

# Younger Than 55 Years Old and *BRAF* V600E Mutation are Risk Factors for Lymph Node Metastasis in Papillary Thyroid Carcinomas $\leq 1.0$ cm but Not in $> 1.0$ cm

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**Background:** Studies on the relationship between *BRAF* V600E mutation and the clinicopathologic features of papillary thyroid carcinoma (PTC), risk of lymph node metastasis in papillary thyroid microcarcinoma (PTMC) have shown inconsistent results.

**Methods:** In this retrospective analysis, clinicopathological data of the patients were collected, and molecular testing was done for *BRAF* V600E mutation. PTC patients are divided into  $PTC \leq 1.0$ cm (PTMC) and  $PTC > 1.0$ cm, and the relationship between *BRAF* V600E mutation and clinicopathologic features was analyzed respectively.

**Results:** Of the 520 PTC patients, 432 (83.1%) were female and 416 (80.0%) were  $< 55$  years old. *BRAF* V600E mutation was detected in 422 (81.2%) tumour samples of PTC. There was no significant difference in the frequency of *BRAF* V600E mutation between different age groups. There were 250 (48.1%) patients with PTMC and 270 (51.9%) patients with  $PTC > 1.0$ cm. *BRAF* V600E mutation was significantly associated with bilateral cancer (23.0% vs 4.9%,  $P=0.005$ ) and lymph node metastasis (61.7% vs 39.0%,  $P=0.009$ ) in PTMC patients, while *BRAF* V600E mutation was significantly associated with bilateral cancer (24.9% vs 12.3%,  $P=0.048$ ) in  $PTC > 1.0$ cm patients. Logistic regression analysis showed that, after adjusting for gender, Hashimoto's thyroiditis and calcification, we found that younger age ( $< 55$  years old) (OR: 2.384, 95% CI: 1.241–4.579,  $P=0.009$ ) and *BRAF* V600E mutation (OR: 2.213, 95% CI: 1.085–4.512,  $P=0.029$ ) were significantly associated with lymph node metastasis in PTMC, similar results were not obtained in  $PTC > 1.0$ cm.

**Conclusion:** Younger age ( $< 55$  years old) and *BRAF* V600E mutation was independent risk factor for lymph node metastasis in PTMC.

**Keywords:** papillary thyroid carcinoma, papillary thyroid microcarcinoma, *BRAF*, lymph node metastasis

## Introduction

Thyroid cancer is the most common malignancy of the endocrine system, and accounts for about 3% of all malignancies in the human.<sup>1</sup> The pathological types of thyroid cancer mainly include papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), medullary thyroid carcinoma (MTC), and anaplastic thyroid cancer (ATC). Among them, PTC is the most common.<sup>2</sup> While the incidence of thyroid cancer has varied in different populations around the world over the past few decades, the overall trend of PTC incidence has been increasing.<sup>3</sup>

PTC originates from the follicular epithelial cells of endoderm origin. The disease usually progresses slowly and the prognosis is relatively good.<sup>4</sup> At present, the treatment methods for PTC mainly include surgical total thyroidectomy/subtotal thyroidectomy, radiofrequency ablation of radioactive  $I^{131}$  and thyroid hormone therapy, etc., all of which can achieve varying degrees of effect.<sup>5</sup> Studies have found that although PTC is often inert, vascular invasion is rare, but the

rate of lymph node metastasis is high, and some PTC cases have the risk of progression and/or recurrence.<sup>6,7</sup> Regional lymph node metastasis is present in 40–90% of PTC patients upon diagnosis, and 15% of cases with lymph node metastasis exhibit aggressive tumor behavior, which is reflected in regional invasion, distant metastasis, treatment tolerance, and increased mortality.<sup>8</sup> Papillary thyroid microcarcinoma (PTMC) is a PTC with the largest tumor diameter  $\leq 1$  cm, and its incidence is about 50% of the total incidence.<sup>9</sup> Most PTMC patients have low malignant degree and slow progression, but some of them show highly invasive manifestations, the most common being lymph node metastasis in the lateral neck.<sup>10</sup> Surgery is the main treatment for PTC, however, prophylactic lymph node dissection is controversial. In some patients without lymph node metastasis, routine prophylactic lymph node dissection may result in hypoparathyroidism and recurrent laryngeal nerve injury, while pure thyroidectomy in high-risk patients may leave metastatic lymph nodes. In order to selectively perform preventive lymph node dissection in high-risk patients, it is important to identify predictors of lymph node metastasis in patients with PTMC.

Mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway is a highly conserved receptor protein kinase signaling pathway in mammals.<sup>11</sup> MAPK/ERK plays an important role in cell proliferation, differentiation, cell motility and apoptosis, is one of the most important oncogenic signaling pathways related to tumors.<sup>12</sup> The serine/threonine kinase BRAF is an intracellular effector of the MAPK signaling cascade, which helps to carry signals from the extracellular to the nucleus.<sup>13</sup> BRAF is encoded by the proto-oncogene *BRAF* gene, is a member of the RAF protein kinase family.<sup>14</sup> *BRAF* gene mutation causes abnormal activation of MAPK/ERK signaling pathway, which leads to abnormal cell differentiation and proliferation, and is associated with poor prognosis of thyroid cancer.<sup>15</sup> *BRAF* V600E is the replacement of thymine (T) at site 1799 by adenine (A), resulting in the conversion of valine (V) to glutamic acid (E) at the translated amino acid 600 position. *BRAF* V600E mutation account for more than 80% of all *BRAF* gene mutations.<sup>16</sup> Studies on the relationship between *BRAF* V600E mutation and the clinicopathologic features of PTC, risk of lymph node metastasis in PTMC have shown inconsistent results.<sup>17</sup> We conducted a study of consecutive case series of PTC from a hospital in Meizhou, China, in order to evaluate the association between *BRAF* V600E mutation and clinicopathological features of PTC.

## Materials and Methods

### Study Design

In this retrospective analysis, clinicopathological data of the patients were collected, and molecular testing was done for *BRAF* V600E mutation. PTC patients are divided into  $PTC \leq 1.0$ cm (PTMC) and  $PTC > 1.0$ cm, and the relationship between of *BRAF* V600E mutation and clinicopathologic features was analyzed, respectively.

### Subjects

A total of 520 PTC patients were recruited from Meizhou People's Hospital, between January 2018 and December 2021. Inclusion criteria: (1) the patients had complete demographic and clinical data; (2) histologically confirmed diagnosis met the diagnostic criteria for PTC; (3) the tumor localization, size, disease stage, Hashimoto's thyroiditis, calcification, lymph node metastasis and other conditions of PTC have been determined through clinical examination. Exclusion criteria: (1) patients without PTC; (2) patients with dysfunction of vital organs; (3) patients with serious cardiovascular and cerebrovascular diseases, autoimmune diseases, pregnancy, etc. This study was supported by the Ethics Committee of the Meizhou People's Hospital.

### *BRAF* V600E Mutation Analysis

Ten pieces of formalin-fixed and paraffin-embedded (FFPE) slices (5  $\mu$ m thick per slice) were placed into a 1.5mL EP tube. After FFPE slices were deparaffinized, DNA was extracted by Tissue DNA separation Kit (Amoy Diagnostics, Xiamen, China). *BRAF* V600E mutation was detected by real-time amplification refractory mutation system (ARMS)-PCR with the *BRAF* V600E Mutation Fluorescence PCR Diagnostic Kit (Amoy Diagnostics, Xiamen, China). PCR was performed with the following procedure: 95°C for 5 min, followed by 15 cycles (95°C for 25s, 64°C for 20s and 72°C for 20s) and 31 cycles (95°C for 25s, 60°C for 35s and 72°C for 20s). When the internal control signal of the sample to be

tested should have an obvious amplification curve and the cycle threshold (Ct) value is 13–21, if the FAM signal has an obvious amplification curve and Ct <28, *BRAF* V600E was positive, if Ct ≥28, *BRAF* V600E was negative.

## Statistical Analysis

Relevant information and medical records of these participants were collected. Clinical information, including gender, age, tumor localization, maximum tumor diameter, disease stage, Hashimoto's thyroiditis, and calcification was collected. SPSS Statistical Software Version 21.0 (IBM Inc., State of New York, USA) was used for data analysis. Association between *BRAF* V600E mutation status and the clinical features of PTC patients was evaluated by Fisher's exact test. Logistic regression analysis was applied to assess the interactions between *BRAF* V600E mutation and these covariates in risk assessment of lymph node metastasis of PTMC.  $P < 0.05$  was set as statistically significant.

## Results

### Association Between *BRAF* V600E Mutation and Clinicopathological Features of PTC Patients

Of the 520 PTC patients, 432 (83.1%) were female and 416 (80.0%) were younger than 55 years of age. There were 178 (34.2%), 232 (44.6%) and 110 (21.2%) patients with left, right and bilateral thyroid cancer, respectively. The main disease stage was I–II ( $n = 470$ , 90.4%). There were 64 cases (12.3%) with Hashimoto's thyroiditis and 176 cases (33.8%) with calcification (Table 1).

*BRAF* V600E mutation was detected in 422 (81.2%) tumour samples of PTC. There was no significant difference in the frequency of *BRAF* V600E mutation between different age groups (Figure 1). There was no statistically significant association between *BRAF* V600E mutation and some clinicopathological features of PTC (such as gender, age, history of smoking, history of alcohol consumption, tumor maximum diameter, disease stage, T-stage, Hashimoto's thyroiditis, and calcification). In addition, *BRAF* V600E mutation was significantly associated with bilateral cancer (23.9% vs 9.2%,  $P = 0.004$ ). *BRAF* V600E mutation was more common in patients with lymph node metastasis ( $P = 0.037$ ). The *BRAF* V600E mutation was more common in patients with a moderate or high risk of postoperative recurrence ( $P = 0.025$ ) (Table 1).

**Table 1** Association Between *BRAF* V600E Mutation and Clinicopathological Features of PTC Patients

Clinicopathological Features	Total (n=520)	<i>BRAF</i>		P value
		Wild Type (n=98)	V600E (n=422)	
Gender				
Male	88(16.9%)	17(17.3%)	71(16.8%)	1.000
Female	432(83.1%)	81(82.7%)	351(83.2%)	
Age				
<55	416(80.0%)	80(81.6%)	336(79.6%)	0.678
≥55	104(20.0%)	18(18.4%)	86(20.4%)	
History of smoking				
Never	510(98.1%)	97(99.0%)	413(97.9%)	0.696
Ever or Current	10(1.9%)	1(1.0%)	9(2.1%)	
History of alcohol consumption				
Never	514(98.8%)	98(100.0%)	416(98.6%)	0.600
Ever or Current	6(1.2%)	0(0)	6(1.4%)	
Cancer site				
Left side	178(34.2%)	41(41.8%)	137(32.5%)	0.004
Right side	232(44.6%)	48(49.0%)	184(43.6%)	
Both sides	110(21.2%)	9(9.2%)	101(23.9%)	

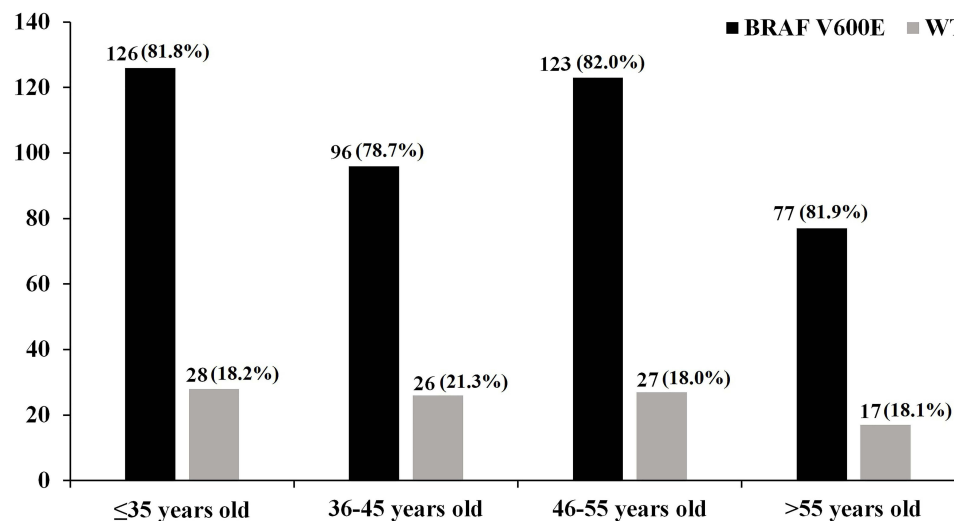
(Continued)

**Table I** (Continued).

Clinicopathological Features	Total (n=520)	BRAF		P value
		Wild Type (n=98)	V600E (n=422)	
Maximum tumor diameter				
≤1cm	250(48.1%)	41(41.8%)	209(49.5%)	0.180
>1cm	270(51.9%)	57(58.2%)	213(50.5%)	
Disease stage				
I-II	470(90.4%)	90(91.8%)	380(90.0%)	0.705
III-IV	50(9.6%)	8(8.2%)	42(10.0%)	
T-stage				
T1-T2	406(78.1%)	80(81.6%)	326(77.3%)	0.416
T3-T4	114(21.9%)	18(18.4%)	96(22.7%)	
N-stage				
N0	194(37.3%)	46(46.9%)	148(35.1%)	0.037
N1	326(62.7%)	52(53.1%)	274(64.9%)	
Hashimoto's thyroiditis				
No	456(87.7%)	83(84.7%)	373(88.4%)	0.393
Yes	64(12.3%)	15(15.3%)	49(11.6%)	
Calcification				
No	344(66.2%)	60(61.2%)	284(67.3%)	0.286
Yes	176(33.8%)	38(38.8%)	138(32.7%)	
Risk of recurrence				
Low	192(36.9%)	47(48.0%)	145(34.4%)	0.025
Moderate	222(42.7%)	31(31.6%)	191(45.3%)	
High	106(20.4%)	20(20.4%)	86(20.4%)	

### Association Between *BRAF* V600E Mutation and Clinicopathological Features of PTMC and PTC Patients with Maximum Tumor Diameter >1.0cm

In this study, there were 250 patients (48.1%) with PTMC. Among them, 208 cases (83.2%) were female and 201 cases (80.4%) were under 55 years old. There were 200 cases (80.0%) of unilateral thyroid cancer and 50 cases (20.0%) of bilateral thyroid cancer; 234 patients (93.6%) with stage I-II; 27 patients (10.8%) with Hashimoto's thyroiditis, and 69 patients (27.6%) with calcification (Table 2). There was no statistically significant association between *BRAF* V600E mutation and

**Figure 1** The frequency of *BRAF* V600E mutation between different age groups.

clinicopathological features of PTMC (such as gender, age, history of smoking, history of alcohol consumption, disease stage, T-stage, Hashimoto's thyroiditis, and calcification). In addition, *BRAF* V600E mutation was significantly associated with bilateral cancer (23.0% vs 4.9%,  $P=0.005$ ), and lymph node metastasis (61.7% vs 39.0%,  $P=0.009$ ) in PTMC patients (Table 2).

There were 270 (51.9%) PTC patients with maximum tumor diameter >1.0 cm in this study. Among them, 224 cases (83.0%) were female and 215 cases (79.6%) were <55 years old. There were 210 cases (77.8%) with unilateral thyroid cancer and 60 cases (22.2%) with bilateral thyroid cancer; 236 patients (87.4%) with stage I–II; 37 patients (13.7%) with Hashimoto's thyroiditis, and 107 patients (39.6%) with calcification (Table 3). There was no statistically significant association between *BRAF* V600E mutation and clinicopathological features of PTMC, including gender, age, history of smoking, history of alcohol consumption, disease stage, T-stage, lymph node metastasis, Hashimoto's thyroiditis, and calcification). In addition, *BRAF* V600E mutation was significantly associated with bilateral cancer (24.9% vs 12.3%,  $P=0.048$ ) in PTC patients with tumor diameter >1.0 cm (Table 3).

**Table 2** Association Between *BRAF* V600E Mutation and Clinicopathological Features of Papillary Thyroid Microcarcinoma (PTMC) ( $\leq 1.0$  cm) Patients

Clinicopathological Features	PTMC ( $\leq 1$ cm) (n=250)	BRAF		P value
		Wild Type (n=41)	V600E (n=209)	
Gender				
Male	42(16.8%)	7(17.1%)	35(16.7%)	1.000
Female	208(83.2%)	34(82.9%)	174(83.3%)	
Age				
<55	201(80.4%)	32(78.0%)	169(80.9%)	0.830
$\geq 55$	49(19.6%)	9(22.0%)	40(19.1%)	
History of smoking				
Never	243(97.2%)	41(100.0%)	202(96.7%)	0.603
Ever or Current	7(2.8%)	0(0)	7(3.3%)	
History of alcohol consumption				
Never	247(98.8%)	41(100.0%)	206(98.6%)	1.000
Ever or Current	3(1.2%)	0(0)	3(1.4%)	
Cancer site				
Unilateral	200(80.0%)	39(95.1%)	161(77.0%)	0.005
Bilateral	50(20.0%)	2(4.9%)	48(23.0%)	
Disease stage				
I–II	234(93.6%)	40(97.6%)	194(92.8%)	0.483
III–IV	16(6.4%)	1(2.4%)	15(7.2%)	
T-stage				
T1–T2	229(91.6%)	39(95.1%)	190(90.9%)	0.543
T3–T4	21(8.4%)	2(4.9%)	19(9.1%)	
N-stage				
N0	105(42.0%)	25(61.0%)	80(38.3%)	0.009
N1	145(58.0%)	16(39.0%)	129(61.7%)	
Hashimoto's thyroiditis				
No	223(89.2%)	33(80.5%)	190(90.9%)	0.058
Yes	27(10.8%)	8(19.5%)	19(9.1%)	
Calcification				
No	181(72.4%)	29(70.7%)	152(72.7%)	0.849
Yes	69(27.6%)	12(29.3%)	57(27.3%)	
Risk of recurrence				
Low	124(49.6%)	27(65.9%)	97(46.4%)	0.058
Medium	92(36.8%)	9(21.9%)	83(39.7%)	
High	34(13.6%)	5(12.2%)	29(13.9%)	

**Table 3** Association Between *BRAF* V600E Mutation and Clinicopathological Features of PTC Patients with Maximum Tumor Diameter >1.0cm

Clinicopathological Features	PTC with >1 cm (n=270)	BRAF		P value
		Wild Type (n=57)	V600E (n=213)	
Gender				
Male	46(17.0%)	10(17.5%)	36(16.9%)	1.000
Female	224(83.0%)	47(82.5%)	177(83.1%)	
Age				
<55	215(79.6%)	48(84.2%)	167(78.4%)	0.363
≥55	55(20.4%)	9(15.8%)	46(21.6%)	
History of smoking				
Never	267(98.9%)	56(98.2%)	211(99.1%)	0.511
Ever or Current	3(1.1%)	1(1.8%)	2(0.9%)	
History of alcohol consumption				
Never	267(98.9%)	57(100.0%)	210(98.6%)	1.000
Ever or Current	3(1.1%)	0(0)	3(1.4%)	
Cancer site				
Unilateral	210(77.8%)	50(87.7%)	160(75.1%)	0.048
Bilateral	60(22.2%)	7(12.3%)	53(24.9%)	
Disease stage				
I-II	236(87.4%)	50(87.7%)	186(87.3%)	1.000
III-IV	34(12.6%)	7(12.3%)	27(12.7%)	
T-stage				
T1-T2	177(65.6%)	41(71.9%)	136(63.8%)	0.276
T3-T4	93(34.4%)	16(28.1%)	77(36.2%)	
N-stage				
N0	89(33.0%)	21(36.8%)	68(31.9%)	0.527
N1	181(67.0%)	36(63.2%)	145(68.1%)	
Hashimoto's thyroiditis				
No	233(86.3%)	50(87.7%)	183(85.9%)	0.831
Yes	37(13.7%)	7(12.3%)	30(14.1%)	
Calcification				
No	163(60.4%)	31(54.4%)	132(62.0%)	0.360
Yes	107(39.6%)	26(45.6%)	81(38.0%)	
Risk of recurrence				
Low	68(25.2%)	20(35.1%)	48(22.5%)	0.120
Medium	130(48.1%)	22(38.6%)	108(50.7%)	
High	72(26.7%)	15(26.3%)	57(26.8%)	

## Logistic Regression Analysis of Risk Factors of Lymph Node Metastasis of PTC

To investigate the effect of *BRAF* V600E mutation, gender, age, bilateral, Hashimoto's thyroiditis, and calcification on lymph node metastasis in all PTC, PTMC, and PTC patients with >1.0cm, respectively, we performed a univariate analysis to measure the association between these parameters and the presence of lymph node metastasis. The results showed that younger age (<55 years old) (OR: 2.385, 95% CI: 1.261–4.509,  $P=0.007$ ), bilateral (OR: 2.147, 95% CI: 1.091–4.226,  $P=0.027$ ), and *BRAF* V600E mutation (OR: 2.520, 95% CI: 1.268–5.007,  $P=0.008$ ) were significantly associated with lymph node metastasis in PTMC, but not PTC patients with >1.0cm. Finally, we performed a multivariate regression logistic analysis in order to investigate whether these features could be considered as independent predictors of lymph node metastasis. After adjusting for gender, Hashimoto's thyroiditis and calcification, we found that younger age (<55 years old) (OR: 2.384, 95% CI: 1.241–4.579,  $P=0.009$ ) and *BRAF* V600E mutation (OR: 2.213, 95% CI: 1.085–4.512,  $P=0.029$ ) were still significantly associated with lymph node metastasis in PTMC (Table 4).

**Table 4** Logistic Regression Analysis of Risk Factors of Lymph Node Metastasis

Variables	All PTC				PTMC ( $\leq 1.0$ cm)				PTC with $> 1.0$ cm			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Gender (Female/Male)	0.906(0.465–1.768)	0.773	0.966(0.487–1.919)	0.922	0.728(0.366–1.450)	0.367	0.663(0.318–1.381)	0.272	1.103(0.566–2.152)	0.773	1.035(0.521–2.055)	0.922
Age ( $<55/\geq 55$ , years old)	1.623(0.882–2.984)	0.119	1.640(0.876–3.071)	0.122	2.385(1.261–4.509)	0.007	2.384(1.241–4.579)	0.009	1.623(0.882–2.984)	0.119	1.640(0.876–3.071)	0.122
Cancer site (Bilateral/Unilateral)	1.320(0.703–2.477)	0.388	1.262(0.666–2.389)	0.475	2.147(1.091–4.226)	0.027	1.982(0.971–4.042)	0.060	1.320(0.703–2.477)	0.388	1.262(0.666–2.389)	0.475
Hashimoto's thyroiditis (No/Yes)	1.282(0.625–2.632)	0.498	1.338(0.645–2.774)	0.434	1.322(0.594–2.944)	0.494	1.201(0.512–2.819)	0.674	1.282(0.625–2.632)	0.498	1.338(0.645–2.774)	0.434
Calcification (Yes/No)	0.950(0.566–1.595)	0.847	1.001(0.591–1.695)	0.998	0.848(0.485–1.483)	0.563	0.807(0.447–1.459)	0.478	0.950(0.566–1.595)	0.847	1.001(0.591–1.695)	0.998
BRAF V600E mutation (Yes/No)	1.244(0.676–2.290)	0.483	1.257(0.675–2.341)	0.472	2.520(1.268–5.007)	0.008	2.213(1.085–4.512)	0.029	1.244(0.676–2.290)	0.483	1.257(0.675–2.341)	0.472

**Abbreviations:** OR, odds ratio; CI, confidence interval.



## Discussion

In this study, 520 patients with PTC were collected to analyze the relationship between the *BRAF* V600E mutation status and the clinicopathologic features of the patients. Risk factors for lymph node metastasis in all PTC, PTMC, and PTC>1.0cm patients were also analyzed respectively. The results showed that younger age (<55 years old) and *BRAF* V600E mutation was independent risk factor for lymph node metastasis in PTMC. *BRAF* gene is an important member of RAF protease family. *BRAF* V600E mutation has been considered as an important molecular marker of PTC,<sup>14</sup> but the mutation rate of *BRAF* V600E in PTC varies in different countries, ranging from about 30% to 90%.<sup>18–20</sup> In a study from Korea, the prevalence of the *BRAF* V600E mutation was 84.0%.<sup>21</sup> *BRAF* V600E mutation prevalence was 38.46% in PTC in a Filipino population.<sup>22</sup> The frequency of *BRAF* V600E mutation increased with age,<sup>23</sup> but not showed in this study. *BRAF* V600E is considered to have a higher specificity in differentiating PTC from benign lesions.<sup>24</sup> Several studies have reported on the relationship between *BRAF* gene mutation status and the clinicopathologic features of PTC, but their conclusions are not entirely consistent.

In this study, *BRAF* V600E mutation was significantly associated with bilateral cancer and lymph node metastasis in PTC patients, but not gender, age, tumor localization, maximum tumor diameter, disease stage, Hashimoto's thyroiditis, and calcification. *BRAF* V600E mutation was significantly associated with the bilaterality of PTC,<sup>25</sup> our results are consistent with this study. In addition, no significant association between *BRAF* V600E mutation and age, gender and tumor localization was detected.<sup>26,27</sup> Studies showed that *BRAF* V600E mutation was associated with lymph node metastasis but not with other clinicopathological features.<sup>28,29</sup> *BRAF* V600E mutation was not associated with central lymph node metastasis in PTC patients.<sup>30</sup> In addition, *BRAF* V600E mutation was associated with age,<sup>31,32</sup> advanced tumor stage,<sup>33,34</sup> and a reduced prevalence of Hashimoto's thyroiditis<sup>34</sup> in PTC patients, however, similar results were not obtained in this study.

PTC is characterized by early lymph node metastasis, among which occult metastasis is the direct cause of recurrence, which greatly increases the probability of recurrent laryngeal nerve and parathyroid gland injury.<sup>35</sup> Surgery is the main treatment for PTC patients, however, prophylactic lymph node dissection is controversial. In some patients without lymph node metastasis, routine prophylactic lymph node dissection may result in hypoparathyroidism and recurrent laryngeal nerve injury, while pure thyroidectomy in high-risk patients may leave metastatic lymph nodes. PTMC is one of the subtypes of PTC, although most of them are less invasive and have a well prognosis, the incidence of lymph node metastasis is still not low. Some studies have confirmed that the incidence of central lymph node metastasis and cervical lymph node metastasis in PTMC patients was 30.2–48.6% and 4.6%–12.2%, respectively.<sup>36–38</sup> However, whether there are some factors affecting lymph node metastasis in PTMC has not been fully elucidated.

In this study, *BRAF* V600E mutation was an independent risk factor for lymph node metastasis in PTMC. *BRAF* V600E mutation was related to the lymph node metastasis of PTMC.<sup>39–42</sup> The *BRAF* V600E mutation was associated with central lymph node metastasis when the tumor was less than 0.5 cm.<sup>43</sup> In addition, *BRAF* V600E mutation was associated with lymph node metastasis of PTMC, however the proportion of lymph node metastasis in PTMC patients with *BRAF* V600E mutation and Hashimoto's thyroiditis is significantly reduced.<sup>44</sup> Moreover, *BRAF* V600E mutation was not associated with lymph node metastasis of PTMC.<sup>45–48</sup> Younger age (<55 years old) was an independent risk factor for lymph node metastasis in PTMC in this study. Studies have showed that age  $\leq 30$  years old,<sup>43</sup> age < 45 years old,<sup>36,49,50</sup> < 50 years old<sup>51</sup> and < 55 years old<sup>52,53</sup> was an independent predictor of central lymph node metastasis. There are also studies that have found that age was not associated with lymph node metastasis of PTMC.<sup>54,55</sup> On the contrary, LNM was more common in older patients (age >55 years old,<sup>56</sup> age  $\geq 45$  years old<sup>57</sup>). In addition, independent risk factors for LNM in patients with PTMC included age <45 years, nodule size  $\geq 6$ mm, tall cell variant of PTC, extrathyroidal extension, and angioinvasion.<sup>58</sup> Bilaterality and gross extrathyroidal extension (ETE) were independent influencing factors of LNM in PTMC patients.<sup>59</sup> Calcification,<sup>52,60</sup> male,<sup>50,53,61</sup> and bilaterality<sup>62</sup> have been found to be risk factors for lymph node metastasis, however, similar results were not obtained in this study.

In tumor-node-metastasis (TNM) classification, *BRAF* V600E is not related to T-stage except for N-stage. There was no correlation between *BRAF* V600E mutation and T stage or disease stage of PTC. A study has showed that *BRAF* V600E mutation was associated with T4 stage.<sup>31</sup> It suggests that the TNM stage and disease stage of PTC may also be related to other factors, except for the mutation status of *BRAF* gene. In patients with thyroid carcinoma, the expression



of naked cuticle homolog 2 (NKD2) gradually increases with the increase of TNM classification, and the high expression of NKD2 may be related to the progression and poor prognosis of thyroid carcinoma.<sup>63</sup> The patients with higher expression levels of programmed death ligand 1 (PD-L1) and phosphoinositide-dependent protein kinase 1 (PDK1) had higher rates of TNM III–IV, lymph node metastasis, and recurrence.<sup>64</sup> PD-1 is a cell surface inhibitory receptor, which plays an important physiological role in the maintenance of peripheral tolerance and characteristics of tumor cells.<sup>65</sup> PD-L1 can be used as a potential prognostic biomarker for disease recurrence in PTC patients.<sup>66</sup> At present, oncologists and pathologists are constantly improving indications, scoring and reporting systems for PD-L1 immunohistochemical tests.<sup>67</sup> In addition, study has also shown that TNM stage is related to noncoding RNA.<sup>68</sup>

In summary, the differences in these results may be related to the differences in sample size and tumor heterogeneity, and the relationship between *BRAF* V600E and clinical features needs to be further verified with the data from multiple populations and multiple research centers. This study had several limitations. First, as a single-center study taking place in urban Meizhou in China, which is limited by geographical selection bias and small number of study cases. Future studies should include patients from multiple institutions. Second, some patients with thyroid nodules  $\leq 1.0$  cm were not biopsied. Therefore, the conclusion that PTMC carrying the *BRAF* V600E mutation has a higher risk of lymph node metastasis may not fully represent the true situation. Third, this study only tested for the *BRAF* V600E mutation and did not analyze other mutations. There is evidence suggesting other gene mutations, such as tumor protein p53 (*TP53*) and telomerase reverse transcriptase (*TERT*) mutations,<sup>69</sup> contribute even more to lymph node metastasis. Future studies should analyze more genetic variants and the interaction between these genetic variations. Lastly, this study could not evaluate the long-term clinical outcomes of PTC patients with the *BRAF* V600E mutation. In the future, the prognostic value of the *BRAF* V600E mutation should be further evaluated based on long-term outcomes.

In conclusion, younger age (<55 years old) and *BRAF* V600E mutation was independent risk factor for lymph node metastasis in PTMC. In other words, lymph node dissection and routine excision of lymph and adipose tissue are important for young PTMC patients with preoperative *BRAF* V600E mutation. However, the need for prophylactic lymph node dissection in the patients with *BRAF* V600E mutation and with tumor diameter >1.0cm needs to be reassessed. On the other hand, the results of this study also suggest that molecular detection of the *BRAF* gene can help guide treatment decisions and identify PTMC at high risk of lymph node metastasis.

## Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Medicine, Meizhou People's Hospital. All participants signed informed consent in accordance with the Declaration of Helsinki.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that they have no competing interests.

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