BRAF V600E mutation and Its Association with Clinicopathological Features of Papillary Thyroid Cancer: A Meta-Analysis

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Background: There is conflicting literature regarding the association of the BRAF V600E mutation and aggressive clinicopathological features of papillary thyroid cancer (PTC). Nevertheless, some propose that BRAF status be incorporated into the management of patients with PTC, specifically recommendations regarding lymph node dissection. We therefore performed a meta-analysis to examine the relationship between BRAF and clinicopathological features of PTC.

Methods: A literature search was performed within PubMed and EMBASE databases using the following Medical Subject Headings (MeSH) and keywords: “braf,” “mutation,” “thyroid,” “neoplasm(s),” “tumor,” “cancer,” and “carcinoma.” Individual study-specific odds ratios and confidence intervals were calculated, as were Mantel-Haenszel pooled odds ratios for the combined studies.

Results: Thirty-two studies including 6372 patients were reviewed. BRAF mutation was associated with lymph node metastases (LNM), advanced stage, extrathyroidal extension, tumor size, male gender, multifocality, absence of capsule, classic PTC, and tall-cell variant PTC. There was no association with age or vascular invasion. Only two studies were prospective; nine included consecutive patients, whereas one included randomly selected patients; and only two included patients who had undergone routine central lymph node dissection and were thus evaluable for the presence of LNM.

Conclusion: Meta-analysis found that BRAF mutation is associated with LNM, stage, extrathyroidal extension, tumor size, male gender, multifocality, absence of capsule, classic PTC, and tall-cell variant PTC in PTC. However, almost all studies were retrospective and only two of 32 included patients who had undergone routine central lymph node dissection, emphasizing the need for well-designed studies to appropriately examine this association before making important clinical decisions. (J Clin Endocrinol Metab 97: 4559–4570, 2012)

The incidence of thyroid cancer has significantly increased over the past three decades with an annual increase of 6.4% from 1997–2008 and with 11.0 per 100,000 men and women now presenting annually (seer.cancer.gov). The most common form of thyroid cancer is papillary thyroid cancer (PTC), comprising 65–88% of all differentiated thyroid cancers (1). Although the overall 10-yr survival rate for patients with PTC is high (>90%), 5–10% of PTC patients will experience regional recurrences and 10–15% will experience distant metastases with an associated overall 10-yr survival rate of only 40% (2).

Although multiple prognostic staging systems exist to help stratify differentiated thyroid cancers, they generally use final histopathological parameters and therefore cannot be applied preoperatively to determine initial surgical management (3). Furthermore, and relevant to this, the extent of surgical management recommended for patients...
with less advanced disease remains controversial. Some suggest that the optimal surgical management of patients with PTC and no clinically evident lymph node metastases should include a total thyroidectomy with bilateral prophylactic level VI lymph node compartment dissection (4). Others, however, put forth the fact that no prospective randomized trial has been published that demonstrates the benefits of prophylactic central neck dissection in terms of local recurrence or survival rates (5). Because several features of PTC, including histology, grade, metastases, and completeness of surgery, used by the various prognostic systems [AGES (age, grade, extent, size); AMES (age, metastasis, extent, size); OHU (Ohio State University); and TNM (tumor, node, metastasis)] can only be determined postoperatively, the appropriate surgical management of patients with PTC continues to remain debatable (6).

Therefore, there is a great need for more accurate preoperative risk stratification systems to inform the initial management of patients with PTC.

The BRAF V600E mutation composed of a T to A transversion, is found in up to 73.4% of PTCs (7) and results in the substitution of valine for glutamate at position 600 of the b-raf protein. The V600E mutant BRAF constitutively activates the MAPK pathway, thus stimulating tumorigenesis. Since its initial discovery, BRAF V600E has emerged as a promising diagnostic as well as prognostic indicator of PTC.

In 2007, Lee et al. (8) published a meta-analysis examining the clinicopathological significance of BRAF V600E in PTC. They found that BRAF mutation was associated...
FIG. 2. A–L, Study-specific and Mantel-Haenszel pooled OR and 95% CI for the association between BRAF mutation and gender (A), age (B), tumor size (C), lymph node metastases (D), ETE (E), multifocality (F), vascular invasion (G), absence of capsule (H), clinical stage (I), classical variant (J), tall cell variant (K), and follicular variant (L) in patients with PTC.

A systematic literature review was performed using PubMed and EMBASE databases using the following MeSH (Medical Subject Headings) terms and keywords: “braf,” “mutation,” “thyroid,” “neoplasms,” “tumor,” “cancer,” and “carcinoma.” Two reviewers (C.L. and K.L.) used the EndNote reference tool to independently screen and select articles for inclusion (Fig. 1). The review includes studies published before September 2011.

Materials and Methods

A systematic literature review was performed within PubMed and EMBASE databases using the following MeSH (Medical Subject Headings) terms and keywords: “braf,” “mutation,” “thyroid,” “neoplasms,” “tumor,” “cancer,” and “carcinoma.” Two reviewers (C.L. and K.L.) used the EndNote reference tool to independently screen and select articles for inclusion (Fig. 1). The review includes studies published before September 2011.

To further investigate the prognostic value of BRAF mutation in PTC. However, many of these studies report contradictory results regarding the association of the BRAF V600E mutation status and prognosis. Although some studies report that BRAF mutation is associated with multiple aggressive clinicopathological features including higher rate of ETE (9–12) and lymph node metastases (10–13), other studies fail to report any significant association between the mutation and aggressive clinicopathological features (14, 15). Despite these discrepancies that exist in the literature, some have already proposed that BRAF V600E mutation detection be incorporated into the management algorithm of patients with PTC, specifically, that patients who have BRAF-positive tumors on fine-needle aspiration (FNA) undergo prophylactic central lymph node dissection (LND) (16, 17). Because of these recent conflicting reports in the literature and the tremendous implications of basing the surgical management of patients with PTC on a molecular marker, we chose to re-examine the association between BRAF V600E mutation and clinicopathological features of PTC. To accomplish this, we conducted a meta-analysis of the literature that included a total of 32 articles published before September 2011. With regard to lymph node metastases, we also examined studies to determine whether patients underwent prophylactic or therapeutic LNDs, because the latter group would bias the results by including patients who underwent a LND only if they had advanced disease as measured by other preoperative or intraoperative parameters.

with histological subtype, the presence of extrathyroidal extension (ETE), and higher clinical stage but not with age, sex, race, or tumor size. Twelve articles published before July 2006 were included in this study. Since July 2006, numerous additional studies have been performed...
Duplicate articles were removed, and only English full-text articles were included. Titles and abstracts were screened for the terms BRAF mutation and papillary thyroid cancer and any terms associated with disease prognosis: gender, sex, age, size, stage, capsule, multifocality, histological subtype, presence of capsule, ETE, lymph node metastasis, vascular invasion, and clinical stage were identified in the full-text articles. All review articles and single case reports were excluded. In instances where the same study cohort was used in multiple articles, either the most recent or the most appropriately informative single article was included. For example, Kim et al. 2005 (48) was used only for a subset analyses that included microcarcinomas and was not used in the overall analysis because it contained a cohort overlapping with Kim et al. 2006 (32), used in the overall PTC analysis.

The reviewers also independently assessed references of relevant articles and reviews to identify additional studies for inclusion. At each stage of the selection process, discrepancies in article selection between the two reviewers were discussed by study team members and resolved. Individual study-specific odds ratios (ORs) and confidence intervals were calculated, and Mantel-Haenszel pooled ORs for the combined studies were calculated using a fixed-effect model. Because variables used for model adjustment differed substantially across studies, the fixed-effect models were based upon actual counts of patient BRAF status and clinicopathological features rather than combined adjusted ORs. This was done specifically to avoid any adjustment-based imbalance in the pooled results. Thus, unadjusted ORs were calculated for each study and actual count numbers were used to generate the pooled ORs reported in the results and forest plots.

As recommended by Stroup et al. (18), further analyses were performed to assign quality scores to each study. Studies were reviewed and assigned additional points in quality if they were prospective, selected patients consecutively or randomly, and were multi-institutional. For the outcome of lymph node metastasis, studies were also weighted more heavily if they performed routine central LNDs (CLNDs). The scoring system produced a quality rating between 0 and 5. A sensitivity analysis was performed where weights were applied to the original count data based upon the rigor of the study, as scored using the 0–5 rating system. The same unadjusted fixed-effect analyses were used to generate Mantel-Haenszel pooled ORs for the combined studies using weighted counts. Stata version 11.2 (StataCorp, College Station, TX) was used for all analyses.

**Results**

Thirty-two studies comprising 6372 patients were included in the meta-analysis, and 3244 (50.9%) of these
patients had BRAF mutation-positive PTCs. The earliest study was published in September 2003, and the latest study was published in September 2011. The largest study by Basolo et al. (10) included 1060 patients, and the smallest study by Nakayama et al. (19) included 40 patients. Not all studies reported on all variables examined in the meta-analysis (Table 1); therefore, only studies that reported the variable of interest were analyzed for BRAF association with that variable. Only two of 32 studies were prospective (20, 21), whereas the rest were either retrospective or not specified. Nine of 33 studies comprised consecutive patients (9, 10, 13, 16, 20–24), whereas one examined randomly selected patients (12); the remainder did not specify.

Gender

Thirty studies, including 5238 patients, were analyzed for the association between BRAF mutation and gender. Of 992 male patients, 512 (51.6%) were BRAF mutation positive, and 2046 (48.2%) of 4246 female patients were BRAF mutation positive. There was a significant association between BRAF mutation and male gender [OR = 1.22; 95% confidence interval (CI) = 1.05–1.41] (Fig. 2A and Table 2).

Age

Nine studies, including 2015 patients, were analyzed for the association between BRAF mutation and age. Of 1045 patients 45 yr or older, 482 (46.1%) were BRAF mutation positive, and 472 (48.7%) of 970 patients younger than 45 yr old were BRAF mutation positive. No significant association was found between BRAF mutation and age (OR = 0.91; 95% CI = 0.76–1.10) (Fig. 2B and Table 2).

Tumor size

Four studies, including 2029 patients, analyzed the association between BRAF mutation and size of the PTC. Of 1159 tumors greater than 1.0 cm, 578 (49.9%) were positive for the BRAF mutation, and 397 (45.6%) of 870 tumors less than or equal to 1.0 cm in size were positive for the BRAF mutation. There was a significant association between BRAF mutation and tumor size greater than 1.0 cm (OR = 1.57; 95% CI = 1.29–1.92) (Fig. 2C and Table 2).

Lymph node metastases

Thirty-one studies, including 5895 patients, were analyzed for the association between BRAF mutation and lymph node metastasis. Of 2230 lymph node-positive pa-
patients, 1293 (58.9%) tested positive for the BRAF mutation, and 1688 (46.1%) of 3665 lymph node-negative patients tested positive for the BRAF mutation. A significant association was found between BRAF mutation and the presence of lymph node metastases (OR = 1.72; 95% CI = 1.53–1.94) (Fig. 2D and Table 2).

**Multifocality**

Eighteen studies, including 3585 patients, were analyzed for the association between BRAF mutation and multifocal PTC. Of 1378 patients with multifocal disease, 736 (53.4%) were BRAF mutation positive, and 1054 (47.8%) of 2207 patients with unifocal disease were BRAF mutation positive. A significant association exists between BRAF mutation and multifocality (OR = 1.30; 95% CI = 1.13–1.49) (Fig. 2F and Table 2).

**Vascular invasion**

Four studies, including 505 patients, were analyzed for the association between BRAF mutation and vascular invasion. Of 89 patients with vascular invasion, 45 (50.6%) were BRAF mutation positive, and 78 (43.3%) of 180 patients with no vascular invasion were BRAF mutation positive. There was no significant association found between BRAF mutation and vascular invasion (OR = 1.23; 95% CI = 0.76–2.01) (Fig. 2G and Table 2).

**Absence of tumor capsule**

Four studies, including 1457 patients, were analyzed for the association between BRAF mutation with absence of tumor capsule. Of 959 patients lacking a tumor capsule, 499 (52.0%) were BRAF mutation positive, and 166 (33.3%) of 498 patients with encapsulated PTC were BRAF mutation positive. There was a significant association between BRAF mutation and absence of tumor capsule in patients with PTC (OR = 2.07; 95% CI = 1.64–2.61) (Fig. 2H and Table 2).

**Clinical stage**

Twenty-two studies, including 5014 patients, were analyzed for the association between BRAF mutation and...
PTC (CPTC). Of 1226 CPTC patients, 604 (49.3%) were BRAF mutation positive, and 118 (28.6%) of 412 patients with other subtypes of PTC, including follicular, tall cell, Warthin-like tumor, macrofollicular, diffuse sclerosing, cribriform-morular, columnar, oncocytic, microcystic, and solid/trabecular variants, were BRAF mutation positive. A significant association exists between BRAF mutation and CPTC (OR = 3.23; 95% CI = 2.43–4.29) (Fig. 2J and Table 3).

Seven studies, including 1235 patients, were analyzed for the association of BRAF mutation and tall cell variant of PTC (TCVPTC). Of 79 TCVPTC, 59 (74.7%) patients were BRAF mutation positive, and 483 (41.8%) of 1156 with other subtypes of PTC were BRAF mutation positive. A significant association exists between BRAF mutation and TCVPTC (OR = 4.27; 95% CI = 2.53–7.23) (Fig. 2K and Table 3).

Nine studies, including 1525 patients, were analyzed for the association of BRAF mutation and follicular variant of PTC (FVPTC). Of 194 FVPTC, 48 (24.7%) patients were BRAF mutation positive, and 711 (53.4%) of 1331 non-FVPTC patients were BRAF mutation positive. A significant association exists between BRAF mutation and non-FVPTC (OR = 0.23; 95% CI = 0.16–0.34) (Fig. 2L and Table 3).

**Microcarcinoma**

Six studies presented clinicopathological data corresponding to papillary microcarcinoma (tumor size ≤ 1 cm). In six studies, including 1249 patients, BRAF mutation was significantly associated with lymph node metastases (OR = 1.54; 95% CI = 1.14–2.10). In five studies, including 1102 patients, BRAF mutation was significantly associated with ETE (OR = 2.79; 95% CI = 2.10–3.72). In three studies, including 975 patients, BRAF mutation was significantly associated with clinical stage (III/IV vs. I/II) (OR = 1.72; 95% CI = 1.21–2.42). In four studies, including 1038 patients, BRAF mutation was significantly associated with multifocal disease (OR = 1.39; 95% CI = 1.06–1.82). Association between BRAF mutation and patient age or gender was not significant (Table 2).

**Histological subtype**

Nine studies, including 1638 patients, were analyzed for the association of BRAF mutation and classical variant of BRAF mutation and advanced clinical stage (OR = 1.82; 95% CI = 1.58–2.10) (Fig. 2I and Table 2).

**FIG. 2.** Continued.
Additional analyses

To investigate the presence of publication bias, a funnel plot of effects calculated from individual studies examining the association between \textit{BRAF} mutation and lymph node metastases was performed. Because small studies with negative results do indeed exist in the literature, there is no strong indication of publication bias among the set of studies included in this meta-analysis. Weighting the studies by quality, as described above, did not significantly change our initial results.

Discussion

The majority of prognostic factors currently used to stage and manage patients with PTC depend on final histopathological evaluation that is available only postoperatively. \textit{BRAF} V600E mutation has been proposed as a potential preoperative tool for risk stratification in patients with PTC, guiding whether the patient should or should not undergo prophylactic CLND (10, 16, 26). However, discrepancies exist among studies that have attempted to determine the association between \textit{BRAF} mutation and poor prognosis. This meta-analysis, encompassing 6372 patients in total, found that \textit{BRAF} V600E mutation is associated with several of the variables used in prognostic staging systems, including male gender, classical variant subtype, larger tumor size, multifocality, ETE, regional lymph node metastasis, absence of tumor capsule, and advanced clinical stage (III/IV vs. I/II) (6) (Fig. 3). \textit{BRAF} V600E mutation, however, was not associated with advanced age ($\geq$45 yr) or vascular invasion.

With respect to study design of the manuscripts included in the meta-analysis, however, there were several limitations. Only two of the 32 studies (20, 21) evaluated their cohorts prospectively, and only nine studies included consecutive patients (9, 10, 13, 16, 20–24), whereas Guan \textit{et al}. (12) examined randomly selected patients. Because the majority of studies, including Basolo \textit{et al}. (10), who studied 1060 patients, did not analyze consecutive patients, there may exist a possible bias toward larger tumors, because these would be more readily available for collection and genetic analysis. This limitation also allows for selection bias toward patients with better-documented disease. For example, patients with known PTC on FNA would more likely undergo LND than those in whom the diagnosis was unclear (e.g. FVPTC), and therefore would more likely have metastases identified. Indeed, a subset analysis of the two prospective studies found no significant association between \textit{BRAF} mutation and lymph node metastases. Of the two studies, only So \textit{et al}. (21) found a significant association between \textit{BRAF} mutation and lymph node metastases.

Of 32 studies, So \textit{et al}. (21) and Lee \textit{et al}. (25) were the only ones to report the use of routine bilateral central neck
TABLE 2. Association of BRAF mutation with clinicopathological features of all PTCs and of micro-PTCs

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>OR</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>All PTCs</td>
<td></td>
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<tr>
<td>Male gender</td>
<td>1.22</td>
<td>1.05–1.41</td>
</tr>
<tr>
<td>Age</td>
<td>0.91</td>
<td>0.76–1.10</td>
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<tr>
<td>Size</td>
<td>1.57</td>
<td>1.29–1.92</td>
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<tr>
<td>Lymph node metastasis</td>
<td>1.72</td>
<td>1.53–1.94</td>
</tr>
<tr>
<td>ETE</td>
<td>2.60</td>
<td>2.27–2.99</td>
</tr>
<tr>
<td>Multifocality</td>
<td>1.30</td>
<td>1.13–1.49</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>1.23</td>
<td>0.76–2.01</td>
</tr>
<tr>
<td>Absence of capsule</td>
<td>2.07</td>
<td>1.64–2.61</td>
</tr>
<tr>
<td>Advanced clinical stage</td>
<td>1.82</td>
<td>1.58–2.10</td>
</tr>
<tr>
<td>Microcarcinomas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>1.1</td>
<td>0.80–1.50</td>
</tr>
<tr>
<td>Age</td>
<td>0.63</td>
<td>0.46–0.87</td>
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<tr>
<td>Lymph node metastasis</td>
<td>1.54</td>
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<td>1.21–2.42</td>
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* Statistically significant for aggressive prognostic features.

Discrepancies in their sensitivities, may contribute to the differing results. The majority of studies used direct sequencing (7, 11–15, 19–23, 27–35). Other methods of mutation detection included fluorescence melting-curve analysis (9, 16), single-strand conformation polymorphism (SSCP) (10, 24, 36–39), restriction fragment length polymorphism analysis (25, 40, 41), mutation allel-specific amplification (MASA) (38, 42), and shifted termination assay (STA) (17). With regard to the different methods, Sapio et al. (38) compared DNA sequencing, SSCP, and MASA to determine which detection method was most sensitive. Using a mixed sample of wild-type and BRAF mutant DNA, they found that SSCP and DNA sequencing were equally sensitive, able to detect BRAF V600E mutation at concentrations down to 60%. MASA was the most sensitive, able to detect BRAF mutation down to a concentration of 20%. Shackelford et al. (43) demonstrated that the STA was capable of detecting low-copy-number heterogeneous clinical samples with a higher sensitivity compared with single-base primer-extension methods. When STA was applied to the detection of the BRAF mutation in a clinical study by Shackelford et al. (43), the STA was able to correctly identify BRAF mutation status in all 90 samples, whereas PCR restriction enzyme analysis misclassified 10 wild-type samples as mutant and direct sequencing misclassified one mutant sample as wild type. Because direct sequencing methods will also read wild-type DNA mixed within the clinical sample, a misreading will occur any time there is greater than 80% wild-type DNA and less than 20% mutant DNA (43). Conversely, STA can detect mutant DNA in concentrations as low as 1% (43). Xing et al. (26) evaluated the utility of STA in the detection of BRAF mutation in FNA specimens and found that STA exhibited 100% sensitivity and specificity compared with direct DNA sequencing. Given the heterogeneous nature of FNA specimens, STA seems to be the most sensitive method for BRAF mutation detection at the preoperative level. The majority of past studies used direct sequencing, which can give false-negative readings if the mutant DNA concentrations fall below 20%.

Our meta-analysis showed that prevalence of BRAF mutation is highest in the TCVPTC (74.7%) and lowest in the FVPTC (24.7%), with an intermediate prevalence in the CPTC (49.3%). This is not surprising because TCVPTC is known to behave more aggressively compared with CPTC, with higher rates of ETE, older age at presentation, higher risk of locoregional and distant relapse, and decreased survival (44, 45). With other major prognostic factors controlled for, TCVPTC has been shown to be an independent prognostic factor for disease-specific death (44). Compared with CPTC, FVPTC exhibits less
aggressive behavior, with lower rates of cervical lymph node metastases and ETE (46). With the exception of four studies (12, 14, 24, 32) that focused on only CPTC, the majority of studies included in this meta-analysis either did not specify or explicitly included a mixture of different histological subtypes of PTC. Because different subtypes have varying disease patterns and \( \text{BRAF} \) mutation prevalence, the results of these past studies may be skewed depending on the composition of the tumor collection analyzed. Furthermore, current criteria for diagnosing FVPTC are often not uniformly agreed upon as Elsheikh et al. (47) have demonstrated both significant interobserver and intraobserver variation in the diagnosis of FVPTC. Complete agreement among experts in diagnosing FVPTC was observed in only 13% of cases, and intraobserver agreement ranged from 17–100%. The inclusion of misdiagnosed follicular adenomas, which would exhibit extremely low rates of \( \text{BRAF} \) mutation, might also skew the results toward a stronger association between \( \text{BRAF} \) mutation and aggressive features. Therefore, a histological homogeneous population would be more informative.

In summary, meta-analysis of the literature shows \( \text{BRAF} \) V600E mutation correlates with poor prognostic features of PTC. Most studies, however, use disparate methods of \( \text{BRAF} \) mutation detection, do not evaluate only patients who have undergone routine CLND, and include a heterogeneous mix of PTC subtypes. Before one recommends that \( \text{BRAF} \) mutation be incorporated into the management algorithm of thyroid cancer, additional well-designed prospective trials that include only patients who have undergone routine CLND are needed to address these limitations, particularly with regard to recommending prophylactic LNDs in patients who have \( \text{BRAF} \)-positive tumors.

**Acknowledgments**

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References

4. Mazaferri EL 2009 What is the optimal initial treatment of low-risk papillary thyroid cancer (and why is it controversial)? Oncology (Williston Park) 23:579–588
27. Kim SK, Song KH, Lim SD, Lim YC, Yoo YB, Kim JS, Hwang TS 2009 Clinical and pathological features and the BRAF(V600E) mutation in patients with papillary thyroid carcinoma with and without concurrent Hashimoto thyroiditis. Thyroid 19:137–141


42. Xu X, Quiros RM, Gattuso P, Ain KB, Prinz RA 2003 High prevalence of BRAF gene mutation in papillary thyroid carcinomas and thyroid tumor cell lines. Cancer Res 63:4561–4567


