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ORIGINAL RESEARCH

Co-existence of *BRAF*^{V600E} and *TERT* promoter mutations in papillary thyroid carcinoma is associated with tumor aggressiveness, but not with lymph node metastasis

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Background: Mutations of *BRAF*^{V600E} and *TERT* promoters are associated with thyroid cancer development. This study further investigated association of these mutations with clinicopathological characteristics from patients with papillary thyroid carcinoma (PTC).

Methods: Tumor tissues from 342 PTC patients were obtained for DNA extraction and polymerase chain reaction amplification to detect the *BRAF*^{V600E} mutation using amplification-refractory mutation system-polymerase chain reaction. *TERT* promoter mutations were assessed using Sanger DNA sequencing. The association of these gene mutations with clinicopathological characteristics was then statistically analyzed.

Results: Two hundred and seventy of 342 (78.9%) PTC patients harbored the *BRAF*^{V600E} mutation, which was associated with older age male patients. Moreover, *TERT* promoter mutations occurred in 12 of 342 (3.5 %) PTC patients, all of whom also had the *BRAF* mutation. One hundred thirty-three patients with papillary thyroid microcarcinoma (PTMC) had no *TERT* mutations. Statistically, the coexistence of *BRAF* and *TERT* promoter mutations were significantly associated with older age, larger tumor size, extrathyroidal extension, and advanced tumor stage, but not with central lymph node metastasis, lateral lymph node metastasis, numbers of lymph node metastasis >5, and numbers of involved/harvested lymph nodes (No. of LNs involved or harvested). The multivariate analyses showed older age (odds ratio [OR], 2.194; 95% CI: 1.117–4.311; p=0.023), larger tumor size (OR, 4.100; 95% CI: 2.257–7.450; p<0.001), and multiplicity (OR, 2.240; 95% CI: 1.309–3.831; p=0.003) were all independent predictors for high prevalence of extrathyroidal extension. However, there was no statistical association with any clinicopathological characteristics except for Hashimoto thyroiditis in PTMC.

Conclusion: The current study demonstrated that the coexistence of *BRAF* and *TERT* promoter mutations were associated with the PTC aggressiveness, although these mutations were not associated with PTC lymph node metastasis or with PTMC.

Keywords: papillary thyroid carcinoma, *TERT* promoter mutation, *BRAF* mutation, clinicopathological features

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Introduction

Thyroid cancer is the most frequently occurring endocrine malignancy, with an increasing rate of incidence over the last 3 decades.[1] Histological classification of thyroid cancer mainly includes papillary, follicular, Hürthle cell, medullary, and anaplastic carcinomas. Papillary, follicular, and Hürthle cell carcinomas are derived from the

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follicular epithelium and produce thyroglobulin, and are considered as well-differentiated thyroid carcinomas.[2] Papillary thyroid carcinoma (PTC) accounts for up to 85% of all thyroid cancers.[3] Generally, PTC has an excellent prognosis with a relatively low mortality rate, but a small portion of PTC patients suffers from an aggressive form of the disease with tumor invasion and metastasis.[4] Accurate identification of this group of PTC patients is of crucial importance to optimizing individualized PTC treatment. However, current PTC risk stratification is based on conventional clinicopathological factors, which are often insufficient to accurately identify the high-risk PTC patients at an early stage. Thus, better biomarkers could help identify patients with aggressive PTC for more aggressive treatment options.

Toward this end, accumulating evidence indicates that gene mutations are involved in the development and progression of PTC.[5,6] Mutation of the proto-oncogene *BRAF* at V600E, is the most prevalent mutation in PTC, being present in ~29%–83% of PTC patients.[5] *BRAF* protein is a member of the rapidly accelerated fibrosarcoma kinase family of growth signal transduction protein kinases and plays a role in regulating activity of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase signaling pathway to promote cell growth, differentiation, and anti-apoptosis. Thus, the *BRAF*^{V600E} mutation was shown to be a potent MAPK activator and occurs in many human cancers, including thyroid cancer.[7] Previous studies showed that the *BRAF*^{V600E} mutation was associated with 1 or more aggressive PTC clinical characteristics, such as older age, extrathyroidal extension (ETE), lymph node metastasis, aggressive histological subtypes, impaired iodine uptake, or tumor recurrence.[8–10] However, other studies reported no statistical association of the *BRAF*^{V600E} mutation with these high-risk clinicopathological characteristics.[11–13]

Additionally, *TERT* is the catalytic subunit of the enzyme telomerase, which maintains telomere ends by addition of the telomere repeat TTAGGG. TERT plays a role in cell senescence, aberrant expression of which is associated with human cancer development.[14] Mutations of the *TERT* promoter arise in certain types of human cancer, including melanoma, bladder cancer, glioblastoma, and thyroid cancer, and frequently occur as 2 recurrent somatic mutation hotspots, that is, chr5:1,295,228 C > T (C228T) and chr5:1,295,250 C > T (C250T).[15–18] Mutations of the *TERT* promoter were shown to be a promising indicator of PTC aggressiveness and poor prognosis, and were consistently validated in several previous studies.[15,19] In addition, Liu et al first reported a link between mutations of the *TERT* promoter and *BRAF*^{V600E} in PTC, and subsequent studies further demonstrated the coexistence of *TERT* promoter and *BRAF*^{V600E} mutations in thyroid cancers and their association with the most aggressive PTC clinical factors and worse prognosis.[16,20–23]

In this study, we further investigated the frequency of *BRAF*^{V600E} and *TERT* promoter mutations for association with clinicopathological characteristics in PTC patients. We expected to provide more insightful information for association of *BRAF* and *TERT* promoter mutations with the most aggressive PTC phenotypes.

Materials and methods

Patients and tissue samples

This study included 342 consecutive patients who underwent surgical PTC resection between August 2016 and August 2017 at The Department of Endocrine and Breast Surgery, The First Affiliated Hospital, Chongqing Medical University (Chongqing, China). However, patients who refused to be included for genetic testing or for whom there was a lack of clinical and pathological data were excluded from the study. Among these 342 patients, 251 were clinical lymph node-negative (cN0) PTC patients and 91 were clinical lymph node-positive (cN1) patients, confirmed by fine-needle aspiration and neck ultrasound. Two hundred ninety patients (84.8%) received a total thyroidectomy. The remaining patients underwent lobectomy with isthmectomy. All patients also received central lymph node dissections (CLND), while 322 patients (94.1%) also received lateral lymph node dissections (LLND), including therapeutic (91 of 322) and prophylactic (231 of 322) dissections. Patients were histologically diagnosed with PTC according to the World Health Organization for the diagnosis of PTC.[2] However, the PTC subtypes were not routinely identified in our institution. The tumor/node/metastasis (TNM) staging was defined according to the 8th edition of the American Joint Committee on Cancer.[24] The fresh tissue samples were collected during surgery, snap-frozen in liquid nitrogen, and stored at −80°C.

In this study, paraffin-embedded tissue sections were obtained and re-evaluated to confirm histological diagnosis by 2 pathologists. Their clinicopathological information, including age, sex, tumor size, multifocality, Hashimoto thyroiditis (HT), ETE, central lymph node metastasis (CLNM), lateral lymph node metastasis (LLNM), numbers of lymph node metastasis (LNMN) >5, numbers of involved and harvested lymph nodes (No. of LNs involved or harvested), and TNM stage were collected for data analyses.

Ethics approval

This study was approved by the ethics committee of The First Affiliated Hospital, Chongqing Medical University. The written informed consent, including genetic test and medical record reviews, was obtained from each patient or their next of kin. The data were anonymized for analysis to protect patients' confidentiality.

DNA extraction and mutation analysis

Tumor tissue samples from all PTC patients underwent extraction of genomic DNA using a kit from Amoy Diagnostics Co. Ltd. (Catalog #ADx-TI01, Xiamen, China), according to the manufacturer's protocol. The DNA samples were diluted in dithioerythritol solution and the concentration of the DNA samples was measured using a Nano-100 spectrophotometer (Allsheng Co. Ltd, Hangzhou, China). The final concentration was ~0.4–1.0 ng/μL.

To assess gene mutations in tumor samples of these patients, we first used a human *BRAF*^{V600E} amplification-refractory mutation system-polymerase chain reaction (PCR) kit (Amoy Diagnostics Co. Ltd.). In brief, we amplified the genomic DNA using PCR, conditions of which included 3 phases, that is, 95°C for 5 min, 15 cycles of 95°C for 25 s, 64°C for 20 s, 72°C for 20 s, and then 31 cycles of 93°C for 25 s, 60°C for 35 s, 72°C for 20 s. The 5-carboxyfuorescein (FAM) and 5-hexachloro-fuorescein (HEX) signals were collected at 60°C and the cycle threshold (Ct) values (the number of cycles in each reaction tube when the fluorescent signal reaches the set threshold) of FAM and HEX signals were reached to determine the mutation spot according to the Ct value. The Ct value of the sample >28 was considered as negative, whereas the Ct value of the sample <28 was positive. Furthermore, the *TERT* promoter known as C228T and C250T was also examined by using PCR followed by the direct DNA sequencing. The *TERT* promoter region was amplified with primer pairs according to a previous study.[25] The PCR products were then separated by gel electrophoresis and a targeting DNA band was sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA) and analyzed using the ABI-Prism 3500DX genetic analyzer (Thermo Fisher Scientific).

Statistical analysis

The data for the continuous variables were summarized as mean ± SD, while the categorical data were summarized with frequencies and percentage. We performed the χ^2-test or independent *t*-test to compare the clinicopathological characteristics of PTC patients with tumor DNA mutations and Pearson χ^2-test with the Bonferroni correction or the one-way analysis of variance with least significant difference post hoc test to analyze continuous variables or categorical data in multiple comparisons, respectively. Univariate and multivariate analyses were performed to calculate the odds ratios for ETE and variables. All statistical analyses were assessed using SPSS software version 18.0.0.0 for IBM computers (SPSS Inc., Chicago, IL, USA), and *P*-values <0.05 were considered statistically significant.

Results

Characteristics of PTC patients and *BRAF*^{V600E} and *TERT* promoter mutations

A total of 342 PTC patients were enrolled in this study, which included 99 males and 243 females with the mean age ± SD of 42.4±13.2 years (ranged between 13 and 81 years old). The mean tumor size was 14.8±9.2 mm (ranged between 2.0 and 55.0 mm), resulting in 133 patients (38.8%) being diagnosed with microcarcinoma (tumor maximum diameter ≤10 mm) according to the World Health Organization criteria.[2] All tumor samples from these patients were successfully genotyped for *BRAF*^{V600E} and *TERT* promoter mutations (Figure 1). The data showed that 270 (78.9%) and 12 (3.5%) patients carried the *BRAF*^{V600E} and *TERT* promoter mutations, respectively, while 72 (21.0%) cases had no mutations for either gene. We also found that the *TERT* C228T mutation (10 of 12) was more prevalent than C250T mutation (2 of 12), and that these mutations were mutually exclusive. Patients carrying *TERT* promoter mutations also had the *BRAF*^{V600E} mutation.

Association of *BRAF*^{V600E} or *TERT* promoter mutations with clinicopathological data from PTC patients

We then associated *BRAF*^{V600E} and *TERT* promoter mutations with clinical features from PTC patients and found that the presence of the *BRAF*^{V600E} mutation was associated with older patient age (p=0.018), male patients (p=0.010), and HT (p=0.002; Table 1). However, we did not observe an association with tumor size, ETE, CLNM, LLNM, No. of LNs involved and harvested, and TNM stages (Table 1). The presence of *TERT* promoter mutations were associated with older patient age (p<0.001), larger tumor size (p<0.001), ETE (p<0.001), and advanced disease stages (p<0.001), but not associated with CLNM, LLNM, LNMN >5, or No. of LNs involved and harvested (Table 1).

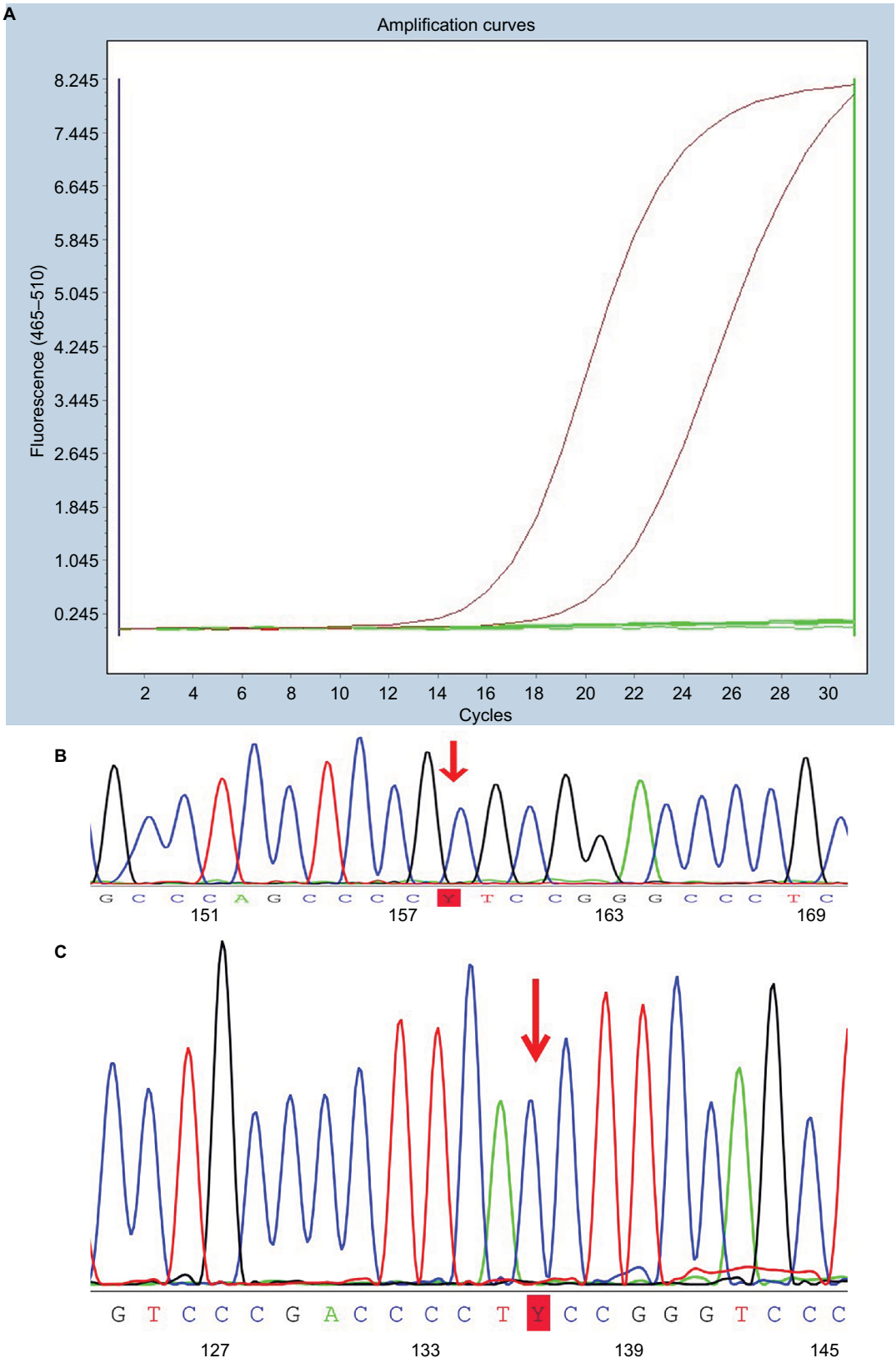

Figure 1 Illustration of polymerase chain reaction data on mutations of *BRAF*^{V600E} and *TERT* promoters.
Notes: (**A**) Amplification plot of PTC with *BRAF*^{V600E} mutation. (**B**) *TERT* promoter C228T mutation identified in a case of PTC. (**C**) *TERT* promoter C250T mutation identified in a case of PTC.
Abbreviation: PTC, papillary thyroid carcinoma.

Table 1 Association of *BRAF*^{V600E} or the *TERT* promoter mutations with clinicopathological characteristics of PTC patients

Variables	*BRAF* mutation, n (%)		*p*-value	*TERT* promoter mutations, n (%)		*p*-value
	Wild-type, n=72	V600E mutation, n=270		Wild-type, n=330	Mutation, n=12	
Age, years (mean ± SD)	39.1±11.9	43.2±13.4	0.018	41.5±12.6	65.1±9.7	<0.001
Sex (F/M, % male)	60/12 (16.6)	183/87 (32.2)	0.010	232/98 (29.6)	11/1 (8.3)	0.109
Tumor size (mean ± SD)	14.6±9.1	15.4±9.7	0.51	14.4±8.8	26.2±12.6	<0.001
Microcarcinoma	25 (34.7)	108 (40.0)	0.51	133 (40.3)	0 (0)	0.005
Multifocality	14 (19.4)	83 (30.7)	0.059	92 (27.8)	5 (41.6)	0.29
HT	22 (30.5)	40 (14.8)	0.002	61 (18.4)	1 (8.3)	0.37
ETE	19 (26.3)	83 (30.7)	0.47	92 (27.8)	10 (83.3)	<0.001
CLNM	50 (69.4)	175 (64.8)	0.46	215 (65.1)	10 (83.3)	0.19
LLNM[a]	40 (59.7)	121 (47.4)	0.074	152 (49.0)	9 (75.0)	0.078
LNMN >5	27 (55.2)	85 (33.3)	0.33	106 (31.8)	6 (50.0)	0.19
No. of LNs harvested	34.9±15.6	36.3±18.5	0.577	35.7±17.7	44.3±24.1	0.104
No. of LNs involved	6.2±8.4	4.9±6.2	0.130	5.1±6.7	6.6±7.4	0.447
TNM stage[b]						
I	67	232		296	3	
II	6	26		28	4	
III	0	7		4	3	
IV	0	5	0.069	2	3	<0.001

Notes: [a]The percentage is only calculated in patients with lateral neck dissection; [b]calculations for stage (I + II) vs (III + IV).
Abbreviations: CLNM, central lymph node metastasis; ETE, extrathyroidal extension; HT, Hashimoto thyroiditis; LLNM, lateral lymph node metastasis; LNMN, numbers of lymph node metastases >5; LN, lymph nodes; PTC, papillary thyroid carcinoma; TNM, tumor/node/metastasis.

Table 2 Association of *BRAF*^{V600E}/*TERT* promoter mutations with clinical characteristics of PTC patients

Characteristics	*BRAF*$^{-}$/*TERT*$^{-}$ (1)	*BRAF*$^{+}$/*TERT*$^{-}$ (2)	*BRAF*$^{+}$/*TERT*$^{+}$(3)	*p*-value	*p*-value	*p*-value
	n=72	n=258	n=12	1 vs 2	1 vs 3	2 vs 3
Age, years (mean ± SD)	39.1±11.9	42.2±12.7	65.0±9.7	0.060[b]	<0.001[b]	<0.001[b]
Sex (F/M, % male)	60/12 (16.6)	172/86 (33.3)	1/11 (8.3)	0.006[c]	0.460[c]	0.070[c]
Tumor size (mean ± SD)	15.4±9.7	14.4±8.6	26.2±12.6	0.26[b]	<0.001[b]	<0.001[b]
Multifocality	14 (19.4)	78 (30.2)	5 (41.6)	0.076[c]	0.088[c]	0.40[c]
HT	22 (30.5)	39 (15.1)	1 (8.3)	0.003[c]	0.11[c]	0.51[c]
ETE	19 (26.3)	73 (28.2)	10 (83.3)	0.75[c]	<0.001[c]	<0.001[c]
CLNM	50 (69.4)	165 (63.9)	10 (83.3)	0.38[c]	0.32[c]	0.16[c]
LLNM[a]	40 (59.7)	112 (46.1)	9 (75.0)	0.068[c]	0.20[c]	0.031[c]
LNMN >5	27 (37.5)	79 (30.6)	6 (50.0)	0.26[c]	0.41[c]	0.15[c]
No. of LNs harvested	34.9±15.6	35.9±18.2	44.3±24.1	0.688[b]	0.096[b]	0.115[b]
No. of LNs involved	6.2±8.4	4.8±6.2	6.6±7.4	0.11[b]	0.854[b]	0.36[b]
TNM stage[d]						
I	27	230	2			
II	6	22	4			
III	0	4	3			
IV	0	2	3	0.19[c]	<0.001[c]	<0.001[c]

Notes: [a]The percentage is only calculated in patients with lateral neck dissection; [b]the one-way analysis of variance test was performed followed by post hoc least significant difference for multiple comparisons; [c]Pearson χ^2-test with Bonferroni correction; the Bonferroni correction compensates for that increase by testing each individual hypothesis at a significance level of a\m, where a is the desired overall alpha level and m is the number of hypotheses. In this table, the trial is testing m=3 hypotheses with a desired a=0.05, then the Bonferroni correction would test each individual hypothesis at a=0.05/3=0.16. [d]calculations for stage (I + II) vs (III + IV).
Abbreviations: CLNM, central lymph node metastasis; ETE, extrathyroidal extension; HT, Hashimoto thyroiditis; LLNM, lateral lymph node metastasis; LNMN, numbers of lymph node metastases >5; LNs, lymph nodes; PTC, papillary thyroid carcinoma; TNM, tumor/node/metastasis.

Association of both *BRAF*^{V600E} and *TERT* promoter mutations with clinical data from PTC patients

Since all *TERT* mutation-positive patients also had the *BRAF* mutation, we divided the 342 PTC patients into 3 groups: *BRAF/TERT*$^{-}$ (no any *BRAF* or *TERT* promoter mutations), *BRAF*$^{+}$/*TERT*$^{-}$ (positive *BRAF* mutation, but negative for *TERT* promoter mutations), and *BRAF*$^{+}$/*TERT*$^{+}$ (positive for both *BRAF* and *TERT* promoter mutations). As shown in Table 2, male patients without HT were more

common in the *BRAF*$^{+}$/*TERT*$^{-}$ group compared with the *BRAF*$^{-}$/*TERT*$^{-}$ group, while the coexistence of *BRAF*^{V600E} and *TERT* promoter mutations were associated with older patient age ($p<0.001$), larger tumor size ($p<0.001$), ETE ($p<0.001$), and advanced disease stage ($p<0.001$), compared with the other 2 groups. However, no statistically significant difference was found in lymph node metastasis among the 3 groups, including CLNM, LLNM, LNMN >5, or No. of LNs involved and harvested. Interestingly, we found that the *BRAF*$^{+}$/*TERT*$^{+}$ group had a significant association with LLNM ($p=0.031$) compared with the *BRAF*$^{+}$/*TERT*$^{-}$ group, although this factor was nonsignificant in the corrected analyses for multiple comparisons.

Association between ETE and clinicopathological characteristics and *BRAF*^{V600E}/*TERT* promoter mutations in PTC

As shown in Table 3, our univariate analyses showed that the presence of ETE was significantly associated with older age, larger tumor size, multifocality, and *BRAF*$^{+}$/*TERT*$^{+}$, while our multivariate analysis revealed that older age (odds ratio [OR], 2.194; 95% CI: 1.117–4.311; $p=0.023$), larger tumor size (OR, 4.100; 95% CI: 2.257–7.450; $p<0.001$), and multiplicity (OR, 2.240; 95% CI: 1.309–3.831; $p=0.003$) were all independent predictors for high prevalence of ETE. Compared with *BRAF*$^{-}$/*TERT*$^{-}$ group, the patients harboring both *BRAF* and *TERT* promoter mutations tended to have a higher ETE risk, although it was not statistically significant ($p=0.069$).

Association of *BRAF*^{V600E}/*TERT* promoter mutations with clinical data from papillary thyroid microcarcinoma (PTMC)

As shown in Table 4, there were 108 of 133 (81.2%) cases of PTMC carrying the *BRAF*^{V600E} mutation, corresponding to 78.9 % of the overall cohort. There was no statistical association with any clinicopathological characteristics except HT. Similarly, in contrast to patients without HT, the prevalence of the *BRAF* mutation in PTMC was significantly lower in patients with HT.

Table 4 Association of the *BRAF*^{V600E} mutation with clinical characteristics of patients with papillary thyroid microcarcinoma

Characteristics	*BRAF* status, number (%)		*p*-value
	Wild-type, n=25	V600E mutation, n=108	
Age, years (mean ± SD)	42.2±10.5	42.0±10.9	0.92
Sex (F/M)	21/4	75/33	0.14
Tumor size (mean ± SD)	7.9±1.3	7.7±1.7	0.69
Multifocality	3 (12.0)	27 (25.0)	0.18
HT	7 (28.0)	10 (9.2)	0.01
ETE	3 (12.0)	14 (12.9)	0.89
CLNM	15 (60.0)	58 (53.7)	0.56
LLNM[a]	8 (38.1)	30 (31.9)	0.67
LNMN >5	4 (16.0)	17 (15.7)	0.97
No. of LNs harvested	30.5±17.9	30.2±15.8	0.58
No. of LNs involved	2.7±3.1	3.3±5.4	0.94
TNM stage[b]			
I	24	97	
II	1	8	
III	0	2	
IV	0	1	0.39

Notes: [a]The percentage is only calculated in patients with lateral neck dissection; [b]calculations for stage (I + II) vs (III + IV).
Abbreviations: CLNM, central lymph node metastasis; ETE, extrathyroidal extension; HT, Hashimoto thyroiditis; LLNM, lateral lymph node metastasis; LNMN, numbers of lymph node metastases >5; LN, lymph nodes; TNM, tumor/node/metastasis.

Discussion

In the current study, we confirmed that the *BRAF*^{V600E} mutation was associated with older age male PTC patients. Furthermore, the coexistence of *BRAF* and *TERT* promoter mutations were significantly associated with high-risk clinicopathological characteristics from PTC patients. In

Table 3 Univariate and multivariate analysis of the clinical characteristics and gene mutations that could be associated with ETE

Characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI) for ETE	*p*-value	OR (95% CI) for ETE	*p*-value
Gender (female vs male)	0.840 (0.500–1.412)	0.510	NA	NA
Age (≥55 vs <55 years)	3.008 (1.678–5.391)	<0.001	2.194 (1.117–4.311)	0.023
Tumor size (>1 vs ≤1 cm)	4.762 (2.667–8.474)	<0.001	4.100 (2.257–7.450)	<0.001
Multifocality (yes vs no)	2.510 (1.528–4.123)	<0.001	2.240 (1.309–3.831)	0.003
HT (yes vs no)	1.150 (0.636–2.077)	0.643	NA	NA
BRAF^{+}/*TERT*$^{-}$[a]	1.101 (0.610–1.986)	0.750	NA	NA
BRAF^{+}/*TERT*$^{+}$[a]	13.947 (2.799–69.504)	<0.001	4.831 (0.886–26.332)	0.069

Note: [a]Comparisons were performed between the *BRAF*$^{-}$/*TERT*$^{-}$ group and each of other two groups (*BRAF*$^{+}$/*TERT*$^{-}$, *BRAF*$^{+}$/*TERT*$^{+}$).
Abbreviations: ETE, extrathyroidal extension; HT, Hashimoto thyroiditis; OR, odds ratio; NA, not available.

particular, the prevalence of the *BRAF*^{V600E} mutation occurred in 78.9% of the 345 patients, which was relatively higher than that of patients from Western countries, but similar to that of Asian patients, including Japanese, Korean, and Chinese.[8,22,26,27] Our data further showed that the *BRAF*^{V600E} mutation was not associated with other aggressive clinicopathological features. To the best of our knowledge, several previous studies suggested that *BRAF* mutations were associated with certain clinicopathological features, such as ETE,[28,29] older age,[25] and tumor size,[26] whereas other studies have shown that presence of the *BRAF* mutation was not associated with any clinicopathological features.[17,18,30] These inconsistent data indicate that additional research on the *BRAF* mutation in combination with other gene mutations could be needed to predict aggressive PTC phenotypes. For example, the finding of intra-tumor heterogeneity of the *BRAF* mutation and percentage of mutant *BRAF* alleles in PTC might partially explain this inconsistency.[31] In our current study, we found that the presence of the *BRAF* mutation was highly associated with a low rate of the concurrent HT, which is consistent with previous studies.[32,33] The concurrent HT could antagonize PTC progression.[34,35] Kim et al also showed HT as an independent predictor for less aggressive *BRAF*-negative and *BRAF*-positive PTCs,[36] although the underlying mechanism remains to be elucidated.

Furthermore, our current study showed that the overall prevalence of *TERT* promoter mutations was 3.5% in these PTC patients, which was lower than the average range between 4.2% and 25%.[37] Two previous Chinese and Korean studies showed a remarkably lower mutation rate of the *TERT* promoter than that of studies from Western countries.[25,26] This difference in prevalence might be due to a larger size of the PTC lesion, and the relatively smaller tumor size might result in the low frequency of the *TERT* promoter mutations in our current study, although exceptions do occur. For instance, Lee et al reported a noticeably higher rate (14.5%) of *TERT* promoter mutation in 207 Korean PTC patients.[20] However, the patients from that study and our current one that used the same methodology to detect the *TERT* promoter mutations did have similar age and tumor size, which did not support the association of the *TERT* promoter mutation prevalence with tumor size. One possible reason is because *TERT* promoter mutation rate is different in different subtypes of PTC. Specifically, the prevalence of *TERT* promoter mutations was highest in tall-cell PTC compared with that of conventional PTC and follicular variant PTC.[23] In our current study, we did not have data on the PTC variants, which could be one of the limitations to our current work. Our current data did show that PTC patients with *TERT* promoter mutations were significantly associated with aggressive clinical features and advanced TNM stage, which is consistent with previous studies.[15,16,18] In addition, we did not detect any *BRAF*$^{-}$/*TERT*$^{+}$ PTCs. Such a combination of gene mutations is rare in PTC patients.[25,27,38] Previous studies, including a number of meta-analyses, demonstrated that concurrent *BRAF* and *TERT* promoter mutations enhanced PTC aggressiveness.[16,20–22,25,26,38,39] Our current work also showed a similar result, which confirmed a strong incremental effect of the combined *BRAF* and *TERT* promoter mutations on promotion of PTC progression. Indeed, activation of the *TERT* promoter can generate the binding motifs for the E-twenty six (ETS) transcription factors, which could, in turn, activate the MAPK signaling pathway.[40] The MAPK pathway activated by *BRAF*^{V600E} was able to upregulate the ETS and led to *TERT* overexpression.[41] These studies could help us to explain why mutations of both genes had synergistic or additive effects on PTCs, although further study is needed to elucidate this.

In the current study, we performed more extensive lymph node dissection in most patients compared with previous studies to further assess whether the association of these 2 gene mutations with PTC lymph node metastasis.[16,22,25,27] This is because occult lymph node metastasis to the central or lateral neck compartment occurs in 42.9%–55% of cN0 PTC patients. Thus, the extent of lymph node resection is critical to discover such a metastasis.[42,43] Xing et al observed that *BRAF*^{V600E} and the *TERT* promoter mutations were both significantly associated with PTC lymph node metastasis with therapeutic CLND and LLND.[16] In contrast, other studies have failed to reproduce such data, although these data were used for prophylactic CLND and therapeutic LLND.[22,30] The most important value of our current study is that 92.0% (231 of 251) of cN0 patients received prophylactic CLND + LLND, and as many subclinical metastatic lymph nodes as possible were dissected to accurately assess the relationship between lymph node metastasis and genetic mutations. Although there was a higher incidence of lymph node metastasis in *BRAF*$^{+}$/*TERT*$^{+}$ group, there was no association between gene mutations and lymph node metastasis. Thus, the coexistence of *BRAF* and *TERT* promoter mutations cannot be used as a useful predictor of PTC lymph node metastasis. Nevertheless, a larger sample size is needed to verify our current finding due to the low prevalence of *TERT* promoter mutations.

The definition of ETE refers to tumor extension beyond the thyroid capsule into the adjacent tissues, which is an important negative prognostic factor and closely associated

with tumor recurrence and patients' mortality. Previous studies reported that *BRAF*$^{+}$/*TERT*$^{+}$ PTC patients had a higher prevalence of ETE, but none of these studies conducted multivariate analysis.[16,21,25] Interestingly, our current study showed that *BRAF*$^{+}$/*TERT*$^{+}$ was not an independent influencing factor for ETE, and the possible reason may be because association of *BRAF*$^{+}$/*TERT*$^{+}$ with older age and larger tumor size weaken the influence of gene mutation on ETE in multivariate analysis. In addition, we analyzed the association of the *BRAF* and *TERT* promoter mutations with PTMCs, which was prevalent according to a previous study.[44] We found that the presence of the *BRAF*^{V600E} mutation was not associated with any high-risk PTMC clinicopathological features. However, we did not detect any *TERT* promoter mutations in PTMC, which was different from the previous study in a European cohort (4.7%).[45] The reason for this discrepancy is unknown, but may be due to differences in ethnicity, region, and methodologies used to detect the *TERT* promoter mutations. The previous studies and our current one did show that mutations of these genes were not associated with any aggressive PTMC behaviors.

Our current study does have some limitations. For example, we do not have follow-up data on these patients, since all patients were recently admitted to our hospital. Moreover, our current study was unable to provide data on association of more extensive surgical approaches (e.g., prophylactic lymph node dissection) with the traditional surgical approaches (e.g., therapeutic lymph node dissection), or prognosis of patients stratified by these 2 gene mutations. In addition, due to the smaller PTMC sample size, such a conclusion may lack the credibility. There is a selection bias due to not all cN0 patients undergoing prophylactic LLND, which might neglect occult lymph node metastasis.

Conclusion

In summary, we demonstrated the coexistence of *BRAF*^{V600E} and *TERT* promoter mutations were associated with PTC clinicopathological aggressiveness, but did not associate with lymph node metastasis, and were not useful to identify the aggressive PTMCs. These results provide evidence to settle the dispute over the roles of *BRAF*^{V600E} and *TERT* promoter mutations in lymph node metastasis of PTC and tailor the individual treatment of PTC patients.

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Disclosure

The authors report no conflicts of interest in this work.

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