)	0.9 (0.05–15.55)	25.30 (0.52–1241)
	RAS+ *TERT* mutations	6/6 (100)	1173 (526.99–2610.97)	39.04 (10.56–144.3)	106.76 (15.30–744.49)	89.88 (8.89–909.14)	138.0 (5.75–3313)
BRAF/RAS	No mutation	6/87 (6.9)	22.15 (9.94–49.25)	1			
	BRAF V600E/RAS mutation only	25/189 (13.2)	34.86 (23.36–52.01)	1.77 (0.72–4.31)	1.74 (0.71–4.24)	1.3 (0.48–3.5)	1.24 (0.42–3.66)
	TERT mutation only	1/5 (20)	154.27 (21.73–1095.16)	5.82 (0.64–52.64)	6.24 (0.65–60.10)	0.90 (0.05–15.55)	25.30 (0.52–1241)
	BRAF V600E/*RAS*+ *TERT* mutations	13/25 (52)	184.2 (106.96–317.28)	8.17 (3.09–21.58)	12.44 (3.33–46.56)	13.16 (2.39–72.42)	14.71 (2.79–77.61)

The "*BRAF*" group excluded patients with *RAS* mutations, the "*RAS*" group excluded patients with *BRAF* mutation, and the "*BRAF/RAS*" group included all the patients regardless of the *BRAF* and *RAS* mutation status. Hazard ratio and 95% CI were calculated using Cox regression for the comparison of the indicated mutation group with the group harboring neither mutation. Adjustment 1 was made for patient age at diagnosis and sex. Adjustment 2 was made for patient age at diagnosis, sex, multifocality, tumor size, extrathyroidal invasion and lymph node metastasis. Adjustment 3 was made for patient age at diagnosis, sex, multifocality, tumor size, extrathyroidal invasion, lymph node metastasis and medical center.

Table 3

Impact of *RAS* or *TERT* promoter mutation or their coexistence on clinicopathologic outcomes of PTC

Characteristic	No Mutation	*RAS* Mutation Only		*TERT* Mutation Only		*RAS+TERT* Mutation	
	No. (Percent)	No. (Percent)	P	No. (Percent)	P	No. (Percent)	P
Sample size	109	42		5		6	
Age at diagnosis, Median (IQR), y	46 (33–59)	41 (32–50)	.10	49 (37–68)	.52	62 (58–65)	.024
Sex, male	27/109 (24.8)	9/42 (21.4)	.83	0/5 (0)	.34	4/6 (66.7)	.044
Multifocality	53/103 (51.5)	18/42 (42.9)	.37	0/5 (0)	.06	2/6 (33.3)	.44
Tumor size, Median (IQR), cm	2.9 (1.9–4)	2.3 (1.8–2.9)	.13	4 (3.0–5.0)	.68	4.4 (2.4–6.2)	.22
Extrathyroidal invasion	17/104 (16.3)	5/38 (13.2)	.80	1/5 (20)	1	2/6 (33.3)	.28
AJCC tumor stage							
stage I	66/108 (61.1)	31/42 (73.8)		3/5 (60)		0/6 (0)	
stage II	18/108 (16.7)	4/42 (9.5)	.59	1/5 (20)	.31	1/6 (16.7)	<.001
stage III	19/108 (17.6)	6/42 (14.3)		0/5 (0)		1/6 (16.7)	
stage IV	5/108 (4.6)	1/42 (2.4)		1/5 (20)		4/6 (66.7)	
stage III + stage IV	24/108 (22.2)	7/42 (16.7)	.51	1/5 (20)	1	5/6 (83.3)	.004
AJCC_T							
T1	31/107 (29.0)	16/42 (38.1)		1/5 (20)		1/6 (16.7)	
T2	46/107 (43.0)	18/42 (42.9)	.43	2/5 (40)	.06	2/6 (33.3)	.09
T3	30/107 (28.0)	8/42 (19.0)		1/5 (20)		2/6 (33.3)	
T4	0/107 (0)	0/42 (0)		1/5 (20)		1/6 (16.7)	
T3 + T4	30/107 (28.0)	8/42 (19.0)	.61	2/5 (40)	.62	3/6 (50)	.36
AJCC_N, N1	38/92 (41.3)	8/38 (21.1)	.043	3/5 (60)	.65	2/6 (33.3)	1
AJCC_M, M1	0/54 (0)	0/26 (0)	1	0/4 (0)	1	2/2 (100)	<.001
Distant metastasis	1/50 (2)	0/23 (0)	1	0/4 (0)	1	4/4 (100)	<.001
Tumor recurrence	6/87 (6.9)	3/34 (8.8)	1	1/5 (20)	.33	6/6 (100)	<.001

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Characteristic	No Mutation	*RAS* Mutation Only		*TERT* Mutation Only		*RAS*+*TERT* Mutation	
	No. (Percent)	No. (Percent)	P	No. (Percent)	P	No. (Percent)	P
Mortality rate	3/108 (2.8)	1/42 (2.4)	1	1/5 (20)	.17	0/6 (0)	1
Total follow-up, Median (IQR), month	30 (17–46)	23 (15–48)	.53	13 (6–28)	.15	25 (23–40)	.87

This table summarizes the analyses in 162 patients with PTC negative for *BRAF* V600E mutation. IQR, interquartile range; AJCC, American Joint Committee on Cancer. AJCC_M refers to distant metastasis status at the diagnosis of thyroid cancer; M1, presence of metastasis. The group “Distant metastasis” below the group “AJCC_M” in the table refers to any distant metastasis, including distant metastasis at the diagnosis and distant metastasis discovered during the follow-up time, i.e., metastatic recurrence.

Table 4

Impact of *BRAF* V600E/*RAS* or *TERT* promoter mutation or their coexistence on clinicopathologic outcomes of PTC

Characteristic	No Mutation	*BRAF*/*RAS* Mutation Only		*TERT* Mutation Only		*BRAF*/*RAS*+*TERT* Mutation	
	No. (Percent)	No. (Percent)	P	No. (Percent)	P	No. (Percent)	P
Sample size	109	240		5		34	
Age at diagnosis, Median (IQR), y	46 (33–59)	45 (34–54)	.34	49 (37–68)	.51	66 (58–72)	<.001
Sex, male	27/109 (24.8)	62/240 (25.8)	.94	0/5 (0)	.34	13/34 (38.2)	.13
Multifocality	53/103 (51.5)	107/237 (45.1)	.34	0/5 (0)	.06	14/34 (41.2)	.33
Tumor size, Median (IQR), cm	2.9 (1.9–4)	2.4 (1.5–3.5)	.09	4 (3.0–5.0)	.68	3 (2.4–4.6)	.12
Extrathyroidal invasion	17/104 (16.3)	74/232 (31.9)	.005	1/5 (20)	1	19/34 (55.9)	<.001
AJCC tumor stage							
stage I	66/108 (61.1)	147/239 (61.5)		3/5 (60)		5/34 (14.7)	
stage II	18/108 (16.7)	19/239 (7.9)	.07	1/5 (20)	.31	3/34 (8.8)	<.001
stage III	19/108 (17.6)	53/239 (22.2)		0/5 (0)		11/34 (32.3)	
stage IV	5/108 (4.6)	20/239 (8.4)		1/5 (20)		15/34 (44.1)	
stage III + stage IV	24/108 (22.2)	73/239 (30.6)	.12	1/5 (20)	1	26/34 (76.4)	<.001
AJCC_T							
T1	31/107 (29.0)	78/240 (32.5)		1/5 (20)		5/34 (14.7)	
T2	46/107 (43.0)	74/240 (30.8)	.08	2/5 (40)	.06	8/34 (23.5)	<.001
T3	30/107 (28.0)	82/240 (34.2)		1/5 (20)		13/34 (38.2)	
T4	0/107 (0)	6/240 (2.5)		1/5 (20)		8/34 (23.5)	
T3 + T4	30/107 (28.0)	88/240 (36.7)	.14	2/5 (40)	.62	21/34 (61.7)	<.001
AJCC_N, N1	38/92 (41.3)	107/218 (49.1)	.26	3/5 (60)	.65	19/33 (57.6)	.21
AJCC_M, M1	0/54 (0)	3/146 (2.1)	.56	0/4 (0)	1	4/21 (19.0)	.005
Distant metastasis	1/50 (2)	8/126 (6.3)	.45	0/4 (0)	1	7/21 (33.3)	<.001
Tumor recurrence	6/87 (6.9)	25/189 (13.2)	.15	1/5 (20)	.33	13/25 (52)	<.001

Characteristic	No Mutation	*BRAF*/*RAS* Mutation Only		*TERT* Mutation Only		*BRAF*/*RAS*+*TERT* Mutation	
	No. (Percent)	No. (Percent)	P	No. (Percent)	P	No. (Percent)	P
Mortality rate	3/108 (2.8)	5/232 (2.2)	.71	1/5 (20)	.17	5/34 (14.7)	.019
Total follow-up, Median (IQR), month	30 (17–46)	35 (19–60)	.18	13 (6–28)	.15	42 (17–52)	.23

IQR, interquartile range; AJCC, American Joint Committee on Cancer. AJCC_M refers to distant metastasis status at the diagnosis of thyroid cancer; M1, presence of metastasis. The group “Distant metastasis” below the group “AJCC_M” in the table refers to any distant metastasis, including distant metastasis at the diagnosis and distant metastasis discovered during the follow-up time, i.e., metastatic recurrence.