RESEARCH

Clinical significance of the *BRAF*^{V600E} mutation in PTC and its effect on radioiodine therapy

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Abstract

The goal of this study was to explore the relationship of the *BRAF*^{V600E} mutation with clinicopathologic factors and evaluate the effect of radioactive iodine (RAI) therapy in a large group of intermediate- and high-risk papillary thyroid cancer (PTC) patients with the *BRAF*^{V600E} mutation and without distant metastases. We collected data for PTC patients who underwent total or near-total thyroidectomy and RAI treatment in our hospital from January 2014–December 2017. There were 1220 PTC patients who met the criteria, and the *BRAF*^{V600E} mutation was observed in 979 of them (80.2%). Multivariate analysis identified that the *BRAF*^{V600E} mutation remained independently associated with age at diagnosis, and bilaterality (OR = 1.023, 95% CI = 1.012–1.039, $P < 0.001$; OR = 1.685, 95% CI = 1.213–2.341, $P = 0.002$, respectively). In addition, the patients with bilateral PTCs had a higher prevalence of extrathyroid invasion, capsular invasion and fusion of metastatic lymph nodes than the unilateral PTC patients. The response to RAI therapy was evaluated in both the entire series and the patients with a high recurrence risk; no significant difference was discerned between the *BRAF*^{V600E} mutation and the wild-type groups ($P = 0.237$ and $P = 0.498$, respectively). To summarize, our results confirmed that PTC patients with the *BRAF*^{V600E} mutation exhibit more aggressive characteristics. In addition, the patients with bilateral PTC have a higher incidence of extrathyroid invasion. Moreover, *BRAF*^{V600E} mutation PTC patients did not show a poorer clinical response after postsurgical RAI therapy, suggesting that RAI therapy may improve the general clinical outcome of these patients.

Key Words

- papillary thyroid carcinoma
- *BRAF*^{V600E} mutation
- bilaterality
- RAI therapy
- clinical response

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Introduction

Papillary thyroid cancer (PTC) is a common endocrine malignancy, accounting for approximately 90% of all thyroid cancers. There are several histological variants of PTC, with conventional PTC constituting the majority of cases (1, 2). PTC incidence has increased rapidly in recent years, largely due to improvements in healthcare and diagnostic technology such as early detection with ultrasonography and fine-needle aspiration biopsy (3, 4, 5). PTC generally has a high cure rate following initial treatment, behaving in a relatively indolent manner and responding well to therapy. However, a small number of patients will experience recurrence during the follow-up period, with reported recurrence rates varying from 1 to 40%, especially in patients with intermediate to high recurrence risk (6, 7, 8, 9). Therefore, identifying the specific features of PTC that correlate with tumor behavior and prognosis has become an important consideration in clinical management. Several previous studies have

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reported several clinicopathological characteristics associated with increased aggressiveness and poor prognosis, including gender, age at diagnosis, tumor size, multifocality, extrathyroidal extension and lymph node metastasis.

In recent years, an increasing number of molecular genetic characteristics associated with invasiveness and clinical management have been uncovered, including the *BRAF*^{V600E} mutation, *TERT* promoter mutations, *RAS* mutations and RET/PTC and PAX8/PPARγ rearrangements (10, 11). The *BRAF*^{V600E} mutation has drawn particular attention worldwide, given that it is the most frequent and specific genetic alteration in PTC and is involved in the tumorigenesis of PTC through the constitutive activation of the mitogen-activated protein kinase (MAPK) signaling transduction pathway (12). In addition, some studies have demonstrated a strong association between the *BRAF* mutation and both PTC recurrence and PTC-related mortality (9, 13). Nevertheless, several other studies have found that *BRAF* status was not associated with negative prognostic features and poorer outcome. For instance, Gandolfi *et al*. (14) found that the occurrence and percentage of the *BRAF*^{V600E}-mutated allele was not preferentially associated with poor prognostic factors, based on the observation of 132 cases of well-differentiated PTC with follow-up periods ranging from 13–372 months. Thus, they suggest reevaluating the role of the *BRAF*^{V600E} mutation as a negative prognostic marker in PTC. Therefore, there still exists controversy as to whether the *BRAF*^{V600E} mutation is a negative prognostic indicator. Furthermore, some studies (15) indicated that the *BRAF*^{V600E} mutation significantly reduces sodium-iodide symporter (NIS) expression and radioiodine uptake ability and influences RAI therapy to the point of causing RAI-refractory PTC. Jiao *et al*. (16) designed their research concerning RAI therapy and PTC with the *BRAF*^{V600E} mutation and without distant metastases by following 228 PTC patients for 1.03–4.80 years. This study demonstrated that RAI therapy may improve the general clinical outcome in *BRAF*^{V600E} mutation PTC patients without distant metastases. It is worth noting, however, that only 228 cases with few clinicopathological characteristics were enrolled in their study. Therefore, we expanded our study to more than 1000 patients with PTC and added more clinicopathological characteristics. In addition, in the subgroup analysis, we compared the clinicopathologic and prognostic significance of bilateral PTC with that of unilateral PTC.

Materials and methods

Study design and samples

In total, 2012 consecutive patients with differentiated thyroid carcinoma were selected in this retrospective study. All patients had undergone total or near-total thyroidectomy, RAI therapy and suppressive therapy with thyroid-stimulating hormone (TSH) at the Zhujiang Hospital of Southern Medical University (Guangzhou, China) from January 2014 to December 2017. Among the 2012 patients, 792 were excluded for at least one of the following reasons: (1) pathology types other than PTC; (2) lack of detailed clinicopathological characteristics or *BRAF*^{V600E} analysis result; (3) low recurrence risk according to the 2015 guidelines of the American Thyroid Association (ATA); (4) suspicion of distant metastases due to elevated serum thyroglobulin (Tg) level or radiological findings including RAI whole-body scan (RI-WBS), ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT), chest CT or histopathological biopsy and (5) loss to follow-up. Following these exclusions, 1220 PTC patients remained and were enrolled in this study.

All patients undergoing RAI therapy or diagnostic testing were prepared using levothyroxine (LT4) withdrawal in combination with a strict low-iodine diet for a minimum of 3–4 weeks, with the goal of reaching an appropriate TSH level above 30 mIU/L. A total of 832 patients received RAI therapy only once, while another 363 patients received two separate RAI treatments, and 25 patients received three treatments. Patients receiving multiple RAI treatments either had a suspicious lesion that was still visible on RI-WBS or an abnormal rise of thyroglobulin with a negative finding on PET/CT during the follow-up period after the initial RAI therapy. The selection of the RAI therapy dose was based on the 2015 guidelines of the ATA. RAI activities of 35–100 mCi were administered to PTC patients with intermediate recurrence risk but without either aggressive histology (e.g., tall cell, insular cell or columnar cell carcinoma) or vascular invasion for remnant ablation. The other patients with an intermediate-high recurrence risk with more aggressive features received 100–200 mCi of RAI therapy (adjuvant or for persistent disease). Thyroxine therapy was resumed on the fourth day and a post-therapy whole-body scan was performed 2–5 days after therapeutic RAI administration.

Follow-up began at the time of the initial RAI therapy and ranged from 0.67–4.67 years (the mean follow-up period was 2.67 years). During follow-up, the collected

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data included suppressed and stimulated Tg, TgAb, RI-WBS, as well as cervical ultrasonography, chest CT, bone scintigraphic imaging and PET/CT. Based on the data, patients were classified using the AJCC/TNM 8th Edition (17) and the ATA risk classification (18). Response to initial therapy was assessed by the ATA and classified as follows: (1) excellent response; (2) indeterminate response; (3) biochemically incomplete response or (4) structurally incomplete response (18).

The demographic and clinicopathologic characteristics data were collected from our database. All the clinicopathological characteristics and response to RAI therapy were compared between the *BRAF*^{V600E} mutation and wild-type PTC patients. In addition, we designed a subgroup analysis to compare the clinicopathologic and prognostic significance of bilateral PTC with that of unilateral PTC. The clinical outcomes in patients with a high recurrence risk were analyzed separately. This study was approved by the Institutional Review Board of the Zhujiang Hospital of Southern Medical University Ethics Committee and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Consent was obtained from each patient or subject after a full explanation of the purpose and nature of all procedures used.

Image analysis and serological assays

Patients meeting all of the following criteria were diagnosed as having PTC without distant metastases: (1) RI-WBS demonstrated no focal or diffuse RAI uptake in the lungs or bones; (2) negative findings of other imaging examinations, including plain radiography, chest CT, bone scintigraphy and PET/CT and (3) negative Tg and TgAb levels. All images were separately evaluated by two experienced nuclear medicine physicians or two radiologists who were unaware of the clinical findings or any other diagnostic imaging data. During follow-up, serological examinations were regularly performed, generally involving the determination of Tg, TgAb and TSH levels.

Mutational testing

The immunohistochemistry (IHC) analysis was designed to test for the *BRAF*^{V600E} mutation. IHC testing for the *BRAF*^{V600E} mutation was performed on all postoperative thyroidectomy specimens using mouse anti-*BRAF*^{V600E} (clone VE1, 1:4 dilution, Ventana Medical Systems, Tucson, AZ, USA) and the OptiView DAB IHC Detection Kit (Roche) on a Ventana BenchMark XT autoimmunostainer (Ventana Medical Systems). Whole tissue sections (4 µm) were transferred to poly-L-lysine-coated adhesive slides and dried at room temperature for 10 min. Antigen retrieval was performed using the heat-induced epitope retrieval method followed by 20 min at 65°C in an incubator. After incubation in CC1 solution (Roche) for 72 min, primary antibody was added and incubated for another 72 min. A haptenated secondary antibody was added and incubated for a further 12 min. Alpha-hapten-horseradish peroxidase was then added to the sections. After incubation for 10 min, diaminobenzidine was added and incubated for 10 minutes. Negative controls were performed by omitting the primary antibody. Positive controls were melanoma tissue. Slides were evaluated by an experienced pathologist who was blind to the *BRAF* mutational status. Diffuse homogeneous cytoplasmic staining in all tumor cells was considered as positive. Non-specific staining of colloids and equivocally weak or focal cytoplasmic staining was considered as negative.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 for Windows (IBM Corp.). The results were expressed as either a percentage or mean ± S.D. The clinicopathologic features among groups that were categorical variables were compared using Fisher's exact test and Pearson's chi-squared test. A Student's *t* test was used for normally distributed continuous variables, and a Mann–Whitney *U* test was used for non-normally distributed continuous variables. A $P<0.05$ was considered statistically significant.

Results

Clinicopathological characteristics

Demographic data are listed in Table 1. The mean age at diagnosis was 38.6 ± 12.4 years (range 5–76 years); 68.0% (830) of patients were female and 32.0% (390) were male. The histologic cancer types included 1168 (95.7%) classic PTCs, 36 (3.0%) follicular variant PTCs and 16 (1.3%) other aggressive PTC variants (including oxyphilic, diffuse sclerosing and solid). As detailed in Table 1, the mean tumor size was 1.70 ± 1.10 cm (range 0.08–10 cm), with almost half the tumors ranging from 1–2 cm in size, close to 30% ≤1 cm and the remainder >2 cm. Among all of the PTC patients, 1102/1220 (90.3%) presented lymph node metastases. In addition, according to the AJCC/TNM 8th Edition, the distribution among the four tumor stages

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Table 1 Patient characteristics.

Characteristic	No. (%)
Age at diagnosis (years)	
Mean (S.D.), range	38.6 (12.4), 6–76
Sex	
Male/female	390 (32)/830 (68)
Histologic subtype	
Classic PTC	1168 (95.7)
Follicular variant PTC	36 (3)
Other aggressive variants PTC[a]	16 (1.3)
Tumor size[b] (cm)	
Mean ± S.D.	1.70 ± 1.10
Median (range)	1.50 (0.08-10)
Tumor size, *n* (%)	
≤1 cm	387 (31.7)
1–2 cm	554 (45.4)
2–4 cm	240 (19.7)
4 cm	39 (3.2)
Lymph node metastases[c]	1102 (90.3)
BRAF^{V600E} mutation	979 (80.2)
TNM stage, *n* (%)	
I	1080 (88.5)
II	89 (7.3)
III	40 (3.3)
IV	11 (0.9)
Recurrence risk, *n* (%)	
Intermediate	844 (69.2)
High	376 (30.8)
Response to therapy, *n* (%)	
Excellent response	713 (58.4)
Indeterminate response	54 (4.4)
Biochemical incomplete response	290 (23.8)
Structural incomplete response	163 (13.4)

[a]Other aggressive variants include oxyphilic, diffuse sclerosing, solid variant. [b]Tumor size is recorded as the greatest tumor dimension. [c]Lymph node metastasis at the completion of initial surgical treatment.
PTC, papillary thyroid carcinoma; S.D., standard deviation.

was I: 88.5%; II: 7.3%; III: 3.3% and IV: 0.9%. Based on the 2015 ATA risk classification, 39.8% of the patients were high risk and 69.2% were intermediate risk. The various responses to the initial therapy were excellent (713/1220, 58.4%), indeterminate (54/1220, 4.4%), biochemical incomplete (290/1220, 23.8%) and structural incomplete (163/1220, 13.4%).

Clinical and pathologic differences in PTCs with the *BRAF*^{V600E} mutation

Of the 1220 patients who were included in this retrospective study, 979 (80.2%) had the *BRAF*^{V600E} mutation and 241 (19.8%) had the *BRAF*^{V600E} wild type. As shown in Table 2, the *BRAF*^{V600E} mutation PTC patients were older at the time of diagnosis (39.5 ± 12.2 years vs 34.9 ± 12.6 years; $P<0.001$), had a higher prevalence of bilateral PTC (36.3 vs 26.1%; $P=0.003$), had a greater presence of nodular goiter (42.1 vs 34.4%; $P=0.030$) and were diagnosed at a more advanced tumor stage (III/IV vs I/II; $P<0.001$) than the *BRAF*^{V600E} wild-type group. The *BRAF*^{V600E} mutation was most often present in the classic variant PTCs (97.2 vs 89.6%), and less frequently present in the aggressive variant PTCs. In addition, our findings also showed that the presence of Hashimoto's thyroiditis ($P=0.037$) and vascular invasion ($P<0.001$) were negatively correlated to the presence of the *BRAF*^{V600E} mutation, whereas correlations with other variables such as gender, extrathyroid invasion, lymph node metastasis, multifocality and so on were not statistically significant (Table 2). As shown in Table 3, multivariate analysis results further indicated that the *BRAF*^{V600E} mutation remained independently associated with age at diagnosis, and area of primary lesion (OR = 1.023, 95% CI = 1.012–1.039, $P<0.001$; OR = 1.685, 95% CI = 1.213–2.341, $P=0.002$, respectively). In addition, histologic variants exhibited a strong negative relationship with the *BRAF*^{V600E} mutation (OR = 0.411, 95% CI = 0.265–0.638, $P<0.001$).

Clinical and pathologic differences in bilateral PTC

In order to further investigate the clinical and pathologic characteristics of bilateral PTCs, we compared the differences between bilateral PTC and unilateral PTC. As shown in Table 4, among the patients in our study, 418/1220 (34.3%) had bilateral PTC. Females were more likely to present with bilateral PTC than with unilateral PTC (73.0 vs 65.5%; $P=0.008$). Bilateral PTC had a higher prevalence than unilateral PTC of extrathyroid invasion (62.7 vs 53.2%; $P=0.002$), capsular invasion (72.7 vs 67.2%; $P=0.048$) and fusion of metastatic lymph nodes (12.8 vs 7.9%; $P=0.009$). No other clinicopathological features associated with bilaterality were found.

Association between response to RAI therapy and *BRAF*^{V600E} mutation status in PTC patients

We also compared the response to RAI therapy between PTC patients with the *BRAF*^{V600E} mutation and those with the *BRAF*^{V600E} wild type. As shown in Table 5, univariate analysis demonstrated that the response to RAI therapy had no significant association with *BRAF*^{V600E} status in the overall cohort ($P=0.237$). Moreover, since the patients with high recurrence risk accounted for 30.8% (376/1220) of all patients, we designed a subgroup analysis for the high recurrence risk group. Our results, however, revealed that there was no significant difference in treatment

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Table 2 Comparison of various clinicopathologic features between the *BRAF*^{V600E} mutant and wild-type groups.

Variable	*BRAF*^{V600E} mutant group	*BRAF*^{V600E} wild-type group	*P* value
Number (%) of patients	979 (80.2)	241 (19.8)	
Sex, *n* (%)			0.533
Male	317 (32.4)	73 (30.3)	
Female	662 (67.6)	168 (69.7)	
Age at diagnosis (years), mean ± S.D.	39.5 ± 12.2	34.9 ± 12.6	<0.001*
Family history			0.935
Yes	50 (5.1)	125 (5.0)	
No	929 (94.9)	229 (95.0)	
Histologic variants, *n* (%)			<0.001*
Classic PTC	952 (97.2)	216 (89.6)	
Follicular variant PTC	18 (1.8)	18 (7.5)	
Other aggressive variants PTC[a]	9 (0.9)	7 (2.9)	
Tumor size, cm			0.088
Mean ± S.D.	1.66 ± 1.12	1.79 ± 1.12	
Range	0.10-10.00	0.08-8.00	
Tumor size[b], *n* (%)			0.137
≤1 cm	324 (33.1)	63 (26.1)	
1–2 cm	441 (45.0)	113 (46.9)	
2–4 cm	183 (18.7)	57 (23.7)	
4 cm	31 (3.2)	8 (3.3)	
Multifocality, *n* (%)			0.161
Yes	484 (49.4)	107 (44.4)	
No	495 (50.6)	134 (55.6)	
Area of primary lesion, *n* (%)			0.003*
Bilaterality	355 (36.3)	63 (26.1)	
Unilateral	624 (63.7)	178 (73.9)	
Capsular invasion, *n* (%)			0.700
Yes	674 (68.8)	169 (70.1)	
No	305 (31.2)	72 (29.9)	
Extrathyroidal invasion, *n* (%)			0.675
Yes	550 (56.2)	139 (57.7)	
No	429 (43.8)	102 (42.3)	
Vascular invasion, *n* (%)			<0.001*
Yes	77 (7.9)	51 (21.2)	
No	901 (92.1)	190 (78.8)	
Neurological invasion, *n* (%)			0.947
Yes	62 (6.3)	15 (6.2)	
No	916 (93.7)	226 (93.8)	
Gross extrathyroidal extension, *n* (%)			0.124
Yes	280 (28.6)	57 (23.7)	
No	699 (71.4)	184 (76.3)	
With Hashimoto thyroiditis, *n* (%)			0.037*
Yes	229 (23.4)	72 (29.9)	
No	749 (76.6)	169 (70.1)	
With nodular goiter, *n* (%)			0.030*
Yes	412 (42.1)	83 (34.4)	
No	566 (57.9)	158 (65.6)	
Lymph node metastases (LNs)[c], *n* (%)			0.432
Yes	879 (89.8)	223 (92.5)	
No	54 (5.5)	10 (4.1)	
No neck dissection	46 (4.7)	8 (3.3)	
Extracapsular extension of metastatic lymph nodes, *n* (%)			0.406
Yes	356 (41.4)	98 (44.5)	
No	503 (58.6)	122 (55.5)	
Fusion of metastatic lymph nodes, *n* (%)			0.199
Yes	77 (9.0)	26 (11.8)	
No	782 (91.0)	194 (88.2)	

(Continued)

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Table 2 Continued.

Variable	*BRAF*^{V600E} mutant group	*BRAF*^{V600E} wild-type group	*P* value
ATA risk stratification, *n* (%)			0.197
Intermediate	669 (68.3)	175 (72.6)	
High	310 (31.7)	66 (27.4)	
The 8th AJCC TNM stage, *n* (%)			0.029*
I/II	932 (95.2)	237 (98.3)	
III/IV	47 (4.8)	4 (1.7)	
Cumulative iodine dose, (mCi)			0.089
Mean ± s.d.	205.3 ± 96.1	218.0 ± 105.8	
Range	35–630	35–628	

[a]Other aggressive variants include oxyphilic, diffuse sclerosing, solid variant. [b]Tumor size is recorded as the greatest tumor dimension. [c]Lymph node metastasis at the completion of initial surgical treatment. *Represents the *P* value <0.05.
PTC, papillary thyroid carcinoma; s.d., standard deviation.

response after RAI therapy between those with and without the *BRAF*^{V600E} mutation (*P*=0.498; Table 6).

Discussion

PTC is generally an indolent disease that usually has a favorable prognosis. Nonetheless, a small number of PTC patients will experience local recurrence, distant metastasis or mortality. According to several previous studies, PTC recurrence develops in up to 20% of patients at the 10-year follow-up and is associated with increased morbidity and possible mortality, although this remains quite controversial (13, 19, 20, 21). Thus, developing methods to predict PTC recurrence has become a research focus in recent years. Numerous reports have demonstrated that some postoperative clinicopathological features, including male gender, extrathyroidal extension, lymph node metastasis, relatively large tumor size and vascular invasion exhibit a strong association with PTC recurrence or mortality (7, 20, 22). Moreover, regarding the risk of recurrence, on the basis of intraoperative findings and the relevant postoperative assessment results, including pathological features and imaging findings, the new 2015 ATA guidelines further divide PTC patients into three types: low, intermediate or high recurrence risk (18).

Table 3 Multivariate logistic regression analysis of *BRAF*^{V600E} mutation in patients with papillary thyroid carcinoma.

Characteristics	OR (95% CI)	*P* value
Age at diagnosis	1.025 (1.012–1.039)	<0.001*
Histologic variants	0.411 (0.265–0.638)	<0.001*
Vascular invasion	0.790 (0.569–1.096)	0.158
Area of primary lesion	1.685 (1.213–2.341)	0.002*
The 8th AJCC TNM stage	1.748 (0.586–5.499)	0.39

*Represents the *P* value <0.05.
OR, odds ratio; CI, confidence interval.

This categorization could help to improve the choice of treatment plan and the clinical management of PTC, especially in patients with intermediate or high recurrence risk.

Recently, gene research at the molecular level has drawn the attention of more and more researchers. Among other findings, their investigations have indicated that the *BRAF*^{V600E} mutation, which, with an incidence ranging from 48.5–75.3% is the most common genetic alteration in PTC, is strongly related to poor prognosis and aggressive clinicopathological characteristics (11, 23, 24, 25). In the current study, our results revealed that the *BRAF*^{V600E} mutation is prevalent in PTC (present in approximately 80.5% of the patients we investigated), especially in classic PTC. This incidence rate is higher than that found in previous studies, something that may be explained by the fact that most of the participants were from the eastern coastal area of China, where residents have relatively high iodine intake from a diet rich in seafood. High dietary iodine intake is strongly associated with the *BRAF*^{V600E} mutation (26). The current study found that the *BRAF*^{V600E} mutation was correlated with older age, histologic variants, bilaterality, less vascular invasion, less Hashimoto's thyroiditis, nodular goiter and relatively advanced TNM stage. Multivariate logistic regression analysis also demonstrated that the association of the *BRAF* mutation with older age, bilaterality, and histologic variants remained independently significant. We did not, however, observe significant differences between the *BRAF*^{V600E} mutation and known unfavorable prognostic indicators, such as extrathyroidal extension, lymph node metastasis, and relatively large tumor size, which had been revealed in most previous studies (23, 24, 27). These inconsistent results may be partly due to the heterogeneity of the *BRAF*^{V600E} mutation within a tumor and the variability of the detection methods. In addition, several recent studies failed to corroborate the above observations,

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Table 4 Comparison of various clinicopathologic features between patients with bilateral and unilateral papillary thyroid carcinoma.

Variable	Bilateral PTCs group	Unilateral PTCs group	*P* value
Number (%) of patients	418 (34.3)	802 (65.7)	
Sex, *n* (%)			0.008*
Male	113 (27.0)	277 (34.5)	
Female	305 (73.0)	525 (65.5)	
Age at diagnosis (years), mean ± S.D.	39.3 ± 12.0	38.3 ± 12.5	0.186
Family history			0.947
Yes	21 (5.0)	41 (5.1)	
No	397 (95.0)	761 (94.9)	
Histologic variants, *n* (%)			0.209
Classic PTC	401 (95.9)	767 (95.6)	
Follicular variant PTC	9 (2.2)	27 (3.4)	
Other aggressive variants PTC[a]	8 (1.9)	8 (1.0)	
Tumor size[b], cm			0.289
Mean ± S.D.	1.73 ± 1.24	1.66 ± 1.06	
Range	0.20-9.00	0.08-10.00	
Tumor size, *n* (%)			0.298
≤1 cm	128 (30.6)	259 (32.3)	
1–2 cm	199 (47.6)	355 (44.3)	
2–4 cm	74 (17.7)	166 (20.7)	
4 cm	17 (4.1)	22 (2.7)	
Capsular invasion, *n* (%)			0.048*
Yes	304 (72.7)	539 (67.2)	
No	114 (27.3)	263 (32.8)	
Extrathyroidal invasion, *n* (%)			0.002*
Yes	262 (62.7)	427 (53.2)	
No	156 (37.3)	375 (46.8)	
Vascular invasion, *n* (%)			0.229
Yes	50 (12.0)	78 (9.7)	
No	368 (88.0)	723 (90.3)	
Neurological invasion, *n* (%)			0.882
Yes	27 (6.5)	50 (6.2)	
No	391 (93.5)	751 (93.8)	
Gross extrathyroidal extension, *n* (%)			0.120
Yes	127 (30.4)	210 (26.2)	
No	291 (69.6)	592 (73.8)	
With Hashimoto thyroiditis, *n* (%)			0.783
Yes	101 (24.2)	200 (24.9)	
No	316 (75.8)	602 (75.1)	
With nodular goiter, *n* (%)			<0.001*
Yes	135 (32.4)	360 (44.9)	
No	282 (67.6)	442 (55.1)	
Lymph node metastases (LNs)[c], *n* (%)			0.695
Yes	374 (89.5)	728 (90.8)	
No	25 (6.0)	39 (4.9)	
No neck dissection	19 (4.5)	35 (4.4)	
Extracapsular extension of metastatic lymph nodes, *n* (%)			0.132
Yes	166 (45.2)	288 (40.4)	
No	201 (54.8)	424 (59.6)	
Fusion of metastatic lymph nodes, *n* (%)			0.009*
Yes	47 (12.8)	56 (7.9)	
No	320 (87.2)	656 (92.1)	
ATA risk stratification, *n* (%)			0.112
Intermediate	277 (66.3)	567 (70.7)	
High	141 (33.7)	235 (29.3)	
The 8th AJCC TNM stage, *n* (%)			0.874
I/II	400 (95.7)	769 (95.9)	
III/IV	18 (4.3)	33 (4.1)	

[a]Other aggressive variants include oxyphilic, diffuse sclerosing, solid variant. [b]Tumor size is recorded as the greatest tumor dimension. [c]Lymph node metastasis at the completion of initial surgical treatment. *Represents the *P* value <0.05.
PTC, papillary thyroid carcinoma; S.D., standard deviation.

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Table 5 Association between response to RAI therapy and *BRAF*^{V600E} status in all PTC patients.

Response to therapy	*BRAF*^{V600E} mutant group	*BRAF*^{V600E} wild-type group	*P* value
Excellent response	572 (58.4%)	141 (58.5%)	0.237
Indeterminate response	134 (13.7%)	29 (12.0%)	
Biochemical incomplete response	48 (4.9%)	6 (2.5%)	
Structural incomplete response	225 (23.0%)	65 (27.0%)	

and the significance of the *BRAF*^{V600E} mutation in PTC remains debatable (14, 28). Thus, it may be more helpful to identify some additional markers associated with the *BRAF*^{V600E} mutation in order to predict the outcome of patients with PTC. Some recent publications have reported that coexisting *TERT* and *BRAF* mutations were even more commonly and more significantly associated with clinicopathological aggressiveness, and might form a novel genetic background defining cases of PTC having the worst clinicopathologic outcomes (9, 11). A recent meta-analysis (29), which included 13 eligible studies incorporating 4347 patients with PTC, 283 of whom had coexistent *BRAF*^{V600E} and *TERT* promoter mutations, corroborated this association. Moreover, PTC-related mortality was significantly higher when these coexistent mutations were present than in the presence of *BRAF*^{V600E} alone (HR=20.07, CI=8.37–48.09). Despite continuing controversy, it is clear that the disease in the *BRAF*^{V600E} mutation group in our study was more aggressive than in the wild-type group.

Moreover, our data indicated that bilateral PTC is actually different from unilateral PTC. Bilateral PTC is generally more prevalent in females and is more likely to exhibit extrathyroidal invasion, capsular invasion and fusion of metastatic lymph nodes. It is worth noting that bilateral PTC has a higher incidence of extrathyroid invasion. Similarly, Wang *et al.* (30) investigated more than 2000 consecutive patients with PTC and reported that patients with bilateral PTC have more aggressive disease features, a higher frequency of the *BRAF* mutation and lower 10-year DFS rates than those with unilateral-multifocal and solitary PTC. The authors also suggested that bilaterality should be regarded as a more progressive state of the disease and should therefore be considered in risk stratification, management guidelines and subsequent follow-up of PTC patients. Although our study did not further distinguish unilateral-multifocal PTC from solitary PTC, which is one of its limitations, we also demonstrated a significant association between the *BRAF*^{V600E} mutation and bilaterality. This finding implies the need for further research in order to investigate this relationship.

PTC is a relatively indolent cancer, even though it tends to recur. Consequently, in recent years, more attention has been focused on the *BRAF*^{V600E} mutation, which has been found to be an independent predictor of recurrence (25, 31, 32, 33). A retrospective multicenter study performed by Xing *et al.* (25), including 2099 PTC patients from 16 medical centers in eight countries, indicated that a significant association between the *BRAF*^{V600E} mutation and PTC recurrence exists in patients with conventionally low-risk disease stage I or II and micro-PTC, as well as those with various subtypes of PTC. In addition, the *BRAF*^{V600E} mutation was associated with poorer recurrence-free probability in Kaplan–Meier survival analyses in various clinicopathologic categories. Thus, the author suggested that the *BRAF*^{V600E} mutation should be regarded as an independent prognostic predictor of PTC recurrence. Moreover, in a meta-analysis of 81 studies that included 25,241 PTC patients, Qing *et al.* (34) also demonstrated that the *BRAF*^{V600E} mutation was a significant predictor of recurrence/persistence (OR=2.33; 95% CI=1.71–3.18). Furthermore, several studies have also found that the *BRAF*^{V600E} mutation is correlated with mortality in patients with PTC (13, 35). Therefore, it is relatively clear that the *BRAF*^{V600E} mutation is associated with a poorer clinical prognosis in PTC patients.

In our current study, since all patients underwent postsurgical RAI therapy, we had the opportunity to further comprehensively evaluate the relationship between the *BRAF*^{V600E} mutation and response to therapy. Unlike the aforementioned studies, however, we did not observe a significant association between the *BRAF*^{V600E} mutation and poorer clinical outcomes in the overall cohort. In addition, we separated all patients into a subgroup that only included the high recurrence risk PTC patients. The results of our subgroup analysis were similar to those of the entire cohort, i.e., the response to RAI therapy in the subgroup was not inferior to that of the wild-type group. One possible reason for this unexpected finding might

Table 6 Association between response to RAI therapy and *BRAF*^{V600E} status in PTC patients with high recurrence risk.

Response to therapy	*BRAF*^{V600E} mutant group	*BRAF*^{V600E} wild-type group	*P* value
Excellent response	148 (47.7%)	32 (48.5%)	0.498
Indeterminate response	42 (13.5%)	5 (7.6%)	
Biochemical incomplete response	19 (6.1%)	3 (4.5%)	
Structural incomplete response	101 (32.6%)	26 (39.4%)	

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be that all patients received postsurgical RAI therapy in our study. According to previous research, radioactive iodine (RAI) therapy, as an important adjuvant treatment following thyroidectomy, could improve overall survival and reduce the likelihood of disease recurrence (36, 37, 38). For example, a large sample cohort study, involving a total of 32,119 patients, showed that RAI therapy following thyroid lobectomy was associated with improved survival at both the 5- and 10-year follow-up (37). Therefore, regarding our unexpected research results, RAI therapy might play a significant role in improving the clinical outcome of *BRAF*^{V600E} mutation PTC patients, especially those with a high recurrence risk. The findings of another study (16) were similar to ours, although a major drawback of their research was a relatively small sample size, consisting of only 228 PTC patients. By contrast, our research cohort was significantly larger (1220 PTC patients), which presumably would be advantageous.

The current study has the following limitations. First, the major shortcoming is that it was a retrospective, single-institution study. Second, since PTC is typically a slowly-progressing disease, our follow-up time (the mean follow-up period was 2.67 years) may not have been long enough to uncover the true prognostic significance of the *BRAF*^{V600E} mutation. Thus, prognostic factors related to the *BRAF*^{V600E} mutation, such as tumor recurrence and survival, have not been adequately examined. Third, since only patients with PTC were enrolled, this study cannot be applied to other types of thyroid cancer, such as medullary thyroid carcinoma, anaplastic thyroid carcinoma and follicular thyroid carcinoma. Therefore, in future analyses, we plan to extend the follow-up time in order to confirm the research results described earlier.

Conclusions

In summary, this large retrospective study confirms that PTC patients with the *BRAF*^{V600E} mutation are more likely to have aggressive characteristics, including older age, tumor bilaterality and more advanced TNM stage. In addition, patients with bilateral PTC have a higher incidence of extrathyroid invasion. Nevertheless, poor clinical outcomes were not observed in intermediate or high recurrence risk PTC patients with the *BRAF*^{V600E} mutation and without distant metastases, suggesting that RAI therapy could improve the general clinical outcome in this patient group. Thus, additional studies with larger patient cohorts and long-term follow-up are warranted in order to confirm these findings.

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

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