

RESEARCH

Relationship between *BRAF* V600E and clinical features in papillary thyroid carcinoma

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Abstract

Objective: To investigate the mutant status of *BRAF* gene and analyze its relationship to epidemiological risk factors and clinical outcomes among patients with papillary thyroid cancer (PTC) in the largest, single-institution Chinese cohort to date.

Methods: The medical records of 2048 PTC patients were reviewed in this retrospective study. Single-factor and multiple logistic regression analyses were applied to identify risk factors for *BRAF* V600E mutation. Survival outcomes including distant metastatic and persistent or recurrent PTC were examined, with a mean follow-up time of 23.4 (5–47) months.

Results: The *BRAF* V600E mutation was present in 83.7% of patients (1715 of 2048). Correlation was found between *BRAF* V600E mutation and several epidemiological features, including age, concomitant hypertension and Hashimoto thyroiditis (HT). For the clinicopathological features, *BRAF* V600E was significantly associated with bilateral multifocality (odds ratio (OR) 1.233, 95% confidence interval (CI) 1.063–1.431, $P < 0.01$) and less lateral lymph node metastases (OR 0.496, 95% CI 0.357–0.689, $P < 0.01$). Smaller tumor size and advanced disease stage were significant in single-factor analyses but became insignificant after multivariate adjustment. No association was found between *BRAF* V600E mutation and extrathyroidal invasion, distant metastatic and disease persistence or recurrence.

Conclusion: Part of epidemiological features are independent risk or protective factors for *BRAF* V600E mutation. The presence of *BRAF* V600E mutation is not an aggressive prognosis on poor clinical outcomes in PTC. However, the high prevalence of *BRAF* V600E may provide guidance for surgery strategy and opportunity for targeted treatment in recurrent and advanced stage disease.

Key Words

- ▶ papillary thyroid cancer
- ▶ *BRAF* V600E
- ▶ epidemiological features
- ▶ clinicopathological features

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Introduction

Thyroid cancer, especially papillary subtype, is the most common malignancy in the endocrine system (1). Papillary thyroid cancer (PTC) can be further classified into conventional variant (CPTC), follicular variant (FVPTC) and other rare variants (2). Despite PTC is usually a well-differentiated thyroid carcinoma with a favorable prognosis, its incidence has been sharply rising in many countries over the last decades (3) (the average incidence

in the USA was 2.4% from 1980 to 1997 and 6.6% from 1997 to 2009 (2)). In addition, recurrence and metastases are common for a small proportion of PTCs who reach advanced disease stages (4). In recent years, molecular markers have received extensive attention to improving risk stratification of PTC (5).

BRAF is the main subtype of RAF kinase and plays a key role in tumorigenesis. The mutation of *BRAF* V600E

could trigger tumorigenesis through constitutively activating MAPK pathway (6). As the most common mutation observed in PTC, *BRAF* V600E has received special attention in various ethnic populations since this protein kinase may contribute to cell proliferation, growth and division. However, due to the limited large cohort evidence, the function of *BRAF* V600E as a biomarker in driving aggressiveness in PTC continues debatable (7, 8). The majority of researches claimed that *BRAF* V600E mutation was associated with poor clinicopathologic outcomes in patients with PTC, such as large tumor size, lymph node metastases, advanced clinical stages and recurrence (9, 10). By contrast, several studies suggested that *BRAF* V600E mutation had no significant association with clinical stage, multicentricity or recurrence (11, 12). These equivocal findings have hindered the fact that whether the mutation had an impact on aggressive behavior of PTC. Furthermore, most researches have focused on the relationship between mutation and clinicopathological characteristics, but the epidemiologic factors related to *BRAF* V600E mutations were rarely studied in previous researches. Here, we investigated epidemiological characteristics that may be associated with the mutation of *BRAF* V600E and then studied the role of *BRAF* V600E mutation in the clinicopathological features of PTC.

Patients and methods

Patient identification and clinicopathologic data collection

This study included 2048 patients (1556 women and 492 men) age 43.14 ± 11.01 years (mean \pm s.d.) who were diagnosed with PTC and underwent surgery between

January 2015 and July 2018 at the Department of the thyroid, breast and vascular surgery in Xijing Hospital. These patients were clinically observed with mean follow-up time of 23.4 months (range 5–47 months) after the initial treatments. All these patients were regularly followed with physical examinations, thyroid function tests and neck ultrasonography every 6–12 months after the initial surgery. If suspicious or indeterminate thyroid nodules or lymph nodes were found, ultrasound-guided fine-needle aspiration cytology (US-FNAC) was used for evaluation. Between January 2015 and July 2018, 2850 patients were diagnosed with thyroid cancer. Among these, 2805 patients (98.42%) were diagnosed with PTC, and 45 patients (1.58%) were diagnosed with other types of thyroid carcinoma. PTC patients without *BRAF* V600E status or lost to follow-up were excluded. The flow diagram of patient included was shown in Fig. 1.

After institutional review board approval and informed patient consenting, we retrospectively collected detailed *BRAF* V600E and clinicopathologic data from institutional patient records. The epidemiological data and clinicopathological features were summarized in Tables 1 and 2, respectively. Patients with alcohol history was defined as patients who drinking more than twice a month and lasting more than 1 year. Patients with smoking history was defined as patients who had a current or past smoking history of ≥ 6 months. Tumor node metastasis (TNM) stages were defined based on the seventh edition of the American Joint Committee on Cancer (AJCC) staging system. Persistent or recurrent disease was defined as the presence of a structural abnormality confirmed by cytological or surgical pathology after the initial surgery. The *BRAF* V600E mutation results had no influence on the treatment decision making.

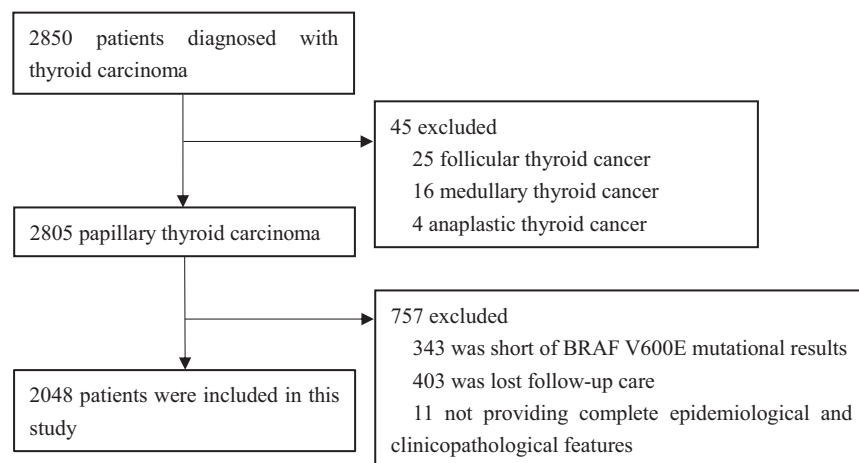


Figure 1
Flow diagram of patient included according to the inclusion and exclusion criteria.

Table 1 Association of *BRAF* V600E with epidemiologic features of all PTC.

	<i>BRAF</i> V600E mutation (-)	<i>BRAF</i> V600E mutation (+)	χ^2	P value
	No. (%)	No. (%)		
Total No. of cases	333 (16.3)	1715 (83.7)		
Age at diagnosis				
≤45	224 (20.3)	881 (79.7)	28.366	<0.001
>45	109 (11.6)	834 (88.4)		
Sex				
Male	74 (15.0)	418 (85.0)	0.707	0.400
Female	259 (16.6)	1297 (83.4)		
Family history of cancer				
Had any family member(s) with history of cancer	27 (11.4)	209 (88.6)	4.550	0.033
None	306 (22.5)	1506 (77.5)		
Presence of history of cancer				
Had any other cancer	5 (12.5)	35 (87.5)	0.424	0.515
None	328 (16.3)	1680 (83.7)		
Presence of smoking history				
Ever	28 (18.1)	127 (81.9)	0.401	0.527
Never	305 (16.1)	1588 (83.9)		
Presence of alcohol history				
Ever	7 (15.2)	39 (84.8)	0.038	0.846
Never	326 (16.3)	1676 (83.7)		
Concomitant diabetes				
Yes	8 (8.6)	85 (91.4)	4.196	0.041
No	325 (16.6)	1630 (83.4)		
Concomitant hypertension				
Yes	18 (7.0)	238 (93.0)	18.300	<0.001
No	315 (17.6)	1477 (82.4)		
Concomitant benign thyroid diseases				
Hyperthyroid				
Yes	7 (24.1)	22 (75.9)	/	0.305
No	326 (16.1)	1693 (83.9)		
Nodular goiter				
Yes	19 (20.4)	74 (79.6)	1.244	0.265
No	314 (16.1)	1641 (83.9)		
HT				
Yes	96 (28.9)	236 (71.1)	46.611	<0.001
No	237 (13.8)	1479 (86.2)		

The chi-square test (χ^2 test) or, for small cell sizes, Fisher's exact test was employed to examine the significance of association between *BRAF* V600E and epidemiologic features. P value <0.05 was treated as statistically significant. Bold indicates statistical significance.

'/' means no χ^2 value because cell sizes were small and Fisher's exact test was employed. '%' is the proportion of patients with or without *BRAF* V600E mutations in the subgroup of patients.

HT, Hashimoto thyroiditis.

Mutational analyses

BRAF V600E mutational analyses were performed by pathologists after surgical treatments of patients. DNA was isolated from formalin-fixed, paraffin-embedded (FFPE) tissue blocks by SDS-proteinase K method and subjected to Amplification Refractory Mutation System (ARMS)- real-time PCR for the detection of *BRAF* V600E mutations. DNA was extracted from each sample via a commercial kit (FFPE DNA reagent, Cat No. ADx-FF01) according to the manufacturer's instructions. Typically, 5 μ m sections (2–4 pieces) were carefully micro-dissected

from FFPE tissue blocks. The sections were initially treated with 1.5 mL xylene/ethanol three times, then digested during an overnight incubation with 20 μ L proteinase K solution and 180 μ L buffer DTL in a 56°C rotating incubator, and DNA purification was performed through QIAgen columns, according to the manufacturer's instructions. Then, the most common T1799A transversion (*BRAF* V600E) mutation was studied. The PCR was used to amplify exon 15 of the *BRAF* gene, which was detected by *BRAF* V600 Mutations Detection Kit (Applied by ADx-BR01, AmoyDx Company, China) according to the manufacturer's instructions. PCR primer sequences were as follows:

Table 2 Relationship of BRAF V600E with clinicopathological features of All PTC.

	BRAF V600E mutation (-)	BRAF V600E mutation (+)	χ^2	P value
	No. (%)	No. (%)		
Total no. of cases	333 (16.3)	1715 (83.7)		
Surgery				
Lobectomy	53 (14.8)	306 (85.2)	0.716	0.397
Total thyroidectomy	280 (16.6)	1409 (83.4)		
Histological type				
CPTC	321 (15.9)	1701 (84.1)	/	<0.001
FVPTC	12 (46.2)	14 (53.8)		
Tumor size				
≤ 2 cm	290 (15.6)	1564 (84.4)	5.488	0.019
> 2 cm	43 (22.2)	151 (77.8)		
Median (quartile), cm	1.0 (0.7–1.5)	0.9 (0.7–1.4)		
Lesions				
Unilateral	260 (18.0)	1184 (82.0)	10.959	0.001
Bilateral	73 (12.1)	531 (87.9)		
Extrathyroidal invasion				
Yes	45 (15.6)	244 (84.4)	0.117	0.732
No	288 (16.4)	1471 (83.6)		
Vascular invasion				
Yes	43 (15.4)	237 (84.6)	0.194	0.660
No	290 (16.4)	1478 (83.6)		
Status of lymph node metastases ^a				
Yes	190 (18.2)	855 (81.8)	3.704	0.054
No	127 (14.9)	727 (85.1)		
Site of lymph node metastases				
Only central	92 (14.0)	564 (86.0)	20.648	<0.001
Only lateral	13 (23.2)	43 (76.8)		
Central and lateral	85 (25.5)	248 (74.5)		
Disease stage (7th edition) ^b				
I + II	282 (17.8)	1301 (82.2)	6.718	0.010
III + IV	48 (12.3)	341 (87.7)		
Distant metastatic				
Yes	1 (20.0)	4 (80.0)	/	1.000
No	332 (16.3)	1711 (83.7)		
Persistent or recurrent disease				
Yes	19 (22.1)	67 (77.9)	2.243	0.134
No	314 (16.0)	1648 (84.0)		

The chi-square test (χ^2 test) or, for small cell sizes, Fisher's exact test was employed to examine the significance of association between BRAF V600E and clinicopathological features. P value <0.05 was treated as statistically significant. Bold indicates statistical significance. Tumor size was summarized with medians (quartile).

'/' means no χ^2 value because cell sizes were small and Fisher's exact test was employed. '%' is the proportion of patients with or without BRAF V600E mutations in the subgroup of patients. 'a' means there are missing cases in 'Status of lymph node metastases'. In patients without BRAF V600E mutations, 16 patients had undetermined lymph node metastasis status. In patients with BRAF V600E mutations, 133 patients had undetermined lymph node metastasis status. 'b' means there are missing cases in 'Disease stage'. In patients without BRAF V600E mutations, the disease stage of three patients cannot be determined. In patients with BRAF V600E mutations, the disease stage of 73 patients cannot be determined. CPTC, conventional papillary thyroid carcinoma; FVPTC, follicular variant papillary thyroid carcinoma.

forward primer, TCTGTAGCAGCCCTCAGTAGCGAAGCA GTGATTTTGGTCTAGCTACAGA; reverse primer, AGC CCTCAGTAGCGAAGCAACTCAGCAGCATCTCAGG. BRAF gene reactions were performed in a final volume of 40 μ L using as template 10–15 ng of genomic DNA, with 1 \times buffer including 14 pmol forward primer, 20 pmol reverse primer, 12.5 pmol dNTPs, 350 pmol MgCl₂, mutant probe 20 pmol and 1 unit of Taq polymerase. Each reaction

included a positive and a negative control sample, and in negative sample, DNA template was substituted by water. PCR recycling started with initial denaturation step at 95°C for 5 min, followed by 40 cycles of denaturation (95°C, 25 s), annealing (64°C, 20 s) and extension (72°C, 20 s) and a last step of 10 min extension at 72°C. PCR efficiency was determined by measuring the Ct value of FAM signal.

Statistical analyses

Data related to histologic characteristics, patient epidemiological data and clinical outcomes were collected. Categorical data were summarized with frequencies and percentages. Continuous data were summarized with means \pm standard deviations (if it is a normal distribution) or medians and quartile (if it is not a normal distribution). The chi-square test (χ^2 test) or, for small cell sizes, Fisher's exact test was employed to examine categorical variables. All *P* values were two-sided, and a *P* value <0.05 was treated as statistically significant. Pooled ORs with their corresponding 95% confidence intervals (95% CIs) were calculated to assess the relationship between *BRAF* V600E mutation and clinicopathological features. All statistical analyses were conducted by the software of SPSS with version 23.0.

Results

BRAF V600E mutation in PTC

There were 2048 patients included in the study with an average age of 43.14 ± 11.01 (range 5–80), and 76.0% of the patients were female (1556 women and 492 men). *BRAF* V600E mutation was found in 1701 of 2022 (84.1%) CPTCs, and 14 of 26 (53.8%) FVPTCs, with an overall prevalence of 83.7% (1715 of 2048). No significant difference of *BRAF* V600E mutation was observed in female and male patients ($P=0.400$). With regard to ages, significant difference of *BRAF* V600E incidence was found between patients aged ≤ 45 and >45 years (79.7 vs 88.4%, $P<0.001$). To further investigate the influence of age on mutational incidence, we divided all patients into children/adolescent group (≤ 25 years) and adults groups of various age ranges (25–35, 35–45, 45–55, >55 years old). As shown in Fig. 2, patients were more prone to be *BRAF* V600E positive with the growth of age.

Association of *BRAF* V600E and epidemiological features in PTCs

To identify epidemiological factors associated with *BRAF* V600E mutation, the relationship between epidemiological features and the mutation was investigated. In the univariate analysis of 2048 PTCs (Table 1), the presence of *BRAF* V600E mutation was found to be significantly associated with several epidemiological features, including age at diagnosis, family history of cancer, concomitant diabetes, hypertension and Hashimoto

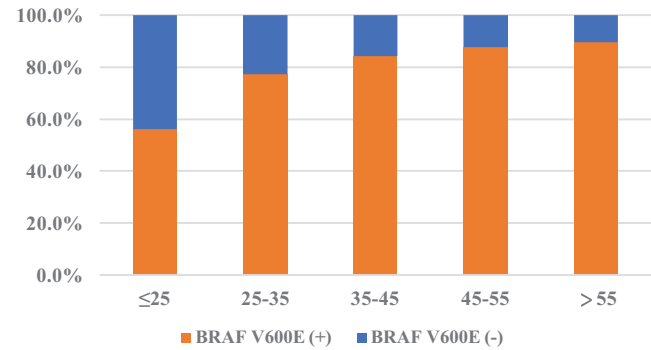


Figure 2

The correlation between the presence of the *BRAF* V600E mutation and the age of patients at the time of diagnosis.

thyroiditis (HT). The incidence of *BRAF* V600E mutations in patients with family cancer history was higher than that in patients without family history of cancer (88.6 vs 77.5%, $P=0.033$). Interestingly, patients with diabetes or hypertension presented higher mutation rates than those who did not have diabetes or hypertension (91.4 vs 83.4%, $P=0.041$; 93.0 vs 82.4%, $P<0.001$). Conversely, the patients concomitant of HT displayed less *BRAF* V600E mutations than patients without HT (71.1 vs 86.2%, $P<0.001$). Similarly, patients with hyperthyroid or nodular goiter showed lower mutation frequency than those who did not have hyperthyroid or nodular goiter, but the association was insignificant. Except the factors mentioned above, no significant association was found between the presence of *BRAF* V600E mutation and other features including gender, presence of history of cancer, presence of smoking and alcohol history.

Relationship of *BRAF* V600E and clinicopathological features in PTCs

In the univariate analysis of all PTCs (Table 2), the presence of *BRAF* V600E was found to be significantly associated with small tumor size ($P=0.019$), bilateral multifocality ($P=0.001$), less central and lateral lymph node metastases simultaneously ($P<0.001$) and advanced disease stage (III and IV) ($P=0.010$). Although there was no significant association, less *BRAF* V600E mutation was found in patients with lymph node metastases than patients without lymph node metastases (81.8 vs 85.1%, $P=0.054$). Furthermore, it was worth noticing that less *BRAF* V600E presented in patients with aggressive lymph node metastases (central and lateral metastases at the same time) than patients with only central or only lateral lymph node metastases (74.5 vs 86.0% or 76.8%, $P<0.001$). No significant association was found between *BRAF*

V600E mutation and another high-risk clinicopathologic characteristics, such as extrathyroidal invasion ($P=0.732$), vascular invasion ($P=0.660$), distant metastatic ($P=1.000$) and PTC persistence or recurrence ($P=0.134$).

Multivariate logistic regression analysis of *BRAF* V600E mutation in PTCs

To further confirm the relationship between *BRAF* V600E and epidemiological or clinicopathological features, multivariate logistic regression analysis was performed (Table 3). The results showed that age ($P=0.002$, OR=1.024, 95% CI=1.009–1.041), concomitant hypertension ($P=0.032$, OR=1.812, 95% CI=1.052–3.120) and lesions ($P=0.006$, OR=1.233, 95% CI=1.063–1.431) were positive independent factors for *BRAF* V600E mutation. In contrast, concomitant HT ($P<0.001$, OR=0.402, 95% CI=0.300–0.538) and lateral lymph node metastases

($P<0.001$, OR=0.496, 95% CI=0.357–0.689) were negative independent factors for *BRAF* V600E mutation. After adjustment for patients' age and sex, the association between *BRAF* V600E mutation and disease stage was not statistically significant ($P=0.771$, OR=1.028, 95% CI=0.856–1.234).

Discussion

This study sought to find the epidemiological factors associated with *BRAF* V600E mutation and clarify the relationship between *BRAF* V600E mutation and clinical outcomes in PTC. Previously, the *BRAF* V600E mutation has been reported as an aggressive prognosis in PTC, although a large cohort study was inadequate and there have been noteworthy inconsistencies in some studies (13, 14). In our analysis, we found a lack of

Table 3 Multivariate logistic regression analysis of *BRAF* V600E mutation of all PTC.

	<i>BRAF</i> V600E mutation (n, %)		P Value	OR	95% CI
	-	+			
Age at diagnosis	39.29 ± 11.44	43.88 ± 10.77	0.002	1.024	1.009–1.041
Sex			0.750	1.051	0.773–1.431
Male	74 (15.0%)	418 (85.0%)			
Female	259 (16.6%)	1297 (83.4%)			
Family history of cancer			0.195	1.352	0.857–2.131
Had any family member(s) with history of cancer	27 (11.4%)	209 (88.6%)			
None	306 (22.5%)	1506 (77.5%)			
Concomitant diabetes			0.428	1.389	0.616–3.129
Yes	8 (8.6%)	85 (91.4%)			
No	325 (16.6%)	1630 (83.4%)			
Concomitant hypertension			0.032	1.812	1.052–3.120
Yes	18 (7.0%)	238 (93.0%)			
No	315 (17.6%)	1477 (82.4%)			
Concomitant HT			<0.001	0.402	0.300–0.538
Yes	96 (28.9%)	236 (71.1%)			
No	237 (13.8%)	1479 (86.2%)			
Tumor size	1.0 (0.7–1.5)	0.9 (0.7–1.4)	0.571	0.950	0.795–1.135
Lesions			0.006	1.233	1.063–1.431
Unilateral	260 (18.0%)	1184 (82.0%)			
Bilateral	73 (12.1%)	531 (87.9%)			
Site of lymph node metastasis			0.346	1.157	0.854–1.568
Central	177 (17.9%)	811 (82.1%)			
Lateral	98 (25.2%)	290 (74.8%)	<0.001	0.496	0.357–0.689
Disease stage (7th edition) ^a			0.771	1.028	0.856–1.234
I + II	282 (17.8%)	1301 (82.2%)			
III + IV	48 (12.3%)	341 (87.7%)			

Age at diagnosis was summarized with means ± standard deviations. Tumor size was summarized with medians (quartile). Multivariate logistic regression analysis was employed to identify risk factors for *BRAF* V600E mutations. P value <0.05 was treated as statistically significant. Bold indicates statistical significance.

'%' is the proportion of patients with or without *BRAF* V600E mutations in the subgroup of patients. 'a' means there are missing cases in 'Disease stage'. In patients without *BRAF* V600E mutations, the disease stage of three patients cannot be determined. In patients with *BRAF* V600E mutations, the disease stage of 73 patients cannot be determined.

HT, Hashimoto thyroiditis; OR, odds ratio; 95% CI, 95% confidence interval.

correlation between *BRAF* V600E mutation and either aggressive clinicopathological features or persistent or recurrent disease.

The incidence of *BRAF* V600E mutation increased in recent years, it seems that *BRAF* V600E mutation gradually become the accompanying phenomenon for PTC patients. Mingzhao Xing *et al.* reported *BRAF* V600E rate of 38.3% in patients between 1990 and 2012 (15). In 2007, Electron *et al.* reported the prevalence of *BRAF* V600E was 46.4% (16). In 2012, Kurtulmus *et al.* showed *BRAF* V600E mutation rate of 39.45% in PTCs (17). The meta-analysis showed *BRAF* V600E mutations occurred in 60.6% of PTCs (update to August, 2015) (6). In 2016, Kim *et al.* reported *BRAF* V600E mutations were presented in 83.7% PTC patients (2789 of 3332) (18), and we found the similar mutation rate in our sample. Compared to previous researches, we update *BRAF* V600E mutation data from 2015 to 2018. The reason why mutation prevalence in our study is higher than the average may ascribe to population aging in recent years and different research methodology. In this study, the *BRAF* V600E mutations were tested from the postoperative tissue samples using the ARMS- real-time PCR method, which was more sensitive and robust at detecting *BRAF* V600E somatic mutations than DNA sequencing on clinical samples (19).

Our study indicated that older age and concomitant hypertension were independent risk factors of *BRAF* V600E mutation. On the contrary, concomitant HT was an independent protective factor of the mutation. Despite being insignificant after multivariate adjustment, the presence of family history of cancer was associated with higher *BRAF* V600E mutation incidence in the univariate analysis, which were in agreement with the data reported in previous study (20). In addition, with the large size of this study, negative correlation between HT and *BRAF* V600E mutation was demonstrated, which was confirmed in a recent smaller study (21). Potential mechanisms and immunological link that might lead to the synchronous appearance of HT and PTC had been investigated (22, 23). If there is more clinical evidence, the correlation between *BRAF* V600E and family history of cancer, hypertension and HT may help to promote the investigation of *BRAF* V600E mutation mechanism. As for the factor of age, plenty of researches had found that incidence of *BRAF* V600E was higher in patients >45 years old (10, 16), but few studies found the positive correlation between *BRAF* V600E and age (Fig. 2). Compared to adults, PTC in pediatric and adolescents presents more lymph node metastases, distant metastases and recurrence (24, 25). Combining the study mentioned above, the phenomenon

that less number of *BRAF* V600E mutations appears in patients aged under 25 years suggested that aggressive features displayed in these patients may not be caused by *BRAF* V600E mutation.

The aggressive role of *BRAF* V600E mutations has been widely investigated in previous studies (6, 8, 10). However, our results did not show that *BRAF* V600E mutation is a biomarker in driving aggressiveness. Given the high and increased prevalence of *BRAF* V600E mutation in recent years (60.6%, on average, in the meta-analysis mentioned above (6), and 83.7% in our study), and the oppositely indolent behavior, the rarity recurrence and mortality of PTC (16 and 3%, respectively) (26, 27), an absence of association between *BRAF* V600E mutation, and these negative events seem logical. In univariate analyses, *BRAF* V600E mutations were significantly associated with smaller tumor, bilateral multifocality, advanced disease stage and less aggressive lymph node metastases in PTCs. Advanced TNM stage showed insignificant after multivariate adjustment. Therefore, bilateral multifocality seems to be the only risk factor associated with *BRAF* V600E. Notably, a recent study showed tumor multifocality has no independent risk prognostic value in outcomes of PTC (28). Other classic risk factors (29), such as extrathyroidal extension, distant metastases and disease recurrence, were insignificantly associated with *BRAF* V600E mutation. Therefore, presence of *BRAF* V600E is not an aggressive role associated with poor clinicopathologic outcomes in PTC. A recent publication has called into question the relationship of *BRAF* V600E mutation and prognosis in PTC. In the analysis of 508 patients with *BRAF* V600E mutation, Henke *et al.* found the mutation is not predictive of long-term outcome in PTC (7).

Although the presence of *BRAF* V600E mutation is not an aggressive prognosis on poor clinical outcomes, surgery strategy and treatment guided by the presence of the mutation may be available. Such therapies might include determining surgery extent and the use of *BRAF* inhibitors. The association between *BRAF* V600E and bilateral lesions and less lateral lymph node metastases was found in our study. If there is more clinical evidence in the future, the status of *BRAF* V600E mutation may be considered as one of the factors for determining the surgery extent besides tumor size, extrathyroidal extension, vascular invasion, lymph node size and willingness of patients (30). Furthermore, considerable patients with *BRAF* V600E mutation will have recurrence since a majority of PTC patients in our cohort (83.7%) had the mutation. We propose that high-risk patients with *BRAF* V600E

mutation use *BRAF* inhibitors, such as sorafenib, lenvatinib and vemurafenib. Those patients might benefit from the targeted therapy, which (sorafenib and lenvatinib) have been approved for metastatic PTC (31).

The greatest strengths of this study are its consecutive large cohort (2048 PTC patients) and its latest research time (from 2015 to 2018). The limitations of this study were as follows. First, a selection bias could occur in this retrospective unicentral research. The large consecutive cohort of patients may help minimize the bias. Second, follow-up time was short in this study (mean 23.4 months, range, 5–47 months). And research on the mechanism of epidemiological features associated with *BRAF* V600E mutation in PTCs should be carried out in the future.

In summary, this was a large consecutive retrospective study that investigated the relationship of *BRAF* V600E mutation with epidemiological and clinicopathological features. Older age and concomitant hypertension were independent risk factors of *BRAF* V600E mutation. Concomitant HT was an independent protective factor for the *BRAF* V600E expression. Bilateral multifocality was the only risk factor associated with *BRAF* V600E but a recent study showed it has no independent risk prognostic value in outcomes of PTC. Other poor clinicopathological features were insignificantly associated with the mutation. Although the presence of *BRAF* V600E mutation is not an aggressive prognosis on poor clinical outcomes, surgery and treatment guided by the presence of the mutation may be available.

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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Ethical approval

Consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used. The study was approved by an independent ethics committee of Xijing Hospital (first affiliated hospital of Fourth Military Medical University).

References

- 1 Pacini F & Castagna MG. Approach to and treatment of differentiated thyroid carcinoma. *Medical Clinics of North America* 2012 **96** 369–383. (<https://doi.org/10.1016/j.mcna.2012.01.002>)
- 2 Pellegriti G, Frasca F, Regalbuto C, Squatrito S & Vigneri R. Worldwide increasing incidence of thyroid cancer: update on

- epidemiology and risk factors. *Journal of Cancer Epidemiology* 2013 **2013** 965212. (<https://doi.org/10.1155/2013/965212>)
- 3 Jung CK, Little MP, Lubin JH, Brenner AV, Wells SA, Jr, Sigurdson AJ & Nikiforov YE. The increase in thyroid cancer incidence during the last four decades is accompanied by a high frequency of *BRAF* mutations and a sharp increase in *RAS* mutations. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** E276–E285. (<https://doi.org/10.1210/jc.2013-2503>)
- 4 Vuong HG, Altibi AM, Abdelhamid AH, Ngoc PU, Quan VD, Tantai MY, Elfil M, Vu TL, Elgebaly A, Oishi N, *et al.* The changing characteristics and molecular profiles of papillary thyroid carcinoma over time: a systematic review. *Oncotarget* 2017 **8** 10637–10649. (<https://doi.org/10.18632/oncotarget.12885>)
- 5 Nikiforov YE & Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. *Nature Reviews: Endocrinology* 2011 **7** 569–580. (<https://doi.org/10.1038/nrendo.2011.142>)
- 6 Zhang Q, Liu SZ, Zhang Q, Guan YX, Chen QJ & Zhu QY. Meta-analyses of association between *BRAF*(V600E) mutation and clinicopathological features of papillary thyroid carcinoma. *Cellular Physiology and Biochemistry* 2016 **38** 763–776. (<https://doi.org/10.1159/000443032>)
- 7 Henke LE, Pfeifer JD, Ma C, Perkins SM, DeWees T, El-Mofty S, Moley JF, Nussenbaum B, Haughey BH, Baranski TJ, *et al.* *BRAF* mutation is not predictive of long-term outcome in papillary thyroid carcinoma. *Cancer Medicine* 2015 **4** 791–799. (<https://doi.org/10.1002/cam4.417>)
- 8 Tufano RP, Teixeira GV, Bishop J, Carson KA & Xing M. *BRAF* mutation in papillary thyroid cancer and its value in tailoring initial treatment: a systematic review and meta-analysis. *Medicine* 2012 **91** 274–286. (<https://doi.org/10.1097/MD.0b013e31826a9c71>)
- 9 Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, Carson KA, Vasko V, Larin A, Tallini G, *et al.* *BRAF* mutation predicts a poorer clinical prognosis for papillary thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6373–6379. (<https://doi.org/10.1210/jc.2005-0987>)
- 10 Kim TH, Park YJ, Lim JA, Ahn HY, Lee EK, Lee YJ, Kim KW, Hahn SK, Youn YK, Kim KH, *et al.* The association of the *BRAF*(V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: a meta-analysis. *Cancer* 2012 **118** 1764–1773. (<https://doi.org/10.1002/cncr.26500>)
- 11 Walczyk A, Kowalska A, Kowalik A, Sygut J, Wypioriewicz E, Chodurska R, Pieciak L & Gozdz S. The *BRAF*(V600E) mutation in papillary thyroid microcarcinoma: does the mutation have an impact on clinical outcome? *Clinical Endocrinology* 2014 **80** 899–904. (<https://doi.org/10.1111/cen.12386>)
- 12 Nam JK, Jung CK, Song BJ, Lim DJ, Chae BJ, Lee NS, Park WC, Kim JS, Jung SS & Bae JS. Is the *BRAF*(V600E) mutation useful as a predictor of preoperative risk in papillary thyroid cancer? *American Journal of Surgery* 2012 **203** 436–441. (<https://doi.org/10.1016/j.amjsurg.2011.02.013>)
- 13 Russo M, Malandrino P, Nicolosi ML, Manusia M, Marturano I, Trovato MA, Pellegriti G, Frasca F & Vigneri R. The *BRAF*(V600E) mutation influences the short- and medium-term outcomes of classic papillary thyroid cancer, but is not an independent predictor of unfavorable outcome. *Thyroid* 2014 **24** 1267–1274. (<https://doi.org/10.1089/thy.2013.0675>)
- 14 Gandolfi G, Sancisi V, Piana S & Ciarrocchi A. Time to re-consider the meaning of *BRAF* V600E mutation in papillary thyroid carcinoma. *International Journal of Cancer* 2015 **137** 1001–1011. (<https://doi.org/10.1002/ijc.28976>)
- 15 Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, Pai S & Bishop J. *BRAF* V600E and tert promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. *Journal of Clinical Oncology* 2014 **32** 2718–2726. (<https://doi.org/10.1200/JCO.2014.55.5094>)

- 16 Kebebew E, Weng J, Bauer J, Ranvier G, Clark OH, Duh QY, Shibru D, Bastian B & Griffin A. The prevalence and prognostic value of BRAF mutation in thyroid cancer. *Annals of Surgery* 2007 **246** 466–470; discussion 70–71. (<https://doi.org/10.1097/SLA.0b013e318148563d>)
- 17 Kurtulmus N, Duren M, Ince U, Cengiz Yakicier M, Peker O, Aydin O, Altioek E, Giray S, Azizlerli H. BRAF(V600E) mutation in Turkish patients with papillary thyroid cancer: strong correlation with indicators of tumor aggressiveness. *Endocrine* 2012 **42** 404–410. (<https://doi.org/10.1007/s12020-012-9651-x>)
- 18 Kim SK, Woo JW, Lee JH, Park I, Choe JH, Kim JH & Kim JS. Chronic lymphocytic thyroiditis and BRAF V600E in papillary thyroid carcinoma. *Endocrine-Related Cancer* 2016 **23** 27–34. (<https://doi.org/10.1530/ERC-15-0408>)
- 19 Ellison G, Donald E, McWalter G, Knight L, Fletcher L, Sherwood J, Cantarini M, Orr M & Speake G. A comparison of ARMS and DNA sequencing for mutation analysis in clinical biopsy samples. *Journal of Experimental and Clinical Cancer Research* 2010 **29** 132. (<https://doi.org/10.1186/1756-9966-29-132>)
- 20 Zhang Q, Song F, Zheng H, Zhu X, Song F, Yao X, Zhang L & Chen K. Association between single-nucleotide polymorphisms of BRAF and papillary thyroid carcinoma in a Chinese population. *Thyroid* 2013 **23** 38–44. (<https://doi.org/10.1089/thy.2012.0228>)
- 21 Zeng RC, Jin LP, Chen ED, Dong SY, Cai YF, Huang GL, Li Q, Jin C, Zhang XH & Wang OC. Potential relationship between Hashimoto's thyroiditis and BRAF(V600E) mutation status in papillary thyroid cancer. *Head and Neck* 2016 **38** (Supplement 1) E1019–E1025. (<https://doi.org/10.1002/hed.24149>)
- 22 Ehlers M & Schott M. Hashimoto's thyroiditis and papillary thyroid cancer: are they immunologically linked? *Trends in Endocrinology and Metabolism* 2014 **25** 656–664. (<https://doi.org/10.1016/j.tem.2014.09.001>)
- 23 Boi F, Pani F & Mariotti S. Thyroid autoimmunity and thyroid cancer: review focused on cytological studies. *European Thyroid Journal* 2017 **6** 178–186. (<https://doi.org/10.1159/000468928>)
- 24 Spinelli C, Rossi L, Piscioneri J, Strambi S, Antonelli A, Ferrari A, Massimino M & Miccoli P. Pediatric differentiated thyroid cancer: when to perform conservative and radical surgery. *Current Pediatric Reviews* 2016 **12** 247–252. (<https://doi.org/10.2174/1573396312666161014092023>)
- 25 Al-Qurayshi Z, Hauch A, Srivastav S, Aslam R, Friedlander P & Kandil E. A national perspective of the risk, presentation, and outcomes of pediatric thyroid cancer. *JAMA Otolaryngology: Head and Neck Surgery* 2016 **142** 472–478. (<https://doi.org/10.1001/jamaoto.2016.0104>)
- 26 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. (<https://doi.org/10.1200/JCO.2014.56.8253>)
- 27 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. (<https://doi.org/10.1001/jama.2013.3190>)
- 28 Wang F, Yu X, Shen X, Zhu G, Huang Y, Liu R, Viola D, Elisei R, Puxeddu E, Fugazzola L, *et al.* The prognostic value of tumor multifocality in clinical outcomes of papillary thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 2017 **102** 3241–3250. (<https://doi.org/10.1210/jc.2017-00277>)
- 29 Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger, *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. (<https://doi.org/10.1089/thy.2015.0020>)
- 30 Wang TS & Sosa JA. Thyroid surgery for differentiated thyroid cancer – recent advances and future directions. *Nature Reviews: Endocrinology* 2018 **14** 670–683. (<https://doi.org/10.1038/s41574-018-0080-7>)
- 31 Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, de la Fouchardiere C, Pacini F, Paschke R, Shong YK, *et al.* Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. *Lancet* 2014 **384** 319–328. ([https://doi.org/10.1016/S0140-6736\(14\)60421-9](https://doi.org/10.1016/S0140-6736(14)60421-9))

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