Original Paper

Meta-Analyses of Association Between *BRAF*^{V600E} Mutation and Clinicopathological Features of Papillary Thyroid Carcinoma

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Key Words

Papillary thyroid carcinoma • *BRAF*^{V600E} • Clinicopathological features • Meta-analysis

Abstract

Background/Aims: The function of *BRAF* V600E as a prognostic biomarker continues controversial by reason of conflicting results in the published articles. **Methods:** A systematical literature search for relevant articles was performed in PubMed, Cochrane Library, Google Scholar, Medline and Embase updated to August 5, 2015. The Chi-square test and *I*² were employed to examine statistical heterogeneity. Pooled ORs with their corresponding 95% confidence intervals (95% CIs) were calculated to assess the relationship between clinicopathological features and *BRAF*^{V600E} mutation. Subgroup analyses by ethnicity were also performed to explore the potential sources of heterogeneity. Furthermore, publication bias was detected using the funnel plot and all statistical analyses were conducted by the software of R 3.12. **Results:** Of 25,241 cases with PTC, 15,290 (60.6%) were positive for *BRAF* mutation and 9,951 (39.4%) were tested negative for *BRAF* mutation. Negative status of *BRAF*^{V600E} mutation negative was significantly associated with gender (OR = 0.90, 95%CI = 0.83-0.97) and concomitant hashimoto thyroiditis (OR = 0.53, 95%CI = 0.43-0.64). By contrast, positive status of *BRAF*^{V600E} mutation was a significant predictor of multifocality (OR = 1.23; 95%CI = 1.14-1.32), extrathyroidal extension (OR = 2.23; 95%CI = 1.90-2.63), TNM stage (OR = 1.67; 95%CI = 1.53-1.81), lymph node metastasis (OR = 1.67; 95%CI = 1.45-1.93), vascular invasion (OR = 1.47; 95%CI = 1.22-1.79) and recurrence/persistence (OR = 2.33; 95%CI = 1.71-3.18). However, there was no significant association between *BRAF*^{V600E} mutation and factors including age > 45 (OR = 0.98; 95%CI = 0.89-1.07), tumor size (OR = 0.84; 95%CI = 0.64-1.09) and distant metastasis (OR = 1.23; 95%CI = 0.67-2.27). **Conclusion:** This meta-analysis confirmed significant associations between *BRAF*^{V600E} mutation and female gender, multifocality, ETE, LNM, TNM stage, concomitant hashimoto thyroiditis, vascular invasion and recurrence/persistence, suggesting the predictive value of *BRAF*^{V600E} mutation for PTC prognosis.

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Introduction

Thyroid carcinoma (TC) is the most common malignant tumor in the endocrine system and it accounts for more than 50% of deaths related to endocrine cancer [1]. Since Papillary thyroid carcinoma (PTC) contributes to about 90% of death resulted from thyroid cancer (TC), growing attention has been paid to PTC which is unable to be detected in the short term due to slow growth [2, 3]. Despite the fact that the overall ten-year survival rate for patients with PTC is about 90%, approximately 5% - 20% of patients suffer from regional or local tumor recurrences and 10% - 15% of them will eventually encounter distant metastasis which significantly reduces the ten-year survival to 40% [4]. Various factors such as tumor size, gender, aging and cancer extension may contribute to poor prognosis of PTC. However, these factors are still debatable in predicting PTC prognostic outcomes including metastasis, recurrence and death [5, 6]. As a member of RAF/RAS/MAPK/MAPK kinase signaling pathway, RAF genes have received special attention since this pathway is associated with the regulation of cell proliferation, growth and division [7, 8]. BRAF is a cytoplasmic protein kinase and it is a main subtype of RAF kinase that could trigger tumorigenesis through the activation of MAPK pathway [9]. PTC patients with BRAF mutation are associated with higher risk of unfavorable clinicopathological characteristics [10]. Besides that, *V600E* transversion is the most common type of BRAF mutation and about 44% of PTC patients were associated with *V600E* mutation.

Recently, *BRAF^{V600E}* mutation has been considered as a biomarker which is related to high risk clinicopathological factors for PTC [11-13]. Some studies claimed that *BRAF^{V600E}* mutation was associated with poor clinicopathologic results such as tumor metastasis, recurrence and advanced clinical stages [14-16]. On the other hand, others suggested that *BRAF^{V600E}* mutation had no significant association with clinical stage, age, gender, multicentricity or recurrence [17, 18]. This inconsistency may result from different study populations and different study design. As a result of this, we undertook a comprehensive meta-analysis in order to ascertain the association between *BRAF^{V600E}* mutation and clinicopathological characteristics.

Materials and Methods

Search Strategy

A systematical review and literature search for relevant articles was performed in PubMed, Cochrane Library, Google Scholar, Medline and Embase with the key terms including "Papillary Thyroid Cancers", "BRAF", "V600E", "T1799A", "Polymorphism, Genetic", "Genetic Mutation", "Clinicopathological Features" and "PTC" (update to August 5, 2015). Apart from that, manual searching for references from cited articles was also carried out to ensure the completeness of article selection. All the included articles were published in English and the process of literature review and searching was independently performed by two authors.

Inclusion and exclusion criteria

We searched for articles which assessed the association between *BRAF^{V600E}* mutation and clinicopathological features in patients with PTC. Studies satisfying the following criteria were included: (1) Patients were diagnosed with PTC; (2) Study must involve the effect of *BRAF^{V600E}* mutation on patients with PTC; (3) Study must evaluate at least one of the clinicopathological features referred below. Furthermore, the most informative or the latest study was selected if multiple studies had the same data source. The following exclusion criteria were applied to studies: 1) duplicated studies; 2) studies not related to the research subject; 3) studies without original data such as case-reports, abstracts, meeting reports and reviews.

Data extraction

Two reviewers carefully reviewed the full text of eligible literatures and independently extracted relevant information into an electronic database. The following information was extracted from each study:

first author; year of publication; country; ethnicity; numbers of cases; clinicopathological features including gender, age, multifocality, extrathyroidal extension (ETE), lymph node metastasis (LNM), TNM stage, tumor size, concomitant hashimoto thyroiditis, distant metastasis, vascular invasion and recurrence/persistence. Any discrepancies between the two reviewers regarding to the extracted data were resolved by a third reviewer. We also contacted the original authors via email if there was any problem in the selected study.

Statistical Analysis

The Chi-square test and I^2 were employed to assess statistical heterogeneity. Significant heterogeneity was presented if P value < 0.10 or $I^2 > 50\%$ [19, 20] and the random-effects model was chosen to calculate the pooled estimates, otherwise a fixed-effect model was selected for meta-analysis. Pooled ORs with their corresponding 95% confidence intervals (95% CIs) were evaluated to assess the relationship between clinicopathological features and *BRAF*^{V600E} mutation. Subgroup analyses by ethnicity (e.g. Caucasians, Asians, African) were also performed to explore potential sources of heterogeneity. Additionally, publication bias was detected using the funnel plot and a P -value < 0.05 indicated significant publication bias. We implemented all statistical analyses using software of R 3.12.

Results

Characteristics of Selected Studies

The literature flow chat (Fig. 1) exhibited the entire selection process of eligible studies. The inclusion and exclusion criteria enabled us to select a total of 81 studies which included 25,241 patients with PTC [10-13, 15, 21-96]. All of the selected studies were performed between 2003 and 2005. Among the 25,241 patients, 15,290 (60.6%) were *BRAF* mutation positive cases. The main characteristics of included studies along with their corresponding clinicopathological features were summarized in Table 1.

Gender

A total of 75 studies with 23,729 PTC patients were included in our meta-analysis in order to assess the association between *BRAF* mutation and gender. We discovered that 11,480 (81.2%) of 14,414 *BRAF*⁺ cases were females and 7,466 (80.2%) of the 9315 *BRAF*⁻ cases were females. As suggested by Fig. 2 and Table 2, significant association was noticed between the negative status of *BRAF* mutation and female gender (OR = 0.90; 95% CI = 0.83-0.97; $P = 0.004$). There was no significant publication bias with respect to gender (Table 2: $P_{\text{publication bias}} = 0.662$).

Fig. 1. Literature flow chart for selecting eligible studies.

Table 1. Characteristics of individual studies included in the meta-analysis. ① Gender; ② Age; ③ Multifocality; ④ Extrathyroidal extension (ETE); ⑤ Lymph node metastasis (LNM); ⑥ TNM stage; ⑦ Tumor size; ⑧ Concomitant hashimoto thyroiditis; ⑨ Distant metastasis; ⑩ Vascular invasion; ⑪ Recurrence/persistence

First author	Year	Country	Ethnicity	No. of cases	Clinicopathological Features
Kwak et al. [92]	2015	Korea	Asian	1945	① ③ ④ ⑥ ⑧ ⑩ ⑪
Na et al. [94]	2015	Korea	Asian	104	① ② ③ ④ ⑤ ⑦ ⑧ ⑩
Jung et al. [90]	2015	Korea	Asian	467	② ④ ⑤ ⑥ ⑩
Wang et al. [95]	2015	Taiwan	Asian	115	① ③ ⑤ ⑥ ⑧ ⑩
Lu et al. [84]	2015	China	Asian	150	① ② ③ ⑤ ⑦
Yang et al. [96]	2015	China	Asian	543	① ② ③ ⑤ ⑥ ⑦
Kim et al. [91]	2015	Korea	Asian	3107	① ② ③ ④ ⑦
Chai et al. [76]	2014	Korea	Asian	137	① ② ③ ④ ⑥ ⑩ ⑪
Pelizzo et al. [86]	2014	Italy	Caucasians	226	① ③ ⑥ ⑪
Russo et al. [87]	2014	Italy	Caucasians	103	① ② ③ ⑤ ⑥ ⑪
Xing et al. [89]	2014	USA	Caucasians	507	③ ⑤ ⑥ ⑩ ⑪
Kim et al. [82]	2014	Korea	Asian	348	① ③ ④ ⑤ ⑩
Khan et al. [81]	2014	India	African	60	① ② ⑤ ⑥ ⑩
Cheng et al. [77]	2014	Taiwan	Asian	50	① ③ ⑥ ⑩
Lukas et al. [85]	2014	USA	Caucasians	137	① ② ③ ⑤ ⑥ ⑦ ⑨
Huang et al. [80]	2014	China	Asian	214	① ② ⑤ ⑥ ⑧
Ahn et al. [75]	2014	Korea	Asian	85	① ② ⑤ ⑧
Lu et al. [93]	2014	China	Asian	292	① ② ③ ⑤ ⑥ ⑦
Liu et al. [83]	2014	China	Asian	132	① ② ③ ⑤ ⑥
He et al. [78]	2014	China	Asian	187	① ② ⑤ ⑥
Shin et al. [88]	2014	Korea	Asian	84	① ③ ④ ⑤
Hong et al. [79]	2014	Korea	Asian	2431	① ④ ⑤
Lee et al. [67]	2013	Korea	Asian	605	① ③ ⑤ ⑥ ⑪
Kleiman et al. [66]	2013	USA	Caucasians	47	① ③ ④ ⑤ ⑩
Virk et al. [73]	2013	USA	Caucasians	129	① ② ③ ④ ⑩
Gouveia et al. [61]	2013	USA	Caucasians	429	① ② ③ ④ ⑤ ⑥ ⑨ ⑩
Choi et al. [11]	2013	Korea	Asian	101	① ② ③ ④ ⑤ ⑧ ⑨
Kim et al. [65]	2013	Korea	Asian	327	① ② ③ ④ ⑤ ⑥ ⑦ ⑧
Jeong et al. [62]	2013	Korea	Asian	211	① ② ③ ⑤ ⑥ ⑦
Kang et al. [64]	2013	Korea	Asian	159	① ④ ⑤ ⑦
Pyo et al. [71]	2013	Korea	Asian	122	① ② ③ ④ ⑤ ⑥
Kang et al. [63]	2013	Korea	Asian	100	③ ④ ⑤ ⑥
Li et al. [68]	2013	USA	Caucasians	315	① ② ③ ④ ⑤ ⑥
Lim et al. [69]	2013	Korea	Asian	2947	① ③ ④ ⑤ ⑥
Mussazhanova et al. [70]	2013	Japan	Asian	23	① ⑤
Zagzag et al. [74]	2013	USA	Caucasians	37	① ③ ④ ⑤
Rossi et al. [72]	2013	Italy	Caucasians	50	⑤
Park et al. [56]	2012	Korea	Asian	143	① ② ③ ④ ⑤ ⑪
Pelttari et al. [57]	2012	Finland	Caucasians	51	① ⑤ ⑪
Elisei et al. [44]	2012	Italy	Caucasians	319	① ② ③ ⑩
Joo et al. [47]	2012	Korea	Asian	148	① ③ ⑥ ⑩
Lee et al. [50]	2012	Korea	Asian	166	① ③ ④ ⑤ ⑥ ⑨
Zheng et al. [60]	2012	China	Asian	512	① ⑨
Wang et al. [58]	2012	USA	Caucasians	208	① ⑤ ⑥ ⑧
Ahn et al. [42]	2012	Korea	Asian	107	① ② ③ ④ ⑥ ⑧
Chakraborty et al. [43]	2012	India	African	86	① ④ ⑤ ⑦
Jeong et al. [46]	2012	Korea	Asian	200	① ② ③ ⑤ ⑦
Moon et al. [54]	2012	Korea	Asian	164	① ③ ⑥ ⑦
Lee et al. [52]	2012	Korea	Asian	429	① ③ ④ ⑤ ⑦
Nam et al. [55]	2012	Korea	Asian	424	① ② ③ ④ ⑤ ⑦
Kim et al. [10]	2012	Korea	Asian	547	① ② ③ ④ ⑤ ⑥ ⑦
Yim et al. [59]	2012	Korea	Asian	35	① ⑥
Finkelstein et al. [45]	2012	USA	Caucasians	56	① ② ⑥
Lee et al. [51]	2012	USA	Caucasians	63	① ② ③ ④ ⑥
Kurtulmus et al. [49]	2012	Turkey	Caucasians	109	① ② ③ ⑤
Marchetti et al. [53]	2012	Italy	Caucasians	85	③ ⑤
Kang et al. [48]	2012	Korea	Asian	299	① ③ ④ ⑤
Pelizzo et al. [40]	2011	Italy	Caucasians	141	① ③ ④ ⑤ ⑥ ⑦
Kim et al. [38]	2011	Korea	Asian	169	① ② ⑤ ⑦
Smith et al. [41]	2011	Australia	Caucasians	76	① ② ⑥
Patel et al. [39]	2011	USA	Caucasians	104	① ③ ④ ⑤
Bommarito et al. [37]	2011	Italy	Caucasians	56	① ③
Jung et al. [34]	2010	Korea	Asian	210	① ② ③ ④ ⑤ ⑧
Basolo et al. [32]	2010	Italy	Caucasians	1060	① ② ③ ④ ⑤ ⑥ ⑦
Zerilli et al. [36]	2010	Italy	Caucasians	27	① ② ③ ④
Lin et al. [35]	2010	China	Asian	61	① ③ ④
Hwang et al. [33]	2010	Korea	Asian	135	① ③ ④
Lee et al. [27]	2009	China	Asian	64	① ④ ⑤ ⑧
Gu et al. [26]	2009	China	Asian	123	① ⑤ ⑥
Moon et al. [28]	2009	Korea	Asian	84	① ④ ⑥
Xing et al. [30]	2009	USA	Caucasians	190	① ③ ④ ⑤ ⑥
Kwak et al. [13]	2009	Korea	Asian	339	① ③ ⑥
Sheu et al. [29]	2009	Germany	Caucasians	221	① ② ③ ④ ⑤
Yip et al. [31]	2009	USA	Caucasians	206	① ③ ④ ⑤
Frasca et al. [24]	2008	Italy	Caucasians	323	③ ⑤ ⑥ ⑪
Guan et al. [25]	2008	China	Asian	38	① ⑤ ⑥
Zuo et al. [23]	2007	Japan	Asian	42	① ② ⑤ ⑥
Jo et al. [22]	2006	Korea	Asian	161	① ⑥ ⑨
Kim et al. [12]	2005	Korea	Asian	60	① ② ③ ④ ⑤ ⑥ ⑪
Kim et al. [21]	2004	Korea	Asian	70	① ⑤
Nikiforova et al. [15]	2003	USA	Caucasians	104	① ④ ⑤ ⑥ ⑨

Fig. 2. Forest plot of OR with respect to gender, age, multifocality, extrathyroidal extension, and tumor size. Subgroup analysis was performed by ethnicity. **Table 2.** Meta-analyses of association between clinicopathological features and *BRAF*^{V600E} mutation. ¹OR: odds ratio, CI: confidence intervals; ²ETE: extrathyroidal extension; ³LNM: lymph node metastasis

Clinicopathological features	OR (95% CI) ¹	<i>P</i> _{odds ratio}	<i>I</i> ²	<i>P</i> _{heterogeneity}	Caucasians	Asians	African	<i>P</i> _{publication bias}
Gender	0.90 (0.83, 0.97)	0.004	33.60 %	0.004	0.91 (0.78, 1.05)	0.89 (0.81, 0.97)	1.18 (0.56, 2.50)	0.662
Age	0.98 (0.89, 1.07)	0.581	43.68 %	0.005	0.91 (0.78, 1.06)	1.01 (0.91, 1.13)	0.68 (0.20, 2.33)	0.124
Multifocality	1.23 (1.14, 1.32)	<.000	33.79 %	0.002	1.29 (1.14, 1.46)	1.20 (1.10, 1.31)	-	0.167
ETE ²	2.23 (1.90, 2.63)	<.000	74.70 %	<.000	2.61 (1.96, 3.47)	2.03 (1.68, 2.44)	10.15 (3.71, 27.77)	0.125
LNM ³	1.67 (1.45, 1.93)	<.000	69.91 %	<.000	2.09 (1.64, 2.68)	1.40 (1.20, 1.63)	5.96 (2.77, 12.80)	0.343
TNM stage	1.67 (1.53, 1.81)	<.000	40.69 %	0.004	2.30 (1.93, 2.74)	1.52 (1.38, 1.67)	0.12 (0.01, 2.26)	0.151
Tumor size	0.84 (0.64, 1.09)	0.240	79.61 %	<.000	0.87 (0.63, 1.21)	0.86 (0.62, 1.20)	0.24 (0.06, 0.96)	0.993
Concomitant hashimoto thyroiditis	0.53 (0.43, 0.64)	<.000	44.97 %	0.048	0.21 (0.08, 0.56)	0.55 (0.45, 0.67)	-	0.368
Distant metastasis	1.23 (0.67, 2.27)	0.510	0.00 %	0.306	1.90 (0.51, 7.12)	1.09 (0.55, 2.18)	-	0.260
Vascular invasion	1.47 (1.21, 1.78)	0.000	0.00 %	0.559	1.49 (1.04, 2.14)	1.43 (1.13, 1.80)	2.47 (0.75, 8.17)	0.268
Recurrence/persistence	2.33 (1.71, 3.18)	<.000	21.28 %	0.468	2.71 (1.88, 3.92)	1.59 (0.89, 2.84)	-	0.339

Age

We synthesized evidence from 38 studies with 11,199 PTC patients for the purpose of assessing the association between *BRAF* mutation and age. We discovered that 2,977 (51.9%) of 5731 *BRAF* mutation positive PTCs were greater than 45 years old whereas 2927 (53.5%) of 5468 *BRAF* mutation negative PTCs were greater than 45 years old. Fig. 2 and Table 2 revealed that no significant association was observed between *BRAF* mutation and age > 45 (OR = 0.98; 95%CI = 0.89-1.07). Besides that, subgroup analysis by ethnicity indicated consistent results and no significant publication bias was detected with respect to age (Table 2; *P* = 0.124).

Multifocality

Among the 56 studies with 19,654 patients, 35.9% (4,131/11,497) of *BRAF* positive mutation cases were multifocal, while 29.6% (2,411/8,157) of *BRAF* mutation negative cases were multifocal PTCs. Significant association was found between the positive status of *BRAF* mutation and multifocality (Fig. 2 and Table 2; OR = 1.23; 95%CI = 1.14-1.32). This significant association was confirmed in the subgroup of Caucasian (OR = 1.29, 95%CI = 1.14-1.46) and Asian (OR = 1.20, 95%CI = 1.10-1.31). Moreover, no significant publication bias was noticed with respect to multifocality (Table 2; *P* = 0.167).

Extrathyroidal Extension

A total of 65 studies with 23,303 patients were included in the meta-analysis. Among these patients, 52.6% (7,473/14,207) of *BRAF* positive mutation cases had extrathyroidal extension whereas 38.0% (3,453/9,096) of *BRAF* negative mutation cases had extrathyroidal extension. Significant association was found between the positive status of *BRAF* mutation and extrathyroidal extension status (Fig. 2 and Table 2; OR = 2.23; 95%CI = 1.90-2.63). Similar association was observed in the subgroup of Caucasian, Asian and African as suggested by subgroup analysis. No significant publication bias was noticed with respect to extrathyroidal extension (Table 2; *P* = 0.125).

Tumor Size

Based on 18 studies comprising of 8,299 patients, we discovered that 58.7% (2326/3965) of *BRAF* mutation positive cases had tumor size > 10 mm, while 64.7% (2805/4334) of *BRAF* mutation negative cases had tumor size > 10 mm. The overall analysis suggested that no significant association existed between *BRAF* mutation and tumor size (Fig. 2 and Table 2; OR = 0.84; 95%CI = 0.64-1.09). There was no significant publication bias presented with respect to tumor size (Table 2; *P* = 0.993).

Lymph Node Metastasis

A total of 59 studies consisting of 16,936 patients were analyzed to evaluate the relationship between lymph node metastasis and *BRAF* mutation. Lymph node metastasis was detected in 5,281 (46.5%) of 11,359 patients with *BRAF* mutation and in 1,858 (33.3%) of 5,577 patients without *BRAF* mutation. Significant association was noticed between the positive status of *BRAF* mutation and lymph node metastasis. *BRAF* mutation was a significant predictor of lymph node metastasis (Fig. 3 and Table 2; OR = 1.67; 95% CI = 1.45-1.93). Moreover, this association was more significant in the subgroup of African (OR = 5.96; 95%CI = 2.77-12.80) compared with the subgroup of Caucasian and Asian. No significant publication bias with respect to lymph node metastasis was presented (Table 2; *P* = 0.343).

TNM Stage

A number of 45 studies including 14,242 patients were analyzed with respect to the effect of *BRAF* mutation on TNM stage. Advanced TNM stage (III/IV) was observed in 3,818 (77.0%) of the 4,958 patients with *BRAF* mutation and in 5,715 (61.6%) of the 9,284 patients without *BRAF* mutation. Significant association was noticed between the positive status of *BRAF* mutation and TNM stage (Fig. 3 and Table 2; OR = 1.67; 95%CI = 1.53-1.81). No significant publication bias existed with respect to TNM stage (Table 2; *P* = 0.151).

Concomitant Hashimoto Thyroiditis

We also performed a meta-analysis based on 11 studies which concerned about the association between *BRAF* mutation and concomitant hashimoto thyroiditis. Among the 3,480 patients with PTC, 459 (17.6%) of 2,604 with *BRAF* mutation had hashimoto thyroiditis and 231 (26.4%) of 876 patients without *BRAF* mutation had hashimoto thyroiditis. Significant association was identified between the negative status of *BRAF* mutation and concomitant hashimoto thyroiditis (Fig. 3 and Table 2; OR = 0.53; 95%CI = 0.43-0.64). No significant publication bias with respect to concomitant hashimoto thyroiditis was presented (Table 2; *P* = 0.368).

Fig. 3. Forest plot of OR with respect to lymph node metastasis, TNM stage, concomitant hashimoto thyroiditis, distant metastasis, vascular invasion, and recurrence/persistence. Subgroup analysis was performed by ethnicity.

Distant Metastasis

Evidence from a total of 7 studies including 1,609 patients was synthesized in order to verify the association between *BRAF* mutation and distant metastasis. Distant metastasis was detected in 32 (3.2%) of 988 patients with *BRAF* mutation and in 19 (3.1%) of 621 patients without *BRAF* mutation. No significant association existed between *BRAF* mutation and distant metastasis (Fig. 3 and Table 2; OR = 1.23; 95%CI = 0.67-2.27). No significant publication bias was presented in studies involving distant metastasis (Table 2; $P = 0.260$).

Vascular invasion

In total, 14 studies with 4,734 patients were analyzed regarding to vascular invasion. Vascular invasion was found in 586 (18.1%) of 3,235 patients with *BRAF* mutation and in 215 (14.3%) of 1,499 patients without *BRAF* mutation. Significant association was indicated between the positive status of *BRAF* mutation and vascular invasion (Fig. 3 and Table 2; OR = 1.47; 95%CI = 1.22-1.79). No significant publication bias was shown for studies investigating vascular invasion (Table 2; $P = 0.268$).

Recurrence/persistence

Finally, we carried out a meta-analysis which evaluated the association between *BRAF* mutation and recurrence/persistence based on 10 studies consisting of 3,905 patients. Recurrence/persistence was observed in 167 (6.4%) of 2,600 patients with *BRAF* mutation and in 71 (5.4%) of 1,305 patients without *BRAF* mutation. There was significant association between recurrence/persistence and the positive status of *BRAF* mutation (Fig. 3 and Table 2; OR = 2.33; 95%CI = 1.71-3.18). There was no significant publication bias presented in studies concerning about the association between *BRAF* mutation and recurrence/persistence (Table 2; $P = 0.339$).

Discussion

This meta-analysis indicated that *BRAF* mutation was significantly associated with several clinicopathological factors including gender, multifocality, ETE, LNM, TNM stage,

concomitant hashimoto thyroiditis, vascular invasion and recurrence/persistence. By contrast, no significant relationship was found between *BRAF* mutation and age, tumor size or distant metastasis.

A similar meta-analysis [9] in 2014 suggested that *BRAF* mutation was associated with several factors including multifocality, ETE, LNM, TNM stage and recurrence. However, our meta-analysis incorporated more studies including seven studies conducted in 2015 and also examined how *BRAF* mutation is related to other factors such as gender, concomitant hashimoto thyroiditis, vascular invasion and tumor size. Another meta-analysis [97] performed in 2012 concluded that *BRAF* mutation was significantly associated with gender, multifocality, ETE, LNM, TNM stage and tumor size whereas age or vascular invasion was not a significant predictor of *BRAF* mutation. Inconsistence existed among similar studies may be attributed to small sample sizes and between-study heterogeneity. In the meta-analysis conducted in 2012, only four studies were analyzed regarding to tumor size and vascular invasion and significant heterogeneity with respect to tumor size was observed.

Since clinicopathological factors including LNM, advanced TNM stage, ETE, male gender and multifocality were reported to be crucial prognostic factors for PTC [98, 99], *BRAF* mutation could be considered as a promising predictor of PTC prognosis. Moreover, *BRAF* mutation may provide additional assistance for tailoring the initial treatment and determining the appropriateness of radioactive iodine adjuvant treatment based on patients' clinical characteristics. For instance, a large study conducted by Xing et al. [100] confirmed the prognostic value of *BRAF* mutation for PTC recurrence.

In this study, concomitant hashimoto thyroiditis was suggested to be a negative predictor of *BRAF* mutation, implying the protective role of hashimoto thyroiditis in PTC. Besides that, several publications reported that PTC patients with hashimoto thyroiditis possessed more favorable prognosis [101, 102] since follicular cells have nuclear changes due to inflammatory responses to PTC. Nevertheless, the anti-tumor effect of thyroid autoimmune response on PTC prognosis should be further investigated.

In spite of the connection between LNM and distant metastasis [103], *BRAF* mutation was significantly associated with LNM alone in our study. Distant metastasis can be described as a rare event which occurred in patients with differentiated thyroid carcinoma [104]. However, our study only incorporated seven studies involving the association between distant metastasis and *BRAF* mutation which may result in a biased result due to limited statistical power. In addition, *BRAF* mutation was hypothesized to be associated with TNM stage which is based on the size of the primary tumor and evidence of metastasis. Our study demonstrated that *BRAF* mutation was positively associated with advanced TNM stage which confirmed that *BRAF* mutation was a risk factor PTC progression.

Moreover, female gender was considered to be a significant negative predictor of *BRAF* mutation particularly in Asian. Jonklaas et al. reported that females with PTC in stage I and II had better prognostic outcomes than males prior to the age of 55 [105] and therefore further studies should be carried out to explain the gender difference in *BRAF* mutation.

According to the extent of invasion beyond the thyroid capsule, ETE can be classified into two groups: maximal and minimal ETE. Some studies have indicated that PTC patients with minimal or microscopic extension had worse prognostic outcomes than those with maximal or macroscopic extension [106-108]. However, ETE or microscopic local invasion is inconsistently defined and utilized among studies and there are a large number of studies which did not concern about various degrees of extrathyroidal extension. Hence, the association between *BRAF* mutation and ETE should be further investigated.

PTC is commonly characterized as a multifocal tumor with an incidence rate of 87% [97]. Multifocal tumors are defined as the presence of two or more carcinoma foci. However, whether the multifocality is contributed by independent primary carcinomas, intraglandular metastatic tumors or a mixture of both has not been elucidated. Kimbrell et al. [109] concluded that small carcinomas can be *BRAF* mutation positive while large ones are usually *BRAF* mutation negative. Therefore, results from this meta-analysis are likely to be affected by the number of carcinoma foci reported in each included studies.

Another limitation may arise from the fact that different specimens including formalin-fixed paraffin-embedded (FFPE) tumor samples, fine-needle aspiration biopsy (FNAB) specimens and frozen samples were used among individual studies which could have substantial influence on the detection of *BRAF* mutation. Besides, conclusions from our study are likely to be affected by some regions with extremely high incidence of *BRAF* mutation. For instance, Korea has been reported as a region with unexpectedly high incidence of *BRAF* mutation. Finally, different molecular methods used for *BRAF* mutation detection is another confounding factor that may contribute to biased results.

For summary, this meta-analysis confirmed the association between *BRAF*^{V600E} mutation and several clinicopathological factors including gender, multifocality, ETE, LNM, TNM stage, concomitant hashimoto thyroiditis, vascular invasion and recurrence/persistence. As a result, *BRAF*^{V600E} mutation status may provide additional information for PTC prognosis.

Disclosure Statement

The authors have not declared any conflicts of interest.

References

- 1 Aschebrook-Kilfoy B, Ward MH, Sabra MM, Devesa SS: Thyroid cancer incidence patterns in the United States by histologic type, 1992-2006. *Thyroid* 2011;21:125-134.
- 2 Ugolini C, Giannini R, Lupi C, Salvatore G, Miccoli P, Proietti A, Elisei R, Santoro M, Basolo F: Presence of *BRAF* V600E in very early stages of papillary thyroid carcinoma. *Thyroid* 2007;17:381-388.
- 3 Pacini F, Castagna MG: Approach to and treatment of differentiated thyroid carcinoma. *Med Clin North Am* 2012;96:369-383.
- 4 Schlumberger MJ: Papillary and follicular thyroid carcinoma. *N Engl J Med* 1998;338:297-306.
- 5 Dean DS, Hay ID: Prognostic indicators in differentiated thyroid carcinoma. *Cancer Control* 2000;7:229-239.
- 6 Deng X, Wu B, Xiao K, Kang J, Xie J, Zhang X, Fan Y: MiR-146b-5p promotes metastasis and induces epithelial-mesenchymal transition in thyroid cancer by targeting ZNF3. *Cell Physiol Biochem* 2015;35:71-82.
- 7 Mercer KE, Pritchard CA: Raf proteins and cancer: B-Raf is identified as a mutational target. *Biochim Biophys Acta* 2003;1653:25-40.
- 8 Hilger RA, Scheulen ME, Strumberg D: The Ras-Raf-MEK-ERK pathway in the treatment of cancer. *Onkologie* 2002;25:511-518.
- 9 Liu X, Yan K, Lin X, Zhao L, An W, Wang C, Liu X: The association between *BRAF* (V600E) mutation and pathological features in PTC. *Eur Arch Otorhinolaryngol* 2014;271:3041-3052.
- 10 Kim SJ, Lee KE, Myong JP, Park JH, Jeon YK, Min HS, Park SY, Jung KC, Koo do H, Youn YK: *BRAF* V600E mutation is associated with tumor aggressiveness in papillary thyroid cancer. *World J Surg* 2012;36:310-317.
- 11 Choi SY, Park H, Kang MK, Lee DK, Lee KD, Lee HS, Kim SW, Lee EN, Hong JC: The relationship between the *BRAF*(V600E) mutation in papillary thyroid microcarcinoma and clinicopathologic factors. *World J Surg Oncol* 2013;11:291.
- 12 Kim TY, Kim WB, Song JY, Rhee YS, Gong G, Cho YM, Kim SY, Kim SC, Hong SJ, Shong YK: The *BRAF* mutation is not associated with poor prognostic factors in Korean patients with conventional papillary thyroid microcarcinoma. *Clin Endocrinol (Oxf)* 2005;63:588-593.
- 13 Kwak JY, Kim EK, Chung WY, Moon HJ, Kim MJ, Choi JR: Association of *BRAF*V600E mutation with poor clinical prognostic factors and US features in Korean patients with papillary thyroid microcarcinoma. *Radiology* 2009;253:854-860.
- 14 Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, Carson KA, Vasko V, Larin A, Tallini G, Tolaney S, Holt EH, Hui P, Umbricht CB, Basaria S, Ewertz M, Tufano AP, Califano JA, Ringel MD, Zeiger MA, Sidransky D, Ladenson PW: *BRAF* mutation predicts a poorer clinical prognosis for papillary thyroid cancer. *J Clin Endocrinol Metab* 2005;90:6373-6379.

- 15 Nikiforova MN, Kimura ET, Gandhi M, Biddinger PW, Knauf JA, Basolo F, Zhu Z, Giannini R, Salvatore G, Fusco A, Santoro M, Fagin JA, Nikiforov YE: BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. *J Clin Endocrinol Metab* 2003;88:5399-5404.
- 16 Namba H, Nakashima M, Hayashi T, Hayashida N, Maeda S, Rogounovitch TI, Ohtsuru A, Saenko VA, Kanematsu T, Yamashita S: Clinical implication of hot spot BRAF mutation, V599E, in papillary thyroid cancers. *J Clin Endocrinol Metab* 2003;88:4393-4397.
- 17 Fugazzola L, Mannavola D, Cirello V, Vannucchi G, Muzza M, Vicentini L, Beck-Peccoz P: BRAF mutations in an Italian cohort of thyroid cancers. *Clin Endocrinol (Oxf)* 2004;61:239-243.
- 18 Puxeddu E, Moretti S, Elisei R, Romei C, Pascucci R, Martinelli M, Marino C, Avenia N, Rossi ED, Fadda G, Cavaliere A, Ribacchi R, Falorni A, Pontecorvi A, Pacini F, Pinchera A, Santeusano F: BRAF(V599E) mutation is the leading genetic event in adult sporadic papillary thyroid carcinomas. *J Clin Endocrinol Metab* 2004;89:2414-2420.
- 19 Higgins JP, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557-560.
- 20 Dinnes J, Deeks J, Kirby J, Roderick P: A methodological review of how heterogeneity has been examined in systematic reviews of diagnostic test accuracy. *Health Technol Assess* 2005;9:1-113.
- 21 Kim KH, Kang DW, Kim SH, Seong IO, Kang DY: Mutations of the BRAF gene in papillary thyroid carcinoma in a Korean population. *Yonsei Med J* 2004;45:818-821.
- 22 Jo YS, Li S, Song JH, Kwon KH, Lee JC, Rha SY, Lee HJ, Sul JY, Kweon GR, Ro HK, Kim JM, Shong M: Influence of the BRAF V600E mutation on expression of vascular endothelial growth factor in papillary thyroid cancer. *J Clin Endocrinol Metab* 2006;91:3667-3670.
- 23 Zuo H, Nakamura Y, Yasuoka H, Zhang P, Nakamura M, Mori I, Miyauchi A, Kakudo K: Lack of association between BRAF V600E mutation and mitogen-activated protein kinase activation in papillary thyroid carcinoma. *Pathol Int* 2007;57:12-20.
- 24 Frasca F, Nucera C, Pellegriti G, Gangemi P, Attard M, Stella M, Loda M, Vella V, Giordano C, Trimarchi F, Mazzone E, Belfiore A, Vigneri R: BRAF(V600E) mutation and the biology of papillary thyroid cancer. *Endocr Relat Cancer* 2008;15:191-205.
- 25 Guan H, Ji M, Hou P, Liu Z, Wang C, Shan Z, Teng W, Xing M: Hypermethylation of the DNA mismatch repair gene hMLH1 and its association with lymph node metastasis and T1799A BRAF mutation in patients with papillary thyroid cancer. *Cancer* 2008;113:247-255.
- 26 Gu LQ, Li FY, Zhao L, Liu Y, Zang XX, Wang TX, Chen HP, Ning G, Zhao YJ: BRAFV600E mutation and X-linked inhibitor of apoptosis expression in papillary thyroid carcinoma. *Thyroid* 2009;19:347-354.
- 27 Lee X, Gao M, Ji Y, Yu Y, Feng Y, Li Y, Zhang Y, Cheng W, Zhao W: Analysis of differential BRAF(V600E) mutational status in high aggressive papillary thyroid microcarcinoma. *Ann Surg Oncol* 2009;16:240-245.
- 28 Moon HJ, Kwak JY, Kim EK, Choi JR, Hong SW, Kim MJ, Son EJ: The role of BRAFV600E mutation and ultrasonography for the surgical management of a thyroid nodule suspicious for papillary thyroid carcinoma on cytology. *Ann Surg Oncol* 2009;16:3125-3131.
- 29 Sheu SY, Grabelius F, Schwertheim S, Handke S, Worm K, Schmid KW: Lack of correlation between BRAF V600E mutational status and the expression profile of a distinct set of miRNAs in papillary thyroid carcinoma. *Horm Metab Res* 2009;41:482-487.
- 30 Xing M, Clark D, Guan H, Ji M, Dackiw A, Carson KA, Kim M, Tufaro A, Ladenson P, Zeiger M, Tufano R: BRAF mutation testing of thyroid fine-needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer. *J Clin Oncol* 2009;27:2977-2982.
- 31 Yip L, Nikiforova MN, Carty SE, Yim JH, Stang MT, Tublin MJ, Lebeau SO, Hodak SP, Ogilvie JB, Nikiforov YE: Optimizing surgical treatment of papillary thyroid carcinoma associated with BRAF mutation. *Surgery* 2009;146:1215-1223.
- 32 Basolo F, Torregrossa L, Giannini R, Miccoli M, Lupi C, Sensi E, Berti P, Elisei R, Vitti P, Baggiani A, Miccoli P: Correlation between the BRAF V600E mutation and tumor invasiveness in papillary thyroid carcinomas smaller than 20 millimeters: analysis of 1060 cases. *J Clin Endocrinol Metab* 2010;95:4197-4205.
- 33 Hwang J, Shin JH, Han BK, Ko EY, Kang SS, Kim JW, Chung JH: Papillary thyroid carcinoma with BRAFV600E mutation: sonographic prediction. *AJR Am J Roentgenol* 2010;194:W425-430.
- 34 Jung CK, Kang YG, Bae JS, Lim DJ, Choi YJ, Lee KY: Unique patterns of tumor growth related with the risk of lymph node metastasis in papillary thyroid carcinoma. *Mod Pathol* 2010;23:1201-1208.
- 35 Lin KL, Wang OC, Zhang XH, Dai XX, Hu XQ, Qu JM: The BRAF mutation is predictive of aggressive clinicopathological characteristics in papillary thyroid microcarcinoma. *Ann Surg Oncol* 2010;17:3294-3300.

- 36 Zerilli M, Zito G, Martorana A, Pitrone M, Cabibi D, Cappello F, Giordano C, Rodolico V: BRAF(V600E) mutation influences hypoxia-inducible factor-1alpha expression levels in papillary thyroid cancer. *Mod Pathol* 2010;23:1052-1060.
- 37 Bommarito A, Richiusa P, Carissimi E, Pizzolanti G, Rodolico V, Zito G, Criscimanna A, Di Blasi F, Pitrone M, Zerilli M, Amato MC, Spinelli G, Carina V, Modica G, Latteri MA, Galluzzo A, Giordano C: BRAFV600E mutation, TIMP-1 upregulation, and NF-kappaB activation: closing the loop on the papillary thyroid cancer trilogy. *Endocr Relat Cancer* 2011;18:669-685.
- 38 Kim SK, Hwang TS, Yoo YB, Han HS, Kim DL, Song KH, Lim SD, Kim WS, Paik NS: Surgical results of thyroid nodules according to a management guideline based on the BRAF(V600E) mutation status. *J Clin Endocrinol Metab* 2011;96:658-664.
- 39 Patel A, Klubo-Gwiedzinska J, Hoperia V, Larin A, Jensen K, Bauer A, Vasko V: BRAF(V600E) mutation analysis from May-Grunwald Giemsa-stained cytological samples as an adjunct in identification of high-risk papillary thyroid carcinoma. *Endocr Pathol* 2011;22:195-199.
- 40 Pelizzo MR, Boschin IM, Barollo S, Pennelli G, Toniato A, Zambonin L, Vianello F, Piotto A, Ide EC, Pagetta C, Sorgato N, Torresan F, Girelli ME, Nacamulli D, Mantero F, Mian C: BRAF analysis by fine needle aspiration biopsy of thyroid nodules improves preoperative identification of papillary thyroid carcinoma and represents a prognostic factor: A mono-institutional experience. *Clin Chem Lab Med* 2011;49:325-329.
- 41 Smith RA, Salajegheh A, Weinstein S, Nassiri M, Lam AK: Correlation between BRAF mutation and the clinicopathological parameters in papillary thyroid carcinoma with particular reference to follicular variant. *Hum Pathol* 2011;42:500-506.
- 42 Ahn D, Park JS, Sohn JH, Kim JH, Park SK, Seo AN, Park JY: BRAFV600E mutation does not serve as a prognostic factor in Korean patients with papillary thyroid carcinoma. *Auris Nasus Larynx* 2012;39:198-203.
- 43 Chakraborty A, Narkar A, Mukhopadhyaya R, Kane S, D'Cruz A, Rajan MG: BRAF V600E mutation in papillary thyroid carcinoma: significant association with node metastases and extra thyroidal invasion. *Endocr Pathol* 2012;23:83-93.
- 44 Elisei R, Viola D, Torregrossa L, Giannini R, Romei C, Ugolini C, Molinaro E, Agate L, Biagini A, Lupi C, Valerio L, Materazzi G, Miccoli P, Piaggi P, Pinchera A, Vitti P, Basolo F: The BRAF(V600E) mutation is an independent, poor prognostic factor for the outcome of patients with low-risk intrathyroid papillary thyroid carcinoma: single-institution results from a large cohort study. *J Clin Endocrinol Metab* 2012;97:4390-4398.
- 45 Finkelstein A, Levy GH, Hui P, Prasad A, Virk R, Chhieng DC, Carling T, Roman SA, Sosa JA, Udelsman R, Theoharis CG, Prasad ML: Papillary thyroid carcinomas with and without BRAF V600E mutations are morphologically distinct. *Histopathology* 2012;60:1052-1059.
- 46 Jeong D, Jeong Y, Lee S, Lee H, Lee W, Kim H, Park D, Park S, Mu W, Cho HD, Oh MH, Lee SS, Yang SH, Kim CJ: Detection of BRAF(V600E) Mutations in Papillary Thyroid Carcinomas by Peptide Nucleic Acid Clamp Real-Time PCR: A Comparison with Direct Sequencing. *Korean J Pathol* 2012;46:61-67.
- 47 Joo JY, Park JY, Yoon YH, Choi B, Kim JM, Jo YS, Shong M, Koo BS: Prediction of occult central lymph node metastasis in papillary thyroid carcinoma by preoperative BRAF analysis using fine-needle aspiration biopsy: a prospective study. *J Clin Endocrinol Metab* 2012;97:3996-4003.
- 48 Kang G, Cho EY, Shin JH, Chung JH, Kim JW, Oh YL: Role of BRAFV600E mutation analysis and second cytologic review of fine-needle aspiration for evaluating thyroid nodule. *Cancer Cytopathol* 2012;120:44-51.
- 49 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.
- 50 Lee JU, Huang S, Lee MH, Lee SE, Ryu MJ, Kim SJ, Kim YK, Kim SY, Joung KH, Kim JM, Shong M, Jo YS: Dual specificity phosphatase 6 as a predictor of invasiveness in papillary thyroid cancer. *Eur J Endocrinol* 2012;167:93-101.
- 51 Lee KC, Li C, Schneider EB, Wang Y, Somervell H, Krafft M, Umbricht CB, Zeiger MA: Is BRAF mutation associated with lymph node metastasis in patients with papillary thyroid cancer? *Surgery* 2012;152:977-983.
- 52 Lee ST, Kim SW, Ki CS, Jang JH, Shin JH, Oh YL, Kim JW, Chung JH: Clinical implication of highly sensitive detection of the BRAF V600E mutation in fine-needle aspirations of thyroid nodules: a comparative analysis of three molecular assays in 4585 consecutive cases in a BRAF V600E mutation-prevalent area. *J Clin Endocrinol Metab* 2012;97:2299-2306.

- 53 Marchetti I, Iervasi G, Mazzanti CM, Lessi F, Tomei S, Naccarato AG, Aretini P, Alberti B, Di Coscio G, Bevilacqua G: Detection of the BRAF(V600E) mutation in fine needle aspiration cytology of thyroid papillary microcarcinoma cells selected by manual macrodissection: an easy tool to improve the preoperative diagnosis. *Thyroid* 2012;22:292-298.
- 54 Moon WJ, Choi N, Choi JW, Kim SK, Hwang TS: BRAF mutation analysis and sonography as adjuncts to fine-needle aspiration cytology of papillary thyroid carcinoma: their relationships and roles. *AJR Am J Roentgenol* 2012;198:668-674.
- 55 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? *Am J Surg* 2012;203:436-441.
- 56 Park HS, Jung CK, Lee SH, Chae BJ, Lim DJ, Park WC, Song BJ, Kim JS, Jung SS, Bae JS: Notch1 receptor as a marker of lymph node metastases in papillary thyroid cancer. *Cancer Sci* 2012;103:305-309.
- 57 Pelttari H, Schalin-Jantti C, Arola J, Loyttyniemi E, Knuutila S, Valimäki MJ: BRAF V600E mutation does not predict recurrence after long-term follow-up in TNM stage I or II papillary thyroid carcinoma patients. *APMIS* 2012;120:380-386.
- 58 Wang W, Zhao W, Wang H, Teng X, Wang H, Chen X, Li Z, Yu X, Fahey TJ, 3rd, Teng L: Poorer prognosis and higher prevalence of BRAF (V600E) mutation in synchronous bilateral papillary thyroid carcinoma. *Ann Surg Oncol* 2012;19:31-36.
- 59 Yim JH, Kim ES, Choi HJ, Jeon MJ, Han JM, Kim WG, Kim TY, Gong G, Kim SY, Kim WB, Shong YK: Association between STAT1 activity and BRAF mutations in papillary thyroid carcinomas. *J Surg Oncol* 2012;106:719-723.
- 60 Zheng X, Xia T, Lin L, Gao S, Lee Y, Yu Y, Wei S, Gao M: BRAFV600E status and clinical characteristics in solitary and multiple papillary thyroid carcinoma: experience of 512 cases at a clinical center in China. *World J Surg Oncol* 2012;10:104.
- 61 Gouveia C, Can NT, Bostrom A, Grenert JP, van Zante A, Orloff LA: Lack of association of BRAF mutation with negative prognostic indicators in papillary thyroid carcinoma: the University of California, San Francisco, experience. *JAMA Otolaryngol Head Neck Surg* 2013;139:1164-1170.
- 62 Jeong D, Jeong Y, Park JH, Han SW, Kim SY, Kim YJ, Kim SJ, Hwangbo Y, Park S, Cho HD, Oh MH, Yang SH, Kim CJ: BRAF (V600E) mutation analysis in papillary thyroid carcinomas by peptide nucleic acid clamp real-time PCR. *Ann Surg Oncol* 2013;20:759-766.
- 63 Kang SH, Pyo JY, Yang SW, Hong SW: Detection of BRAF V600E mutation with thyroid tissue using pyrosequencing: comparison with PNA-clamping and real-time PCR. *Am J Clin Pathol* 2013;139:759-764.
- 64 Kang SY, Ahn S, Lee SM, Jeong JY, Sung JY, Oh YL, Kim KM: Shifted termination assay (STA) fragment analysis to detect BRAF V600 mutations in papillary thyroid carcinomas. *Diagn Pathol* 2013;8:121.
- 65 Kim YS, Kim JS, Bae JS, Park WC: Clinical implication of the BRAFV600E mutation in papillary thyroid carcinoma. *World J Surg Oncol* 2013;11:99.
- 66 Kleiman DA, Buitrago D, Crowley MJ, Beninato T, Veach AJ, Zanzonico PB, Jin M, Fahey TJ, 3rd, Zarnegar R: Thyroid stimulating hormone increases iodine uptake by thyroid cancer cells during BRAF silencing. *J Surg Res* 2013;182:85-93.
- 67 Lee JI, Jang HW, Kim SW, Kim JW, Oh YL, Chung JH: BRAFV600E mutation in fine-needle aspiration aspirates: Association with poorer prognostic factors in larger papillary thyroid carcinomas. *Head Neck* 2013;35:548-553.
- 68 Li C, Aragon Han P, Lee KC, Lee LC, Fox AC, Beninato T, Thiess M, Dy BM, Sebo TJ, Thompson GB, Grant CS, Giordano TJ, Gauger PG, Doherty GM, Fahey TJ, 3rd, Bishop J, Eshleman JR, Umbricht CB, Schneider EB, Zeiger MA: Does BRAF V600E mutation predict aggressive features in papillary thyroid cancer? Results from four endocrine surgery centers. *J Clin Endocrinol Metab* 2013;98:3702-3712.
- 69 Lim JY, Hong SW, Lee YS, Kim BW, Park CS, Chang HS, Cho JY: Clinicopathologic implications of the BRAF(V600E) mutation in papillary thyroid cancer: a subgroup analysis of 3130 cases in a single center. *Thyroid* 2013;23:1423-1430.
- 70 Mussazhanova Z, Matsuda K, Naruke Y, Mitsutake N, Stanojevic B, Rougounovitch T, Saenko V, Suzuki K, Nishihara E, Hirokawa M, Ito M, Nakashima M: Significance of p53-binding protein 1 (53BP1) expression in thyroid papillary microcarcinoma: association with BRAFV600E mutation status. *Histopathology* 2013;63:726-734.
- 71 Pyo JS, Kang G, Kim DH, Chae SW, Park C, Kim K, Do SI, Lee HJ, Kim JH, Sohn JH: Activation of nuclear factor-kappaB contributes to growth and aggressiveness of papillary thyroid carcinoma. *Pathol Res Pract* 2013;209:228-232.

- 72 Rossi ED, Martini M, Capodimonti S, Lombardi CP, Pontecorvi A, Vellone VG, Zannoni GF, Larocca LM, Fadda G: BRAF (V600E) mutation analysis on liquid-based cytology-processed aspiration biopsies predicts bilaterality and lymph node involvement in papillary thyroid microcarcinoma. *Cancer Cytopathol* 2013;121:291-297.
- 73 Virk RK, Van Dyke AL, Finkelstein A, Prasad A, Gibson J, Hui P, Theoharis CG, Carling T, Roman SA, Sosa JA, Udelsman R, Prasad ML: BRAFV600E mutation in papillary thyroid microcarcinoma: a genotype-phenotype correlation. *Mod Pathol* 2013;26:62-70.
- 74 Zagzag J, Pollack A, Dultz L, Dhar S, Ogilvie JB, Heller KS, Deng FM, Patel KN: Clinical utility of immunohistochemistry for the detection of the BRAF v600e mutation in papillary thyroid carcinoma. *Surgery* 2013;154:1199-1204; discussion 1204-1195.
- 75 Ahn HY, Chung YJ, Kim BS, Kang KH, Seok JW, Kim HS, Park SJ, Cho BY: Clinical significance of the BRAF V600E mutation in multifocal papillary thyroid carcinoma in Korea. *Surgery* 2014;155:689-695.
- 76 Chai YJ, Kim SJ, Kim SC, Koo do H, Min HS, Lee KE, Kim JH, Youn YK: BRAF mutation in follicular variant of papillary thyroid carcinoma is associated with unfavourable clinicopathological characteristics and malignant features on ultrasonography. *Clin Endocrinol (Oxf)* 2014;81:432-439.
- 77 Cheng SP, Hsu YC, Liu CL, Liu TP, Chien MN, Wang TY, Lee JJ: Significance of allelic percentage of BRAF c.1799T > A (V600E) mutation in papillary thyroid carcinoma. *Ann Surg Oncol* 2014;21 Suppl 4:S619-626.
- 78 He G, Zhao B, Zhang X, Gong R: Prognostic value of the BRAF V600E mutation in papillary thyroid carcinoma. *Oncol Lett* 2014;7:439-443.
- 79 Hong AR, Lim JA, Kim TH, Choi HS, Yoo WS, Min HS, Won JK, Lee KE, Jung KC, Park do J, Park YJ: The Frequency and Clinical Implications of the BRAF(V600E) Mutation in Papillary Thyroid Cancer Patients in Korea Over the Past Two Decades. *Endocrinol Metab (Seoul)* 2014;29:505-513.
- 80 Huang FJ, Fang WY, Ye L, Zhang XF, Shen LY, Han RL, Wei Q, Fei XC, Chen X, Wang WQ, Wang S, Ning G: BRAF mutation correlates with recurrent papillary thyroid carcinoma in Chinese patients. *Curr Oncol* 2014;21:e740-747.
- 81 Khan MS, Pandith AA, Azad N, Hussain MU, Masoodi SR, Wani KA, Andrabi KI, Mudassar S: Impact of molecular alterations of BRAF in the pathogenesis of thyroid cancer. *Mutagenesis* 2014;29:131-137.
- 82 Kim MH, Bae JS, Lim DJ, Lee H, Jeon SR, Park GS, Jung CK: Quantification of BRAF V600E alleles predicts papillary thyroid cancer progression. *Endocr Relat Cancer* 2014;21:891-902.
- 83 Liu S, Zhang B, Zhao Y, Chen P, Ji M, Hou P, Shi B: Association of BRAFV600E mutation with clinicopathological features of papillary thyroid carcinoma: a study on a Chinese population. *Int J Clin Exp Pathol* 2014;7:6922-6928.
- 84 Lu H, Qiu T, Ying J, Guo C, Lyn N: [Correlation between BRAF V600E mutation and clinicopathologic features of papillary thyroid carcinoma]. *Zhonghua Bing Li Xue Za Zhi* 2014;43:794-798.
- 85 Lukas J, Drabek J, Dudesek B, Vazan P, Stranska J, Jancik S, Mackova M, Syrucek M, Lukas D, Duskova J, Dundr P, Hintnausova B, Jiskra J: Correlation among the BRAF gene mutation status, clinicopathological features of primary tumour, and lymph node metastasizing of papillary thyroid carcinoma. *Exp Clin Endocrinol Diabetes* 2014;122:268-272.
- 86 Pelizzo MR, Dobrinja C, Casal Ide E, Zane M, Lora O, Toniato A, Mian C, Barollo S, Izuzquiza M, Guerrini J, De Manzini N, Merante Boschini I, Rubello D: The role of BRAF(V600E) mutation as poor prognostic factor for the outcome of patients with intrathyroid papillary thyroid carcinoma. *Biomed Pharmacother* 2014;68:413-417.
- 87 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.
- 88 Shin DY, Kim KJ, Chang S, Kim H, Hwang S, Kim W, Bae J, Park S, Kang SW, Chung WY, Lee EJ: Follicular variant of papillary thyroid carcinoma with B-type Raf showing higher frequency of suspicious sonographic features and multifocality. *Head Neck* 2014.
- 89 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. *J Clin Oncol* 2014;32:2718-2726.
- 90 Jung YY, Yoo JH, Park ES, Kim MK, Lee TJ, Cho BY, Chung YJ, Kang KH, Ahn HY, Kim HS: Clinicopathologic correlations of the BRAFV600E mutation, BRAF V600E immunohistochemistry, and BRAF RNA in situ hybridization in papillary thyroid carcinoma. *Pathol Res Pract* 2015;211:162-170.

- 91 Kim SK, Lee JH, Woo JW, Park I, Choe JH, Kim JH, Kim JS: BRAF V600E mutation: Differential impact on central lymph node metastasis by tumor size in papillary thyroid carcinoma. *Head Neck* DOI:10.1002/hed.24192.
- 92 Kwak HY, Chae BJ, Eom YH, Hong YR, Seo JB, Lee SH, Song BJ, Jung SS, Bae JS: Does papillary thyroid carcinoma have a better prognosis with or without Hashimoto thyroiditis? *Int J Clin Oncol* 2015;20:463-473.
- 93 Lu J, Gao J, Zhang J, Sun J, Wu H, Shi X, Teng L, Liang Z: Association between BRAF V600E mutation and regional lymph node metastasis in papillary thyroid carcinoma. *Int J Clin Exp Pathol* 2015;8:793-799.
- 94 Na JI, Kim JH, Kim HJ, Kim HK, Moon KS, Lee JS, Lee JH, Lee KH, Park JT: VE1 immunohistochemical detection of the BRAF V600E mutation in thyroid carcinoma: a review of its usefulness and limitations. *Virchows Arch* 2015;467:155-168.
- 95 Wang TY, Liu CL, Chen MJ, Lee JJ, Pun PC, Cheng SP: Expression of haem oxygenase-1 correlates with tumour aggressiveness and BRAF V600E expression in thyroid cancer. *Histopathology* 2015;66:447-456.
- 96 Yang LB, Sun LY, Jiang Y, Tang Y, Li ZH, Zhang HY, Bu H, Ye F: The Clinicopathological Features of BRAF Mutated Papillary Thyroid Cancers in Chinese Patients. *Int J Endocrinol* 2015;2015:642046.
- 97 Li C, Lee KC, Schneider EB, Zeiger MA: BRAF V600E mutation and its association with clinicopathological features of papillary thyroid cancer: a meta-analysis. *J Clin Endocrinol Metab* 2012;97:4559-4570.
- 98 Czarniecka A, Kowal M, Rusinek D, Krajewska J, Jarzab M, Stobiecka E, Chmielik E, Zembala-Nozynska E, Poltorak S, Sacher A, Maciejewski A, Zebracka-Gala J, Lange D, Oczko-Wojciechowska M, Handkiewicz-Junak D, Jarzab B: The Risk of Relapse in Papillary Thyroid Cancer (PTC) in the Context of BRAFV600E Mutation Status and Other Prognostic Factors. *PLoS One* 2015;10:e0132821.
- 99 Ji YB, Yoo HS, Song CM, Park CW, Lee CB, Tae K: Predictive factors and pattern of central lymph node metastasis in unilateral papillary thyroid carcinoma. *Auris Nasus Larynx* 2015.
- 100 Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, Elisei R, Bendlova B, Yip L, Mian C, Vianello F, Tuttle RM, Robenshtok E, Fagin JA, Puxeddu E, Fugazzola L, Czarniecka A, Jarzab B, O'Neill CJ, Sywak MS, Lam AK, Riesco-Eizaguirre G, Santisteban P, Nakayama H, Clifton-Bligh R, Tallini G, Holt EH, Sykorova V: Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. *J Clin Oncol* 2015;33:42-50.
- 101 Lee JH, Kim Y, Choi JW, Kim YS: The association between papillary thyroid carcinoma and histologically proven Hashimoto's thyroiditis: a meta-analysis. *Eur J Endocrinol* 2013;168:343-349.
- 102 Anand A, Singh KR, Kushwaha JK, Hussain N, Sonkar AA: Papillary Thyroid Cancer and Hashimoto's Thyroiditis: An Association Less Understood. *Indian J Surg Oncol* 2014;5:199-204.
- 103 Liu FH, Kuo SF, Hsueh C, Chao TC, Lin JD: Postoperative recurrence of papillary thyroid carcinoma with lymph node metastasis. *J Surg Oncol* 2015;112:149-154.
- 104 Kim HJ, Lee JI, Kim NK, Min YK, Kim SW, Chung JH: Prognostic implications of radioiodine avidity and serum thyroglobulin in differentiated thyroid carcinoma with distant metastasis. *World J Surg* 2013;37:2845-2852.
- 105 Jonklaas J, Nogueras-Gonzalez G, Munsell M, Litofsky D, Ain KB, Bigos ST, Brierley JD, Cooper DS, Haugen BR, Ladenson PW, Magner J, Robbins J, Ross DS, Skarulis MC, Steward DL, Maxon HR, Sherman SI, National Thyroid Cancer Treatment Cooperative Study G: The impact of age and gender on papillary thyroid cancer survival. *J Clin Endocrinol Metab* 2012;97:E878-887.
- 106 Ito Y, Kakudo K, Hirokawa M, Fukushima M, Tomoda C, Inoue H, Kihara M, Higashiyama T, Urano T, Takamura Y, Miya A, Kobayashi K, Matsuzuka F, Miyauchi A: Clinical significance of extrathyroid extension to the parathyroid gland of papillary thyroid carcinoma. *Endocr J* 2009;56:251-255.
- 107 Radowsky JS, Howard RS, Burch HB, Stojadinovic A: Impact of degree of extrathyroidal extension of disease on papillary thyroid cancer outcome. *Thyroid* 2014;24:241-244.
- 108 Rivera M, Ricarte-Filho J, Tuttle RM, Ganly I, Shaha A, Knauf J, Fagin J, Ghossein R: Molecular, morphologic, and outcome analysis of thyroid carcinomas according to degree of extrathyroid extension. *Thyroid* 2010;20:1085-1093.
- 109 Kimbrell HZ, Sholl AB, Ratnayaka S, Japa S, Lacey M, Carpio G, Bhatia P, Kandil E: BRAF Testing in Multifocal Papillary Thyroid Carcinoma. *Biomed Res Int* 2015;2015:486391.