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

Qing Zhang, Shao-zheng Liu, Qing Zhang, Yan-xing Guan, Qing-jie Chen, Qin-yao Zhu

Department of Nuclear Medicine, the First Affiliated Hospital of Nanchang University, Nanchang, China

Key Words
Papillary thyroid carcinoma • \textit{BRAF}^{V600E} • Clinicopathological features • Meta-analysis

Abstract

\textbf{Background/Aims:} The function of \textit{BRAF} V600E as a prognostic biomarker continues controversial by reason of conflicting results in the published articles. \textbf{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 \textit{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 \textit{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. \textbf{Results:} Of 25,241 cases with PTC, 15,290 (60.6%) were positive for \textit{BRAF} mutation and 9,951 (39.4%) were tested negative for \textit{BRAF} mutation. Negative status of \textit{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 \textit{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 \textit{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). \textbf{Conclusion:} This meta-analysis confirmed significant associations between \textit{BRAF}^{V600E} mutation and female gender, multifocality, ETE, LNM, TNM stage, concomitant hashimoto thyroiditis, vascular invasion and recurrence/persistence, suggesting the predictive value of \textit{BRAF}^{V600E} mutation for PTC prognosis.

Q. Zhang and S. Liu are first co-authors for this study.
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, BRAFV600E mutation has been considered as a biomarker which is related to high risk clinicopathological factors for PTC [11-13]. Some studies claimed that BRAFV600E 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 BRAFV600E 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 BRAFV600E 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 BRAFV600E 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 BRAFV600E 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: $P_{publication bias} = 0.662$).

![Fig. 1. Literature flow chart for selecting eligible studies.](image-url)
Table 1: Characteristics of individual studies included in the meta-analysis.

<table>
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<tr>
<th>First author</th>
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<th>No. of cases</th>
<th>Clinicopathological Feature</th>
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</table>
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 exited 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).
Distant Metastasis

Evidence from a total of 7 studies including 1,609 patients was synthesized in order to verify the association between \(\text{BRAF} \) mutation and distant metastasis. Distant metastasis was detected in 32 (3.2%) of 988 patients with \(\text{BRAF} \) mutation and in 19 (3.1%) of 621 patients without \(\text{BRAF} \) mutation. No significant association existed between \(\text{BRAF} \) 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 \(\text{BRAF} \) mutation and in 215 (14.3%) of 1,499 patients without \(\text{BRAF} \) mutation. Significant association was indicated between the positive status of \(\text{BRAF} \) 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 \(\text{BRAF} \) 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 \(\text{BRAF} \) mutation and in 71 (5.4%) of 1,305 patients without \(\text{BRAF} \) mutation. There was significant association between recurrence/persistence and the positive status of \(\text{BRAF} \) 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 \(\text{BRAF} \) mutation and recurrence/persistence (Table 2; \(P = 0.339\)).

Discussion

This meta-analysis indicated that \(\text{BRAF} \) 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 \textit{BRAF} mutation and age, tumor size or distant metastasis.

A similar meta-analysis [9] in 2014 suggested that \textit{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 \textit{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 \textit{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 \textit{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], \textit{BRAF} mutation could be considered as a promising predictor of PTC prognosis. Moreover, \textit{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 \textit{BRAF} mutation for PTC recurrence.

In this study, concomitant hashimoto thyroiditis was suggested to be a negative predictor of \textit{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], \textit{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 \textit{BRAF} mutation which may result in a biased result due to limited statistical power. In addition, \textit{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 \textit{BRAF} mutation was positively associated with advanced TNM stage which confirmed that \textit{BRAF} mutation was a risk factor PTC progression.

Moreover, female gender was considered to be a significant negative predictor of \textit{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 \textit{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 \textit{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 \textit{BRAF} mutation positive while large ones are usually \textit{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\textsuperscript{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\textsuperscript{V600E} mutation status may provide additional information for PTC prognosis.

**Disclosure Statement**

The authors have not declared any conflicts of interest.

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