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

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Abbreviations: CI, Confidence interval; CLND, central LND; CPTC, classical variant of PTC; ETE, extrathyroidal extension; FNA, fine-needle aspiration; FVPTC, follicular variant of PTC; MASA, mutation allele-specific amplification; OR, odds ratio; PTC, papillary thyroid cancer; SSCP, single-strand conformation polymorphism; STA, shifted termination assay; TCVPTC, tall cell variant of PTC.

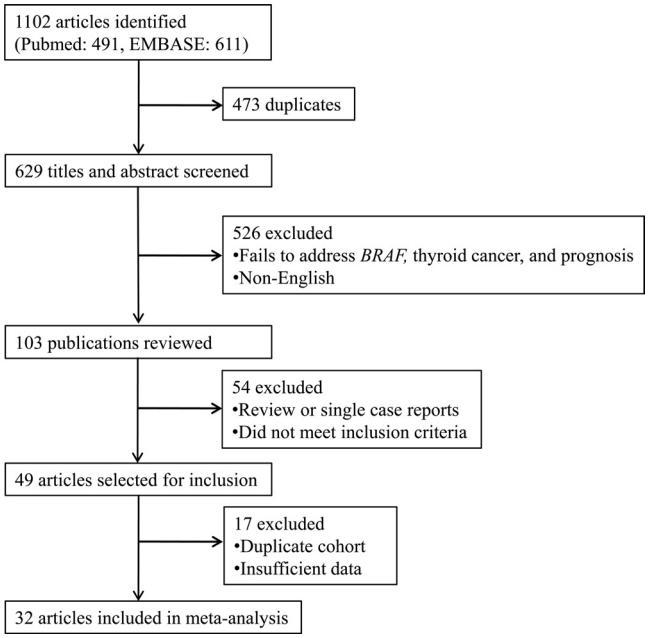


FIG. 1. Article selection process.

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

TABLE 1. A summary of the 32 studies included in the meta-analysis and the associated prognostic factors examined

Study	Total number	*BRAF* mutation (%)	Gender	Age	Size	LNM	ETE	Clinical stage	Histological subtype	Multifocality	Absence of tumor capsule	Vascular invasion
Adeniran *et al.*, 2006 (9)	96	41.7	Y	N	N	Y	Y	Y	Y	N	N	N
Basolo *et al.*, 2010 (10)	1060	44.6	Y	Y	Y	Y	Y	Y	N	Y	Y	N
Czarniecka *et al.*, 2010 (27)	88	43.2	Y	N	N	Y	Y	N	N	Y	N	N
Elisei *et al.*, 2008 (36)	102	37.3	Y	N	N	Y	Y	Y	N	Y	N	Y
Frasca *et al.*, 2008 (11)	323	38.7	Y	N	N	Y	Y	Y	Y	Y	N	N
Fugazzola *et al.*, 2004 (14)	56	32.1	Y	Y	N	Y	N	Y	N	Y	N	N
Goutas *et al.*, 2008 (40)	55	27.3	Y	Y	N	Y	N	Y	N	N	N	N
Guan *et al.*, 2009 (12)	1032	61.9	N	N	N	Y	Y	Y	N	N	N	N
Ito *et al.*, 2009 (15)	631	38.4	Y	N	Y	Y	N	Y	Y	N	N	N
Jo *et al.*, 2006 (28)	161	63.4	Y	N	N	Y	Y	Y	N	N	N	N
Jung *et al.*, 2010 (29)	210	77.1	Y	Y	Y	Y	Y	N	Y	Y	N	N
Kim *et al.*, 2005 (30)	79	81.0	Y	Y	N	Y	N	N	N	N	N	N
Kim *et al.*, 2006 (13)	103	33.0	Y	N	N	Y	N	Y	N	N	N	N
Kim *et al.*, 2006 (32)	203	73.4	Y	N	N	Y	Y	Y	N	Y	N	N
Kim *et al.*, 2009 (31)	101	87.1	Y	N	N	N	N	N	N	N	N	N
Kwak *et al.*, 2009 (22)	339	62.8	Y	N	N	Y	Y	Y	N	Y	N	N
Lee *et al.*, 2009 (25)	64	37.5	Y	N	N	Y	Y	N	Y	N	Y	N
Lee *et al.*, 2006 (33)	100	58.0	Y	N	N	Y	Y	Y	N	N	N	Y
Lin *et al.*, 2010 (34)	61	34.4	Y	N	N	Y	Y	N	N	Y	N	N
Liu *et al.*, 2005 (23)	105	46.7	Y	Y	N	Y	Y	Y	Y	Y	N	N
Nakayama *et al.*, 2007 (19)	40	65.0	Y	N	N	Y	Y	Y	N	N	N	N
Namba *et al.*, 2003 (7)	126	30.2	Y	Y	N	Y	Y	Y	N	N	N	N
O'Neill *et al.*, 2010 (41)	101	59.4	Y	N	N	Y	Y	Y	Y	Y	Y	Y
Oler *et al.*, 2009 (24)	120	48.3	Y	Y	N	Y	Y	N	N	Y	N	N
Pelizzo *et al.*, 2011 (20)	141	69.5	Y	N	Y	Y	Y	Y	Y	Y	N	N
Riesco-Eizaguirre *et al.*, 2006 (37)	67	41.8	Y	N	N	Y	Y	Y	Y	Y	N	N
Sapio *et al.*, 2006 (38)	43	44.2	Y	N	N	Y	N	Y	Y	Y	N	N
So *et al.*, 2011 (21)	71	62.0	N	N	N	Y	N	N	N	N	N	N
Sykorova *et al.*, 2010 (39)	242	33.5	Y	Y	N	Y	N	Y	N	Y	Y	Y
Xing *et al.*, 2009 (12)	190	38.4	Y	N	N	Y	Y	Y	N	Y	N	N
Xu *et al.*, 2003 (42)	56	37.5	Y	N	N	Y	N	N	N	N	N	N
Yip *et al.*, 2009 (16)	206	51.5	Y	N	N	Y	Y	N	Y	Y	N	N
Total (patients, prevalence, number of studies examined)	6372	50.9	30	9	4	31	22	22	11	18	4	4

Y indicates that the study was evaluated for the corresponding prognostic factor; N indicates that the study was not evaluated for the corresponding prognostic factor.

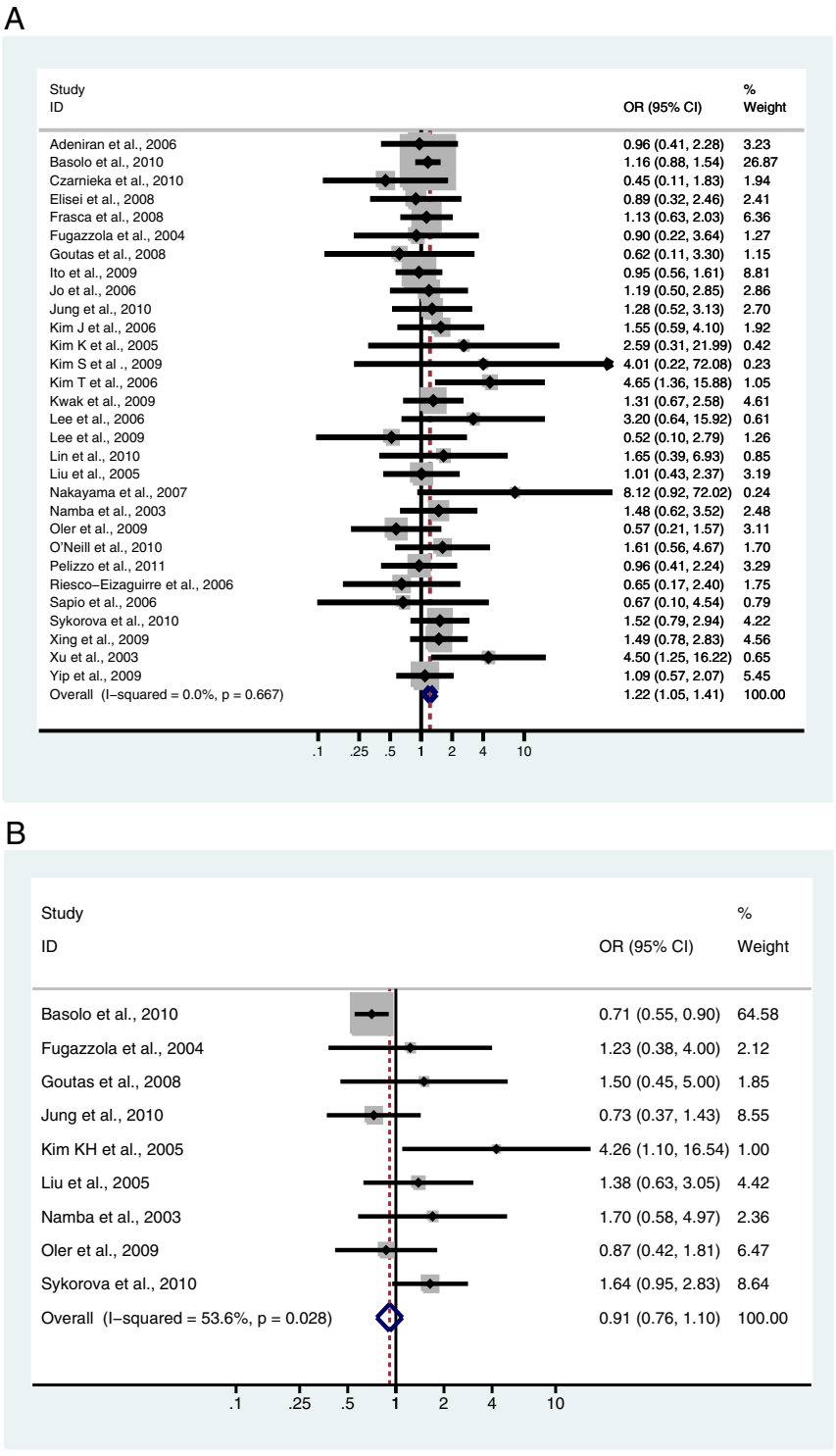
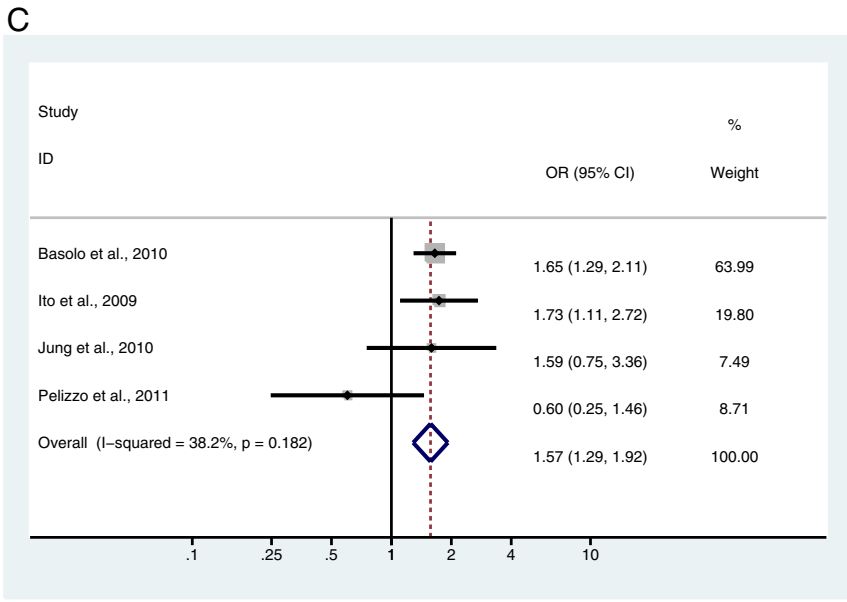


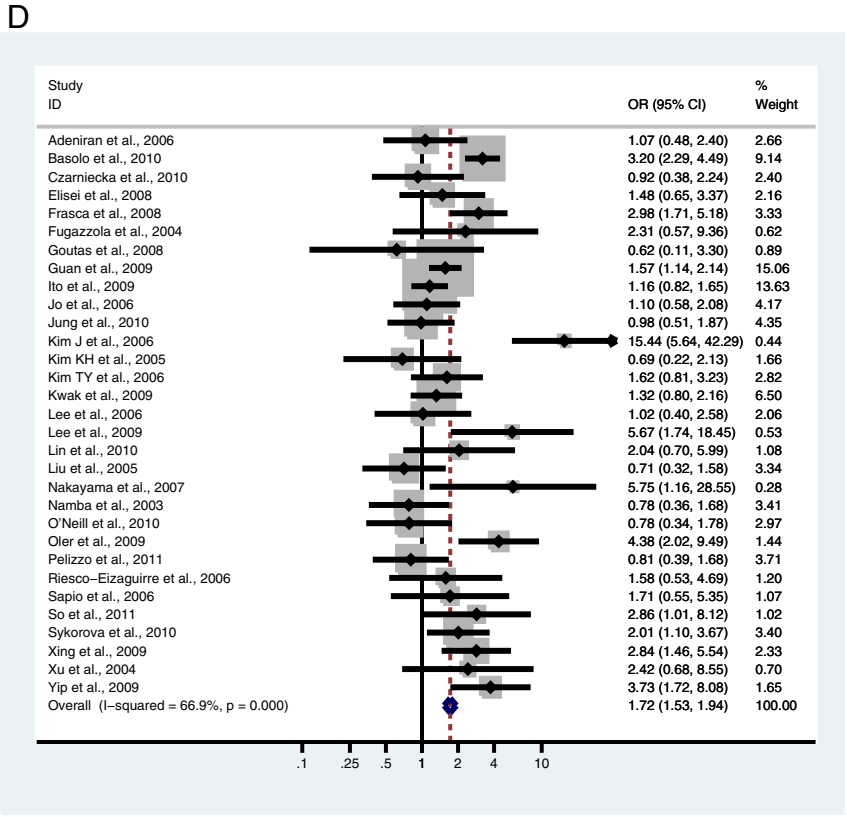
FIG. 2. A–L, Study-specific and Mantel-Haenszel pooled OR and 95% CI for the association between *BRAF* mutation and gender (A), age (B), tumor size (C), lymph node metastases (D), ETE (E), multifocality (F), vascular invasion (G), absence of capsule (H), clinical stage (I), classical variant (J), tall cell variant (K), and follicular variant (L) in patients with PTC.

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 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 a 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 reexamine 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.

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.





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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, multicentricity, extrathyroidal extension, extracapsular invasion, lymph node metastasis, histological subtype, variant, and other clinicopathological features. Full-text articles were then reviewed in their entirety and selected if they studied the prognostic significance of *BRAF* mutation by examining the association between *BRAF* V600E mutation and any clinicopathological features of PTC. Specific features including patient age, gender, tumor size, 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 clinicopathologic 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

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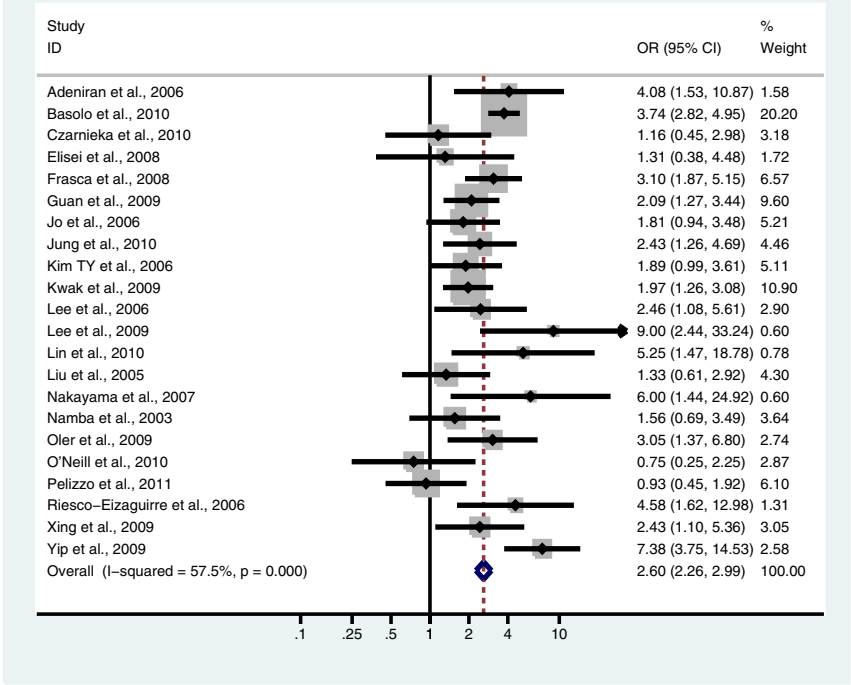

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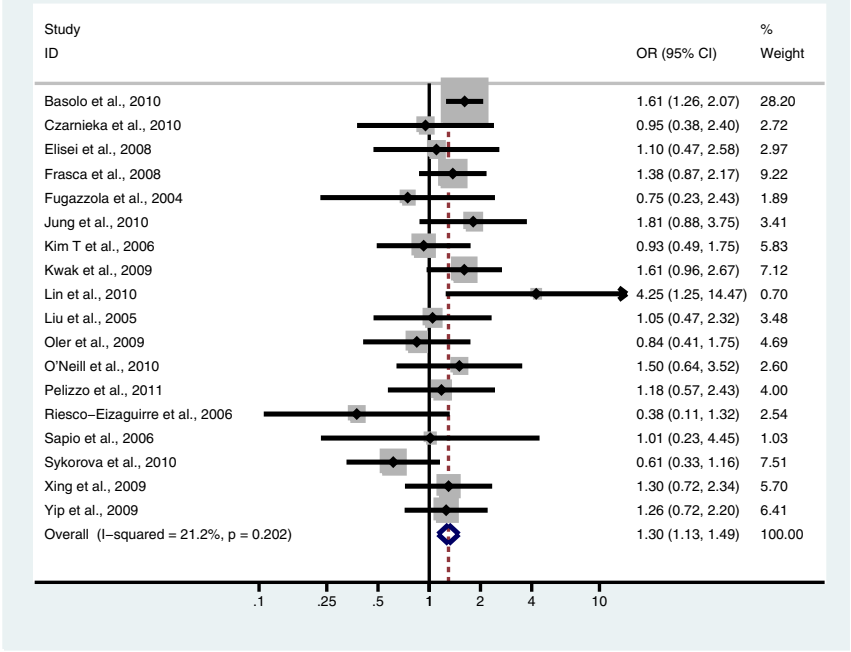

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patients had *BRAF* mutation-positive PTCs. The earliest study was published in September 2003, and the latest study was published in September 2011. The largest study by Basolo *et al.* (10) included 1060 patients, and the smallest study by Nakayama *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-

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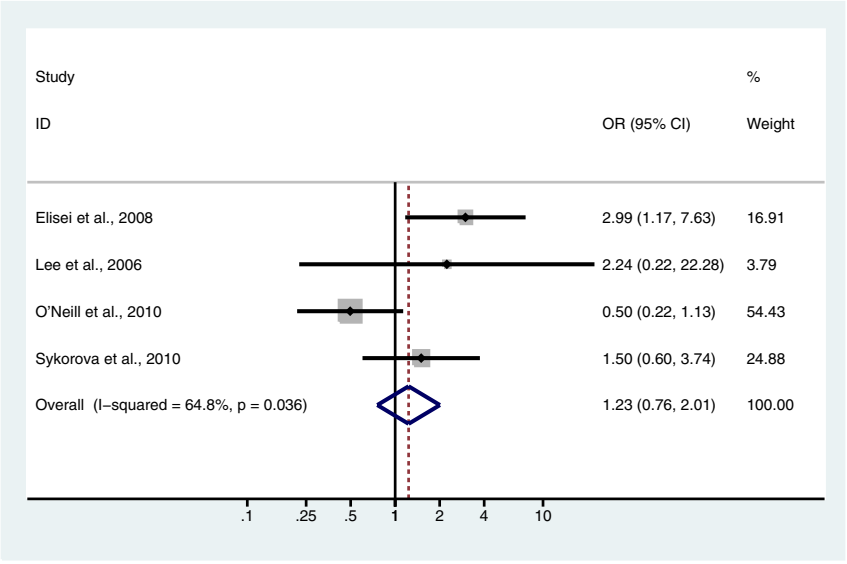

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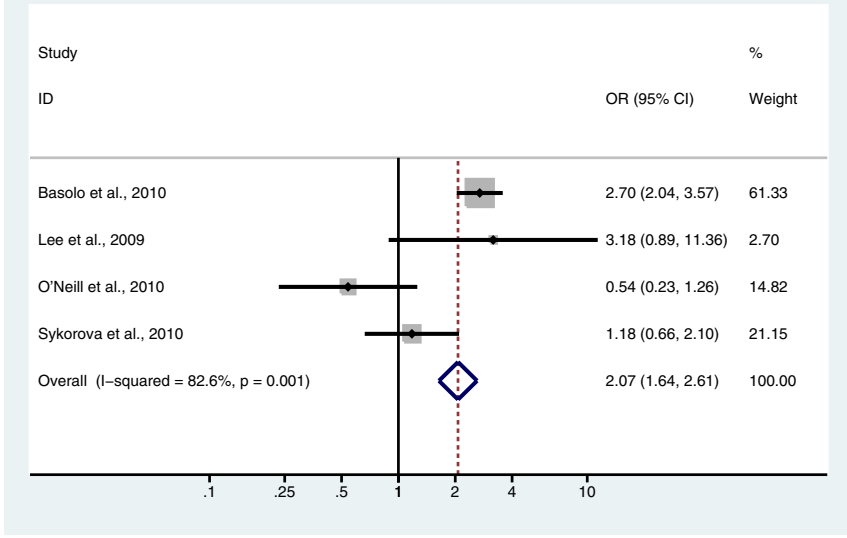

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tients, 1293 (58.9%) tested positive for the *BRAF* mutation, and 1688 (46.1%) of 3665 lymph node-negative patients tested positive for the *BRAF* mutation. A significant association was found between *BRAF* mutation and the presence of lymph node metastases (OR = 1.72; 95% CI = 1.53–1.94) (Fig. 2D and Table 2). Twenty-nine of 32 studies either did not specify the type of LND performed or stated that patients underwent varying extents of thyroidectomies and therapeutic LND. Kim *et al.* (13) included patients who received lymphatic mapping and sentinel LNDs. Only So *et al.* (21) and Lee *et al.* (25) used prospectively collected patients who underwent routine bilateral CLND.

Extrathyroidal extension

Twenty-two studies, including 4668 patients, were analyzed for the association between *BRAF* mutation and ETE. Of 1547 patients with ETE, 1064 (68.8%) were positive for the *BRAF* mutation, and 1414 (45.3%) of 3121 patients with no ETE were positive for the *BRAF* mutation. There was a significant association between *BRAF* mutation and ETE in patients with PTC (OR = 2.60; 95% CI = 2.27–2.99) (Fig. 2E 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 of *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

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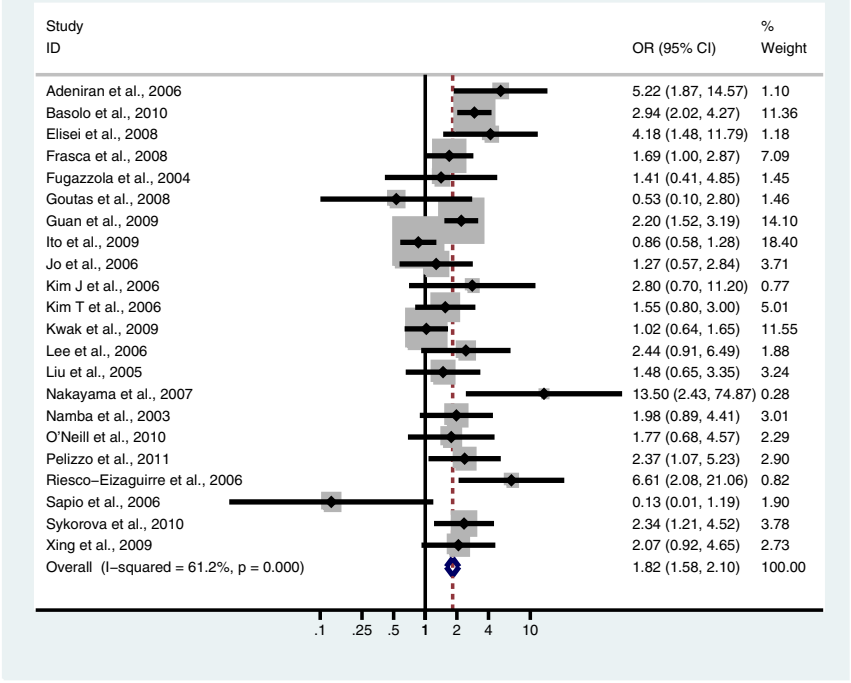

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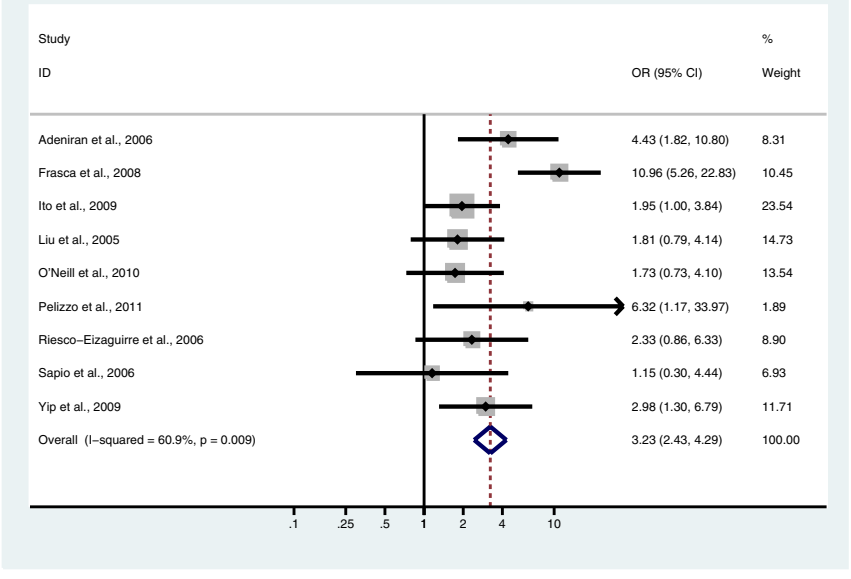

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clinical stage. Of 3813 patients diagnosed with stage I or stage II disease, 1742 (45.7%) were *BRAF* mutation positive, and 731 (60.9%) of 1201 patients diagnosed with stage III or stage IV disease were *BRAF* mutation positive. There was a significant association between *BRAF* mutation and advanced clinical stage (OR = 1.82; 95% CI = 1.58–2.10) (Fig. 2I and Table 2).

Histological subtype

Nine studies, including 1638 patients, were analyzed for the association of *BRAF* mutation and classical variant of 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, micropapillary, 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).

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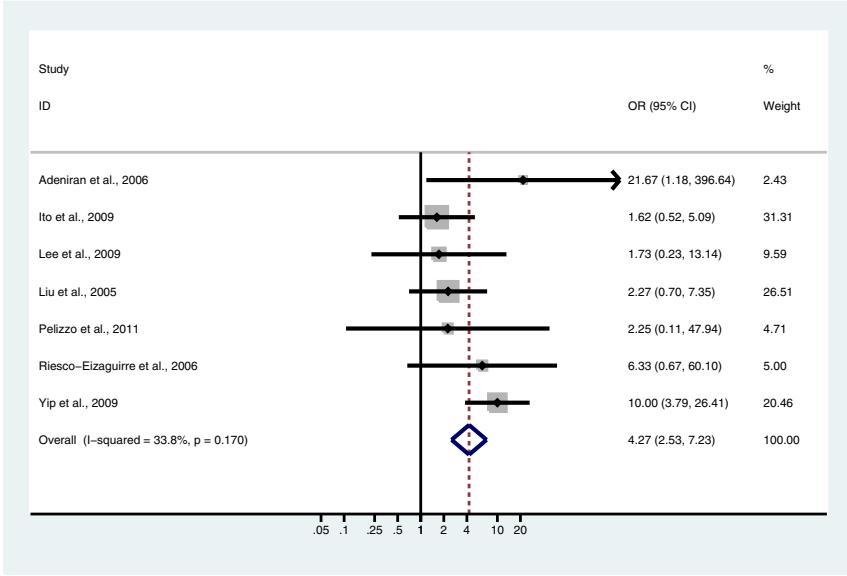

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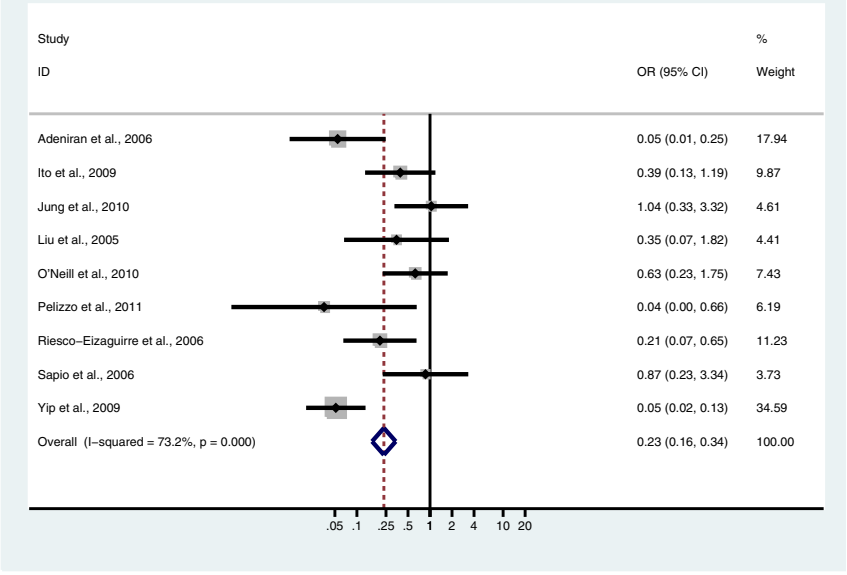

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Additional analyses

To investigate the presence of publication bias, a funnel plot of effects calculated from individual studies examining the association between *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. *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 *BRAF* mutation and poor prognosis. This meta-analysis, encompassing 6372 patients in total, found that *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). *BRAF* V600E mutation, however, was not associated with advanced age (≥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 *et al.* (12) examined randomly selected patients. Because the majority of studies, including Basolo *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 *BRAF* mutation and lymph node metastases. Of the two studies, only So *et al.* (21) found a significant association between *BRAF* mutation and lymph node metastases.

Of 32 studies, So *et al.* (21) and Lee *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

Prognostic factor	OR	95% CI
All PTCs		
Male gender[a]	1.22	1.05–1.41
Age	0.91	0.76–1.10
Size[a]	1.57	1.29–1.92
Lymph node metastasis[a]	1.72	1.53–1.94
ETE[a]	2.60	2.27–2.99
Multifocality[a]	1.30	1.13–1.49
Vascular invasion	1.23	0.76–2.01
Absence of capsule[a]	2.07	1.64–2.61
Advanced clinical stage[a]	1.82	1.58–2.10
Microcarcinomas		
Male gender	1.1	0.80–1.50
Age	0.63	0.46–0.87
Lymph node metastasis[a]	1.54	1.14–2.10
ETE[a]	2.79	2.10–3.72
Multifocality[a]	1.39	1.06–1.82
Advanced clinical stage[a]	1.72	1.21–2.42

[a] Statistically significant for aggressive prognostic features.

dissections for their patient population. So *et al.* (21) demonstrated an association only in tumors 0.5–1 cm in size, and Lee *et al.* (25) in only microcarcinomas. The pathological data included in the other 30 studies to determine the association between LNM and *BRAF* mutation could only have been obtained from those patients who underwent CLND for a suspicious feature, seen on ultrasound for example or noted at the time of surgery, thus biasing the study toward the evaluation of only patients who had significant lymphadenopathy. Those patients who would have undergone neck dissections due to a suspicion on ultrasound, other imaging, or intraoperatively represent only a subset of the target population for the potential use of the *BRAF* biomarker. Were one to suggest using *BRAF* preoperatively, one would by definition be proposing that patients who were positive undergo CLND even without any evidence of significant nodal disease. Furthermore, and on a practical matter, proposing that patients who are *BRAF* positive should undergo routine CLND because 59% are likely to have metastases as opposed to 46% of those who are *BRAF* negative harbor metastases appears to be based upon a statistical result rather than what would be applicable clinically.

Differences in *BRAF* mutation detection methods were also used among the 32 studies analyzed and, given the 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 allele-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%.

TABLE 3. Association of *BRAF* mutation with histological subtype of PTC

	OR	95% CI
Classical	3.23	2.43–4.29
Tall cell	4.27	2.53–7.23
Follicular	0.23	0.16–0.34

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

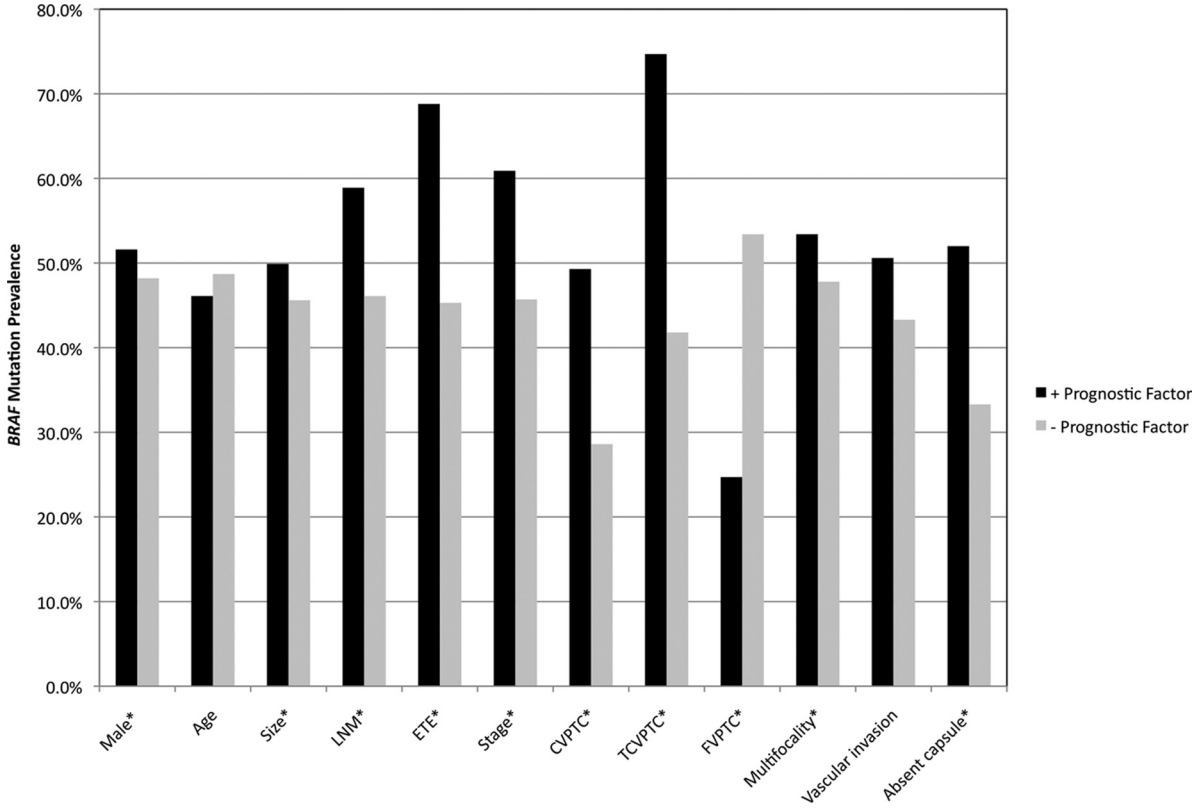

FIG. 3. *BRAF* mutation prevalence among patients with and without specific prognostic factors. The term +prognostic factor indicates *BRAF* mutation prevalence among patients positive for the corresponding prognostic factor, and −prognostic factor indicates *BRAF* mutation prevalence among patients negative for the corresponding prognostic factor. *, Statistically significant association between *BRAF* mutation and aggressive prognostic features.

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 *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 *BRAF* mutation, might also skew the results toward a stronger association between *BRAF* mutation and aggressive features. Therefore, a histological homogeneous population would be more informative.

In summary, meta-analysis of the literature shows *BRAF* V600E mutation correlates with poor prognostic features of PTC. Most studies, however, use disparate methods of *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 *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 *BRAF*-positive tumors.

Acknowledgments

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