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ORIGINAL ARTICLE

Associations of the BRAF V600E Mutation and PAQR3 Protein Expression with Papillary Thyroid Carcinoma Clinicopathological Features

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Abstract

The $BRAF^{V600E}$ mutation is the most prevalent genetic event in patients with papillary thyroid cancer (PTC). However, no study has investigated the expression of PAQR3 in papillary thyroid tissues in relation to the $BRAF^{V600E}$ mutation and the clinicopathological features of PTC patients. Furthermore, the potential associations of the $BRAF^{V600E}$ mutation, PAQR3 expression and clinicopathological parameters in the cancerous tissues of PTC patients have not been investigated. This study was conducted on 60 patients with PTC who were treated surgically at our institution from 2017 to 2018. PCR was used to amplify DNA by the amplification refractory mutation system (ARMS) method to detect $BRAF^{V600E}$ gene mutations. In addition, immunohistochemical techniques were utilized to assess PAQR3 expression in tumor tissue sections. The $BRAF^{V600E}$ mutation was associated with lymph node metastasis (LNM, $p < 0.05$) but not with other clinicopathological features. Low PAQR3 expression was associated with extrathyroidal extension and LNM ($\chi^2 = 7.143$, $p = 0.009$; $\chi^2 = 6.459$, $p = 0.014$, respectively). Furthermore, a statistically significant association was observed between chronic lymphocytic thyroiditis and LNM ($\chi^2 = 5.275$, $p = 0.0250$). A linear relationship between the $BRAF^{V600E}$ mutation and PAQR3 protein expression has not been identified. These factors may be independent risk factors of extrathyroidal extension and LNM in PTC and be used to indicate the invasiveness of PTC tumors. Higher quality, multivariate analyses based on larger samples from around the world are urgently needed to further validate and revise our findings in the future.

Keywords BRAF gene · PAQR3 · Thyroid cancer · Papillary thyroid microcarcinoma · Lymph node metastasis (LNM) · Extrathyroidal extension

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Background

Papillary thyroid carcinoma (PTC) is the most frequent type of thyroid cancer and accounts for approximately 80% of all thyroid cancer cases [1]. PTCs are associated with activating mutations of genes encoding RET or TRK tyrosine kinase receptors as well as RAS genes [2]. Among these genetic abnormalities, BRAF mutations are the most common genetic alteration in thyroid cancer, occurring in approximately 45% of sporadic PTCs [3–5]. Oncogenic activation of the protein kinase BRAF drives tumor growth by promoting mitogen-activated protein kinase (MAPK) signaling [6, 7].

PAQR3, also named Raf kinase trapping to Golgi (RKTG), belongs to the progesterone and adipoQ receptor (PAQR) superfamily [8]. PAQR3 is a type III membrane protein whose N-terminus faces the cytosol, and multiple sequences are responsible for its localization to the Golgi apparatus and Raf-1 interaction. The PAQR3 N-terminus faces one side of the

cytoplasm, while the C-terminus is directed toward the inner cavity of the Golgi matrix, which is responsible for the transmission and regulation of intracellular signals. Because it was initially found to anchor intracellular free Raf kinases to Golgi bodies, RKTG changes the subcellular localization of Raf-1 to the Golgi apparatus and consequently represses Ras/MAPK signaling [9]. Overexpression of PAQR3 inhibits the ERK activation, cell proliferation and transformation of A375 human multiple myeloma (MM) cells that bear the BRaf ^{V600E} mutation, the most common mutation in melanoma [10]. An increasing number of scientific studies have suggested that PAQR3 functions as a tumor suppressor by regulating the biological behaviors of numerous human cancers, including esophageal squamous cell carcinoma [11], human gastric cancers [12] and osteosarcoma [13]. Aberrant de novo or decreased PAQR3 expression has been correlated with the progression and prognosis of human gastric cancers [12], breast cancers [14], hepatocellular carcinoma [15], and esophageal squamous cell carcinoma [16].

However, to our knowledge, the relationship among PAQR-3 expression, the BRAF^{V600E} mutation status and the clinicopathological features of Chinese patients with PTC has not been studied. This study aimed to investigate the incidence of BRAF gene mutations and PAQR3 in patients with PTC and to explore the potential diagnostic and therapeutic value in thyroid cancer.

Materials and Methods

Patients and Analysis of Clinicopathological Parameters

Thyroid tissue specimens (tumor tissues and adjacent thyroid tissues) were collected from PTC patients who underwent surgical treatment at The First Affiliated Hospital of USTC from 2017 to 2018. Patients included in the study were required to meet the following criteria: i) no previous history of thyroid disease or any other common cancer; ii) a final pathological diagnosis of papilla thyroid carcinoma or thyroid micropapilla carcinoma; iii) complete clinical trial data; iv) received either a bilateral or unilateral thyroidectomy and underwent preventive or curative central lymph node dissection (CLND); and v) therapeutic lateral lymph node dissection, including levels II, III, IV and V, in the case of lateral cervical lymph node metastasis (LNM). All protocols were approved by the institutional review board. The patient information and clinicopathological parameters, such as the patient's age at onset, tumor size, lymphocytic thyroiditis, multifocality, extrathyroidal invasion, and presence of LNM, were analyzed retrospectively.

DNA Extraction

DNA was extracted from paraffin sections. Three to five formalin-fixed, paraffin-embedded (FFPE) sections (5–10 μm thick) were obtained from each subject. Tissue sections were deparaffinized by xylene and ethanol and digested overnight at 60 °C in 180 μL of Buffer DTL and 20 μl of Proteinase K Solution. DNA was extracted using the AmoyDx® FFPE DNA Kit (Amoy Diagnostics, China, ADx-FF01) according to the manufacturer's instructions. The DNA samples were held at room temperature for 5 min and then collected by centrifugation and stored at −80 °C until use.

Real-Time PCR-Based Assay to Detect the BRAF^{V600E} Mutation

The DNA samples were fully melted at room temperature and then centrifuged at 12,000×g for 15 s at 4 °C. The sample reaction mixture comprised 35 μl of V600E Reaction Mix and 0.4 μl of BRAF Taq DNA Polymerase. We transferred 35 μl of the above solution mixture into the appropriate PCR tubes, and 5 μL of the DNA sample or 5 μL of ddH2O (no-template control, NTC) was added to the appropriate PCR tubes. For paraffin-embedded samples, we used 10~15 ng of template DNA (2~3 ng/μl) in each PCR tube depending on the storage time needed. The tubes were briefly centrifuged and placed into the real-time PCR instrument. The cycling program was executed according to the manufacturer's instructions (AmoyDx BRAF^{V600E} Mutation Detection Kit, Amoy Diagnostics, China, ADx-BR01/BR02).

Immunochemical Staining and Evaluation

According to the manufacturer's protocol, immunohistochemistry was performed with a PAQR3 primary antibody (sc515831HRP; Santa Cruz, CA, USA) that was diluted 1:100. The results were determined using a blind method, and each slice was counted by two pathologists. The immunoreactive score (IRS) was calculated according to the staining intensity (SI) multiplied by the percentage of stained positive cells (PP). The detailed score standards were as follows: SI (ranging from 0 to 3 scores): 0 was negative, 1 was weak, 2 was moderate and 3 was strong; the percent positivity was scored as '0' (<5%, negative), '1' (5–25%, sporadic), '2' (>25–50%, focal), and '3' (>50%, diffuse). The IRS score ranged from 0 to 9. High PAQR3 expression was defined as IRS > 3, and low PAQR3 expression was defined as IRS ≤ 3.

Statistical Analysis

SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA) was used to perform statistical analyses. Quantitative data are expressed as the mean ± standard deviation (SD). Pearson's chi-square

test or Fisher's exact test was used to determine the relationship between PAQR3 expression and the clinicopathological factors of PTC patients. Associations between the $BRAF^{V600E}$ mutation status and PAQR3 expression were analyzed using the Kruskal-Wallis test or the chi-square test, and correlations between LNM and clinicopathological factors were analyzed using the chi-square test. $P < 0.05$ was regarded as statistically significant.

Results

Associations Between the $BRAF^{V600E}$ Status and Clinicopathological Features of PTC

As shown in Table 1, the expression of genes with the $BRAF^{V600E}$ mutation was detected. Among 60 Chinese patients with PTC, 39 had the $BRAF^{V600E}$ mutation, accounting for 65% of the study population; 11 were males (28%) and 28 were females (72%), with a mean age of 41.8 ± 10.9 years. The mean tumor size was 16.9 ± 9.6 mm. Multiplicity and extrathyroidal extensions were present in 13 (33.3%) and 15 (38.5%) out of the 39 cases, respectively. Among the 21 patients without the $BRAF^{V600E}$ mutation, 4 were males (19.0%) and 17 were females (80.9%), with a mean age of 45.5 ± 12.8 years. The mean tumor size was 14.2 ± 9.2 mm. Multiplicity and extrathyroidal extensions were present in 6 (28.6%) and 10 (47.6%) of the 21 cases, respectively (Table 1). As partially shown in Table 1, the age distribution, gender, tumor size, extrathyroid extension, multifocality and lymphocytic thyroiditis did not differ significantly between patients with and without the $BRAF^{V600E}$ mutation. The figures below show a negative (wild-type BRAF, Fig. 1a) and positive result (mutant-type BRAF, Fig. 1b).

Association Between PAQR3 Expression and Clinicopathological Parameters in Patients with Thyroid Cancer

Analyses of the relationship between PAQR3 expression and various clinicopathological parameters are shown in Table 2. The expression level of PAQR3 was associated with a few patient clinical characteristics (Table 2). The PAQR3 expression status in PTC samples was significantly correlated with the extrathyroidal extension of the tumor ($p < 0.009$). The absence of PAQR3 expression as detected by immunohistochemical analysis was closely correlated with extrathyroid extension. No statistically significant relationships between PAQR3 expression and age, tumor size or multiplicity were observed. The diagnoses of all PTCs in our study were confirmed by hematoxylin and eosin (HE) staining after the operation (Fig. 2a). Immunohistochemical analysis results (Fig. 2b–d) showed that PAQR3 mainly appeared in the cytoplasm and was brown.

Table 1 Associations between the $BRAF^{V600E}$ mutation and clinicopathological features of papillary thyroid carcinomas

Parameter	BRAF mutation + (%)	BRAF mutation -(%)	χ^2/t value	*P* value
No. of cases	39(65.0)	21(35.0)		
Age, years	41.8 ± 10.9	45.5 ± 12.8	t = 1.172	0.246[b]
Age at diagnosis, y			$\chi^2 = 1.425$	0.284[b]
Patients <45, $n = 32$	23(71.9)	9(28.1)		
Patients ≥45, $n = 28$	16(57.1)	12(42.9)		
Gender			$\chi^2 = 0.611$	0.541[b]
Male	11(73.3)	4(26.7)		
Female	28 (62.2)	17(37.8)		
Tumor size (mm)	16.9 ± 9.6	14.2 ± 9.2	t = 1.069	0.290[b]
Multifocality			$\chi^2 = 0.143$	0.778[b]
Present	13(68.4)	6(31.6)		
Absent	26(63.4)	15(36.6)		
Extrathyroidal-extension			$\chi^2 = 0.471$	0.587[b]
Present	15(60.0)	10(40.0)		
Absent	24(68.6)	11(31.4)		
Lymphocytic thyroiditis			$\chi^2 = 0.012$	0.775[b]
Present	14(70.0)	6(30.0)		
Absent	25(62.5)	15(37.5)		

[a] $P < 0.05$, statisticalsignificance

[b] $P > 0.05$, no statistical significance

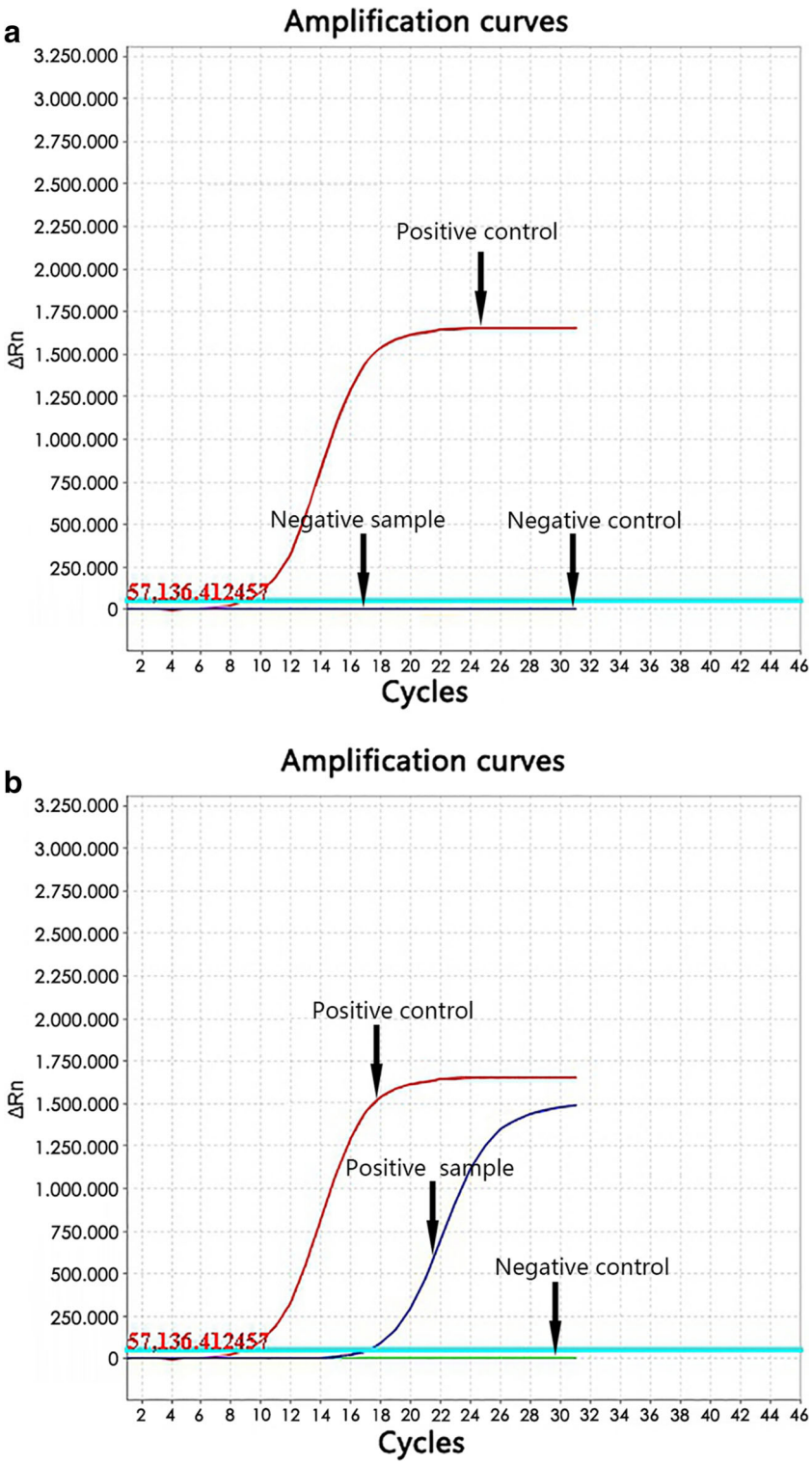

Fig. 1 **a** Sample curve with the wild-type BRAF gene; **b** Sample curve with the mutant BRAF gene

Relationship Between the Clinicopathological Parameters and LNM

The incidence rate of LNM was 65% in patients with PTC. The LNM rate was significantly increased in patients with lymphocytic thyroiditis ($p < 0.05$) and in patients with mutant BRAF tumors ($p < 0.01$). The expression of PAQR3 was negatively correlated with LNM ($p < 0.05$). Among 39 patients with LNM as confirmed by pathology, 28 (71.8%) had low PAQR3-expressing tumors. However, the LNM in PTC was not closely related to gender, patient age, tumor size, multifocality,

Table 2 The relationships between PAQR3 protein expression and the clinicopathological features in the papillary thyroid carcinomas

Clinical parameter	PAQR3 expression(%)			
	Low expression	High expression	χ^2/t value	P value
No. of cases	36(60.0)	24(40.0)		
Age (years)			$\chi^2 = 0.179$	0.793[b]
Patients <45	20(62.5)	12(37.5)		
Patients ≥45	16(57.1)	12(42.9)		
Gender			$\chi^2 = 1.481$	0.362[b]
Male	11(73.3)	4(26.7)		
Female	25(55.6)	20(44.4)		
Tumor size (mm)	17.3 ± 10.4	13.9 ± 7.6	t = 1.381	0.173[b]
Multiplicity			$\chi^2 = 1.849$	0.257[b]
Present	9(47.4)	10(52.6)		
Absent	27(65.9)	14(34.1)		
Extrathyroidal-extension			$\chi^2 = 7.143$	0.009[a]
Present	20(80.0)	5(20.0)		
Absent	16(45.7)	19(54.3)		

[a] $P < 0.05$, statistical significance

[b] $P > 0.05$, no statistical significance

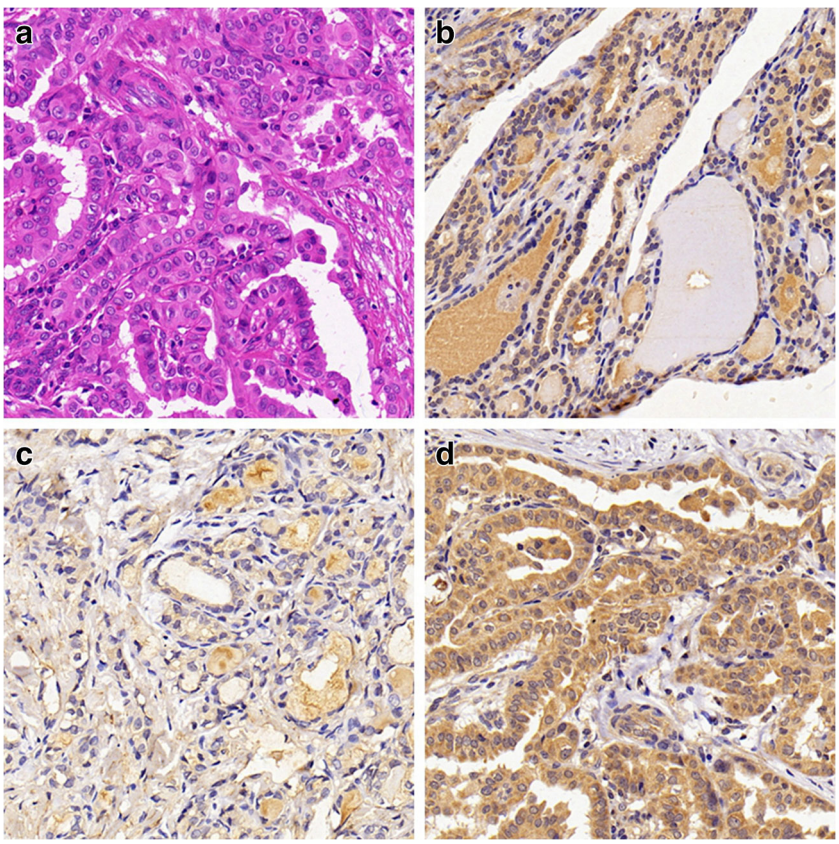

Fig. 2 **a** PTC tissues without immunohistochemical staining; **b** PAQR3 protein expression in peritumoral tissues; **c** Low PAQR3 protein expression in PTC; **d** High PAQR3 protein expression in PTC(A, B, C and D-original magnification: 400×)

bilaterality or extrathyroidal extension. The $BRAF^{V600E}$ gene mutation was positively correlated with LNM ($\chi 2 = 10.28$, $p = 0.002$, Table 3).

Association Between the $BRAF^{V600E}$ Mutation and PAQR3 Expression in the PTC Group

We successfully detected BRAF mutations and PAQR3 expression in 60 patients with thyroid cancer. Thirty-nine out of 60 PTCs (65%) were positive for the $BRAF^{V600E}$ mutation. PAQR3 expression was not significantly associated with the presence of the $BRAF^{V600E}$ mutation ($p > 0.05$), with low expression being found in 21 out of 39 BRAF-mutated PTCs (53.8%) and in 15 out of 21 BRAF wild-type PTCs (71.4%). The correlation coefficient for the association of the two different factors was 0.123 ($p = 0.270$, Table 4).

Discussion

BRAF is a serine/threonine kinase that functions within the Ras-Raf-MEK-MAPK pathway. This pathway normally regulates cell proliferation and survival under the control of growth factors and hormones. Mutations in the BRAF gene have been associated with the development of cancer. Approximately 89% of BRAF mutations are missense mutations that introduce an amino acid substitution at the valine 600 (V600) residue, and approximately 92% of BRAF V600 mutations are V600E (valine to glutamic acid). These mutations cause permanent activation of the BRAF protein even in the absence of growth factors, resulting in excessive cell proliferation and resistance to apoptosis. The $BRAF^{V600E}$ mutation occurs in 40% of papillary thyroid tumors. The search for drugs that block oncogenic BRAF signaling is an active area of pharmaceutical research and development. More recent

Table 3 Associations between lymph node metastasis and clinicopathological parameters of papillary thyroid carcinomas

Parameter	Lymph node metastasis no.(%)		χ^2/t value	P value
	Yes	N0		
No. of cases	39(65)	21(35)		
Age (years)			$\chi^2 = 1.425$	0.284[b]
Patients <45	23(71.9)	9(28.1)		
Patients ≥45	16(57.1)	12(42.9)		
Gender			$\chi^2 = 0.024$	1.000[b]
Male	10(66.7)	5(33.3)		
Female	29(64.4)	16(35.6)		
Tumor size (mm)	12.9 ± 7.7	17.6 ± 10.0	t = −1.910	0.061[b]
Multifocality				
Present	14(73.7)	5(26.3)	$\chi^2 = 0.922$	0.395[b]
Absent	25(61.0)	16(39.0)		
Bilaterality				
Present	13(72.2)	5(27.8)	$\chi^2 = 0.590$	0.560[b]
Absent	26(61.9)	16(38.1)		
Extrathyroidal-extension			$\chi^2 = 0.923$	0.416[b]
Present	18(72.0)	7(28.0)		
Absent	21(60.0)	14(40.0)		
Lymphocytic thyroiditis				
Present	17(85.0)	3(15.0)	$\chi^2 = 5.275$	0.025[a]
Absent	22(55.0)	18(45.0)		
Paqr3 expression			$\chi^2 = 6.459$	0.014[a]
Low expression	28(77.8)	8(22.2)		
High expression	11(45.8)	13(54.2)		
BRAF mutation			$\chi^2 = 10.28$	0.002[a]
Present	31(79.5)	8(20.5)		
Absent	8(38.1)	13(61.9)		

[a] $P < 0.05$, statistical significance

[b] $P > 0.05$, no statistical significance

Table 4 Relationship between the $BRAF^{V600E}$ mutation and PAQR3 expression in the PTC group

Groups	PAQR3				
	Low expression	High expression	Total	P value	K(coefficient)
BRAF gene				0.270[a]	0.123
Mutant type	21	18	39		
Wild-type	15	6	21		
Total	36	24	60		

[a] $P > 0.05$, no statistical significance

studies on select Asian populations have confirmed that the mutation is a common genetic event in these populations.

The prevalence rate of the $BRAF^{V600E}$ mutation varies significantly in different populations, ranging from 29 to 83% in PTC; while the reason for this discrepancy is unclear, geographical, genetic and other factors potentially play roles [17–19]. Our results showed that 65% (39/60) of PTCs harbored the $BRAF^{V600E}$ mutation, which is a moderate incidence rate given the range mentioned above.Numerous studies have evaluated the associations between the BRAFV600E mutation and both clinicopathological factors and poor clinical outcomes in PTC; however, controversial and conflicting results have been obtained for the correlations between the $BRAF^{V600E}$ mutation and clinicopathological factors [20–22].

Furthermore, multiple previous studies have demonstrated associations of the $BRAF^{V600E}$ mutation with an aggressive clinical course and poor clinicopathological features, such as extrathyroidal invasion, LNM, bilaterality and advanced stage [23–26]. However, the clinical significance of this mutation in terms of a prognostic marker in PTC is controversial because several authors reported that this mutation was not correlated with poor clinical outcomes or tumor aggressiveness [27–29]. The reasons for this discrepancy are unclear but may relate to differences in geographic or genetic factors, assay conditions and data analysis. However, the variation in these results may also be due to a lack of prospective studies that reduce the possible impact of selection bias, studies from a single center with small sample sizes, univariate analysis and the various histological subtypes within PTC [30]. Song JY conducted a meta-analysis to evaluate pooled prognostic data and found a significant association between the $BRAF^{V600E}$ mutation and LNM (OR = 1.34; 95% CI: 1.09–1.65; $p = 0.005$) [31]. However, other studies did not demonstrate this correlation. Kurtulmus N et al. indicated that the $BRAF^{V600E}$ mutation was not significantly associated with the presence of LNM ($p = 0.14$), and analysis showed that mutation-positive LNM was significantly associated with an increased metastatic lymph node diameter ($p = 0.01$) [32]. Different institutional surgery protocols for PTC patients may result in bias in demonstrating the association between BRAF mutations and LNM. In the present study, to investigate the prognostic value of the $BRAF^{V600E}$ mutation for LNM, we assessed PTC patients who underwent total thyroidectomy or hemithyroidectomy and routine CLND. We found that 39 of 60 (65%) PTC patients harbored the $BRAF^{V600E}$ mutation, which was not statistically associated with any clinicopathological characteristics except for LNM.

The results of the present study did not demonstrate any significant correlations between the $BRAF^{V600E}$ mutation and the clinicopathological features of PTC, including age, gender, tumor size, multiplicity, LNM and extrathyroidal extension. The conflicting results of these studies might be attributable to variations in the study populations in terms of size, age distribution, histological variants, genetic factors, environmental factors, clinical stage of the PTC patients at the time of initial diagnosis, and methods or criteria used to detect the $BRAF^{V600E}$ mutation [19, 33].

PAQR3, which belongs to the PAQR family, functions as a spatial regulator of RAF1 kinase by sequestrating it to the Golgi. Recent studies have revealed that PAQR3 may play a potential tumor suppressive role in several human tumor types. Studies have confirmed that the expression of PAQR3 was associated with the differentiation degree, lymphatic metastasis and tumor infiltration depth [34]. A study by Zhao C indicated that PAQR3 is a direct target of miR-15b-5p, and overexpression of PAQR3 partially rescued miR-15b-5p-induced cell proliferation, migration and invasion. PAQR3 is a tumor suppressor gene that suppresses proliferation, migration, tumorigenicity, epithelial–mesenchymal transition (EMT) and metastasis in different types of cancers [35].

PAQR3 can also significantly reduce the protein level of Twist1, an essential transcription factor required to initiate EMT and promote tumor cell metastasis and infiltration; this process may be due to PAQR3-inhibited tumor cell metastasis and an infiltration mechanism [36]. PAQR3 is involved in tumor formation and development, participates in the regulation of multiple signaling pathways, and inhibits the invasion and metastasis of tumors. Because PAQR3 plays an important role in tumor suppression, we examined its expression and relationships with the clinicopathological outcomes of thyroid cancer; we also investigated its role in cell invasion as well as the other molecules involved in this process.

Although we did not confirm a correlation between the BRAF mutation and PAQR3 expression, our research showed that they both have certain clinical values in thyroid cancer patients. The V600E mutation of the BRAF gene and the PAQR3 protein can be used to predict extrathyroidal extension and LNM and are thus very valuable for the diagnosis and treatment of thyroid cancer.

In this study, we analyzed PAQR3 expression in a consecutive series of 60 PTCs, focusing on the correlation between PAQR3 and clinicopathological features, such as tumor size, age, gender, multifocality, presence of node metastases, degree of neoplastic infiltration, and presence of lymphocytic thyroiditis. Because the BRAF mutation is considered one of the most important gene alterations found in PTC, we also correlated its activation with the expression of PAQR3. A previous study demonstrated that PAQR3 could bind and translocate cytoplasmic wild-type B-Raf or mutated B-RAF to the Golgi apparatus, resulting in the suppression of B-Raf-stimulated ERK activation. Immunofluorescence and coimmunoprecipitation assays demonstrated that both wild-type B-Raf and B-RafV600E were found to associate with PAQR3 in vitro. PAQR3 may play a suppressive role in human cancers that harbor oncogenic B-Raf mutations via negatively regulating the Ras-Raf-MEK-ERK pathway [10].

In cases with BRAF mutations, PAQR3 seems to negatively regulate the constitutive activation of the RAF/MEK/ERK pathway. In this study, we hypothesized that the common $BRAF^{V600E}$ mutation was associated with PAQR3 expression and proliferation in PTC. In our samples, we found a strong correlation between PAQR3 expression and the degree of neoplastic infiltration. We found low PAQR3 expression to be associated with extrathyroidal extension. We speculate that tumor infiltration of the thyroid capsule may represent a crucial intermediate step in local invasion, and this process may be modulated by PAQR3 expression. More specifically, we found low PAQR3 expression to be associated with both extrathyroidal extension and LNM. Our findings, together with those of other authors, strongly suggest that PAQR3 may be a novel but important molecular marker for PTC patients.

In conclusion, the present study revealed that the $BRAF^{V600E}$ mutation was not significantly correlated with any of the clinicopathological features of PTC except for LNM. Although the $BRAF^{V600E}$ mutation has been reported in 28–70% of PTCs, making it the most frequent genetic abnormality found in PTC, its long-term clinical significance is not fully understood because numerous study results are contradictory. This discrepancy may be due to the heterogeneity of PTC at the molecular level or the overlapping phenotypes due to different genetic variations. Here, we found that PTC patients with LNM expressed lower levels of PAQR3 than those without LNM, and PAQR3 expression was associated with extrathyroidal extension. However, this cross-sectional 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 and low PAQR3 expression should be further evaluated based on long-term outcomes.

Compliance with Ethical Standards

Conflict of Interest Conflict of Interest The authors declare that they have no conflict of interest.

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Disclosure Summary The authors have nothing to disclose.

References

1. Cvejic D, Selemetjev S, Savin S, Paunovic I, Tatic S (2009) Changes in the balance between proliferation and apoptosis during the progression of malignancy in thyroid tumours. Eur J Histochem 53:65–71
2. Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA (2003) High prevalence of braf mutations in thyroid cancer genetic evidence for constitutive activation of the ret/ptc-ras-braf signaling pathway in papillary thyroid carcinoma. Cancer Res 63:1454–1457
3. Gu LQ, Li FY, Zhao L, Liu Y, Zhao YJ (2009) Braf ^{v600e} mutation and x-linked inhibitor of apoptosis expression in papillary thyroid carcinoma. Thyroid 19:347–354
4. Mathur A, Moses W, Rahbari R, Khanafshar E, Duh Q, Clark O et al (2011) Higher rate of braf mutation in papillary thyroid cancer over time. Cancer 117:4390–4395
5. Xing M (2005) Braf mutation in thyroid cancer. Endocr Relat Cancer 12:245–262
6. Whipple CA, Boni A, Fisher JL, Hampton TH, Ernstoff MS (2016) The mitogen-activated protein kinase pathway plays a critical role in regulating immunological properties of BRAF mutant cutaneous melanoma cells. Melanoma Res 26:223–235
7. Ji H, Wang Z, Perera SA et al (2007) Mutations in BRAF and KRAS converge on activation of the mitogen-activated protein kinase pathway in lung cancer mouse models. Cancer Res 67:4933–4939
8. Tang YT, Hu T, Arterburn M, Boyle B, Bright JM, Emtage PC et al (2005) PAQR proteins: a novel membrane receptor family defined by an ancient7-transmembrane pass motif. J Mol Evol 61:372–380
9. Feng L, Xie X, Ding Q, Luo X, He J, Fan F et al (2007) Spatial regulation of Raf kinase signaling by RKTG. Proc Natl Acad Sci 104:14348–14353
10. Fan F, Feng L, He J, Wang X, Jiang X, Zhang Y et al (2008) RKTG sequesters B-Raf to the Golgi apparatus and inhibits the proliferation and tumorigenicity of human malignant melanoma cells. Carcinogenesis 29:1157–1163
11. Bai G, Chu J, Eli M, Bao Y, Wen H (2017) PAQR3 overexpression suppresses the aggressive phenotype of esophageal squamous cell carcinoma cells via inhibition of ERK signaling. Biomed Pharmacother 94:813–819
12. Ling ZQ, Guo W, Lu XX, Zhu X, Hong LL, Wang Z et al (2014) A golgi-specific protein paqr3 is closely associated with the

progression, metastasis and prognosis of human gastric cancers. Ann Oncol 25:1363–1372
13. Ma Z, Wang Y, Piao T, Li Z, Zhang H, Liu Z, Liu J (2015) The tumor suppressor role of PAQR3 in osteosarcoma. Tumor Biol 36: 3319–3324
14. Chen J, Wang F, Xu J, He Z, Lu Y, Wang Z (2016) The role of PAQR3 gene promoter hypermethylation in breast cancer and prognosis. Oncol Rep 36:1612–1618
15. Wu H, Zhang W, Ding Q, Peng G, Zou Z, Liu T et al (2014) Identification of PAQR3 as a new candidate tumor suppressor in hepatocellular carcinoma. Oncol Rep 32:2687–2695
16. Bai G, Yang M, Zheng C, Zhang L, Eli M (2018) Suppressor PAQR3 associated with the clinical significance and prognosis in esophageal squamous cell carcinoma. Oncol Lett 15:5703–5711
17. Sun J, Zhang J, Lu J, Gao J, Liang Z (2016) BRAF V600E and TERT promoter mutations in papillary thyroid carcinoma in Chinese patients. PLoS One 11:e0153319
18. Guo L, Ma YQ, Yao Y et al (2019) Role of ultrasonographic features and quantified BRAFV600E mutation in lymph node metastasis in Chinese patients with papillary thyroid carcinoma. Sci Rep 9:75
19. Li Y, Nakamura M, Kakudo K (2009) Targeting of the BRAF gene in papillary thyroid carcinoma (review). Oncol Rep 22:671–681
20. Nasirden A, Saito T, Fukumura Y, Hara K, Akaike K, Kurisaki-Arakawa A et al (2016) In Japanese patients with papillary thyroid carcinoma, TERTpromoter mutation is associated with poor prognosis, in contrast toBRAFV600E mutation. Virchows Arch 469: 687–696
21. Agnieszka C, Monika K, Dagmara R, Jolanta K, Michal J, Ewa S et al (2015) The risk of relapse in papillary thyroid Cancer (PTC) in the context of BRAFV600E mutation status and other prognostic factors. PLOS ONE 10:e0132821
22. Choi SY, Park HS, Kang MK, Lee DK, Lee KD, Lee HS et al (2013) The relationship between the $BRAF^{V600E}$ mutation in papillary thyroid microcarcinoma and clinicopathologic factors. World J Surg Oncol 11:1–5
23. Liu Z, Lv T, Xie C, Di Z (2018) $BRAF^{V600E}$ gene mutation is associated with bilateral malignancy of papillary thyroid Cancer. Am J Med Sci 356:130–134
24. Qu HJ, Qu XY, Hu Z, Lin Y, Wang JR, Zheng CF et al (2017) The synergic effect of BRAFV600E mutation and multifocality on central lymph node metastasis in unilateral papillary thyroid carcinoma. Endocr J 65:113–120
25. Chakraborty A, Narkar A, Mukhopadhyaya R, Kane S, D'Cruz A, Rajan MGR (2012) BRAF V600E mutation in papillary thyroid carcinoma: significant association with node metastases and extra thyroidal invasion. Endocr Pathol 23:83–93
26. Kang SH, Pyo JY, Yang SW et al (2013) Detection of BRAF V600E mutation with thyroid tissue using pyrosequencing. Am J Clin Pathol 139:759–764
27. Ahn D, Park JS, Sohn JH, Kim JH, Park SK, Seo AN et al (2012) BRAF (V600E) mutation does not serve as a prognostic factor in Korean patients with papillary thyroid carcinoma. Auris Nasus Larynx 39:198–203
28. Kurt B, Yalçın S, Alagöz, Engin et al (2012) The relationship of the BRAF (V600E) mutation and the established prognostic factors in papillary thyroid carcinomas. Endocr Pathol 23:135–140
29. Givens DJ, Buchmann LO, Agarwal AM, Grimmer JF, Hunt JP (2014) BRAF V600E does not predict aggressive features of pediatric papillary thyroid carcinoma. Laryngoscope 124(9):E389–E393
30. Kim SJ, Lee KE, Myong JP, Park JH, Youn YK (2011) BRAF V600E mutation is associated with tumor aggres- siveness in papillary thyroid cancer. World J Surg 36:310–317
31. Song JY, Sun SR, Dong F, Huang T, Wu B, Zhou J (2018) Predictive value of BRAF V600E mutation for lymph node metastasis in papillary thyroid Cancer: a meta-analysis. Curr Med Sci 38: 785–797
32. Kurtulmus N, Ertas B, Saglican Y, Kaya H, Duren M (2016) BRAF V600E mutation: has it a role in cervical lymph node metastasis of papillary thyroid Cancer? Eur Thyroid J 5:195–200
33. Tang KT, Lee CH (2010) BRAF mutation in papillary thyroid carcinoma: pathogenic role and clinical implications. J Chin Med Assoc 73:113–128
34. Li RH, Zhang AM, Li S, Li TY, Wang LJ, Zhang HR et al (2016) PAQR3 gene expression and its methylation level in colorectal cancer tissues. Oncol Lett 12:1773–1778
35. Zhao C, Li Y, Chen G et al (2017) Overexpression of miR-15b-5p promotes gastric cancer metastasis by regulating PAQR3. Oncol Rep 38:352–358
36. Guo W, You X, Xu D, Zhang Y, Wang Z, Man K et al (2016) PAQR3 enhances Twist1 degradation to suppress epithelial-mesenchymal transition and metastasis of gastric cancer cells. Carcinogenesis 37:397–407