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A meta-analysis evaluating the relationship between B-type Raf kinase mutation and cervical lymphatic metastasis in papillary thyroid cancer

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Abstract

Background: B-type Raf kinase (BRAF) mutation is proved to be a critical predictive factor in papillary thyroid cancer (PTC) with aggressive characteristics. However, the association between BRAF mutation and cervical lymphatic metastasis in PTC is controversial.

Methods: We searched papers on the study of BRAF mutation and cervical lymphatic metastasis in PTC patients through PubMed, Web of Science, Embase, and Cochranelibrary. The BRAF (+) cases, BRAF (–) cases, and cervical lymphphatic metastatic cases in both BRAF (+) and BRAF (–) groups were collected. After Quality assessment, statistical Analysis (funnel plot and Harbord evaluation, Random-effect model, heterogeneity, subgroup analysis, sensitivity analysis, and metacum analysis) were done by the Review Manager (RevMan) 5.3 and stata14 statistical software.

Results: There were 78 cross-section studies which met our inclusion criteria. And all of them had no selection bias, publication bias, or any other bias. A significant association existed between BRAF mutation and cervical lymph node metastasis (LNM) (odds ratio [OR] = 1.63; 95% confidence interval [CI]: 1.44–1.84; $P < .05$). Overall, 46 studies were conducted among East Asians. Twenty four articles had provided the data of central lymph node metastasis (CLNM), 11 articles with the data of lateral lymph node metastasis (LLNM), and classic/conventional PTC (CPTC) was analyzed in 10 studies. Subgroup analyses were performed based on ethnicity, metastatic site, and subtype of PTC. Significant association between BRAF (+) mutation and cervical LNM were indicated in East Asians (OR = 1.73; 95% CI: 1.49–2.02; $P < .05$), in non-East Asians (OR = 1.57; 95% CI: 1.26–1.96; $P < .05$), and in CLNM (OR = 1.80; 95% CI: 1.56–2.07; $P < .05$). While no significant association was found in LLNM (OR = 1.37; 95% CI: 0.76–2.48; $P = .29 > .05$) and in CPTC (OR = 1.32; 95% CI: 0.97–1.80; $P = .08 > .05$). We did not find any other major changes when sensitivity analysis was performed. The metacum analysis showed no significant association existed before 2012. While a significant association began to exist between BRAF mutation and LNM from 2012, and this association became stable from 2017.

Conclusions: We consider that a significant association exists between BRAF mutation and cervical LNM. Further meta-analysis on subgroup may reveal some valuable factors between BRAF gene mutation and LNM. And we do not recommend that BRAF (+) as the biomarker for LNM in PTC.

Abbreviations: BRAF = B-type Raf kinase, CIs = confidence intervals, CLNM = central lymph node metastasis, CPTC = classic/conventional PTC, LLNM = lateral lymph node metastasis, LNM = lymphatic metastasis, OR = odds ratio, PTC = papillary thyroid cancer, RevMan = Review Manager.

Keywords: B-type Raf kinase mutation, lymphatic metastasis, meta-analysis, papillary thyroid cancer

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Ethical approval and informed consent is not applicable in this study.

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1. Introduction

The B-type Raf kinase (BRAF) gene, found on chromosome 7q24, encodes a cytoplasmic serinethreonine protein kinase, is the major activator of MAPK signal pathway, and is proved to play a key role in the development of many malignant tumors.[1–3] The study on BRAF gene in thyroid cancers began in the early of 21st century, and BRAF mutation is considered as one of the most important molecular biomarkers in papillary thyroid cancer (PTC). Approximately 99.8% of BRAF mutation in thyroid nodules is associated with thyroid cancer. The average frequency of BRAF mutation in PTC is around 45%, making BRAF mutations the most common defined genetic abnormality in thyroid cancers.[4,5] Studies in the past years showed that BRAF mutation is a critical predictive factor in PTC with aggressive characteristics. BRAF (+) mutation was associated with T stage, extracapsule invasion, RAI refractory, and low overall survival (OS).[6–10] However, the association between BRAF mutation and cervical lymph node metastasis (LNM) in PTC is controversial. Few studies had done researches on the association between BRAF and cervical metastatic sites of LNM, such parapretracheal region (which is defined as central lymph node metastasis) and Level II, III, IV, V (defined as lateral lymph node metastasis). Chen et al,[11] Jeong et al,[12] Lu et al[13] thought there were a significant relationship between BRAF mutation and LNM, while Tuccilli et al,[14] Eloy et al,[15] Guan et al[16] did not agree with them according to their studies. The incidence of thyroid nodules has been rising in recent years. Statistics showed that about 19% to 67% of the population was affected by thyroid nodules, and among which 5% to 15% had malignant nodules.[17] Thyroid carcinoma is the most common endocrine malignancy and accounts for 3% to 4% of all cancers in the United States. The estimated incidence in 2017 is 57,000 (15,000 men, 42,000 women) with 2000 deaths (900 men, 1100 women).[18] The prevalence of cervical central lymph node metastasis (CLNM) can reach as high as 7% to 65%.[19–21] High resolution ultrasound is preferred for the detection of metastatic lymph nodes. However, it is difficult to evaluate the metastatic lymph nodes in PTC because of the narrow central area surrounded by bones. Surgeries including resection of primary tumor and neck dissection are the major treatment of PTC. But neck dissection is associated with the risk of damage to recurrent laryngeal nerve and mistaken removal of parathyroid gland, leading to vocal fold paralysis, dyspnea, or even permanent tracheotomy and spasm due to the permanent hypoparathyroidism. So it will bring great advantages to patients, if an effective method to predict the LNM in PTC can be found. According to the studies before, BRAF has a great possibility to be such a biomarker to predict LNM in PTC.

We searched 476 articles from PubMed, Web of Science, Embase, and Cochranelibrary, which included the relationship between BRAF mutation and LNM. Seventy-eight cross-sectional studies were selected into our study carefully. A strict standard meta-analysis was performed with the largest studies, and subgroup meta-analysis was first done in 3 subgroups: ethnicity, metastatic site, and subtype of PTC. The association between BRAF mutation and cervical LNM were considered and analyzed comprehensively in our study.

2. Materials and methods

Ethical approval was not necessary, because this is a meta-analysis of 78 cross-section studies which have been published without ethical controversies.

2.1. Inclusion criteria

Studies selected into this meta-analysis should be cross-sectional studies investigating the association between BRAF mutation and the risk of cervical LNM in papillary thyroid cancer, and written in English. Papers without the BRAF (+) cases, BRAF (–) cases, and cervical lymphatic metastatic cases in both BRAF (+) and BRAF (–) group should be removed. Papers including people <18 years old were excluded from our study. BRAF mutation should be detected from the primary thyroid tumor, and BRAF mutation got from metastatic sites or blood were also excluded from our study.

2.2. Literature search

We searched PubMed, Web of Science, Embase, and Cochranelibrary up to November 28, 2018 with the search strategy: PubMed: (((((((“Thyroid cancer, papillary”) OR “Papillary thyroid carcinoma”[Title/Abstract]) OR “Papillary Carcinoma Of Thyroid”[Title/Abstract]) OR “Thyroid carcinoma, papillary”[Title/Abstract]) OR “Nonmedullary Thyroid Carcinoma”[Title/Abstract]) OR “Familial Nonmedullary Thyroid Cancer”[Title/Abstract])) AND ((((“Lymphatic Metastasis”) OR “Lymphatic Metastases”[Title/Abstract]) OR “Metastases, Lymphatic”[Title/Abstract]) OR “Metastasis, Lymphatic”[Title/Abstract])) AND (((“BRAF protein, human”) OR “v-raf murine sarcoma viral oncogene homolog B1, human”[Title/Abstract]) OR “B-Raf protein, human”[Title/Abstract]);

Web of science: (“Papillary thyroid carcinoma” OR “thyroid papillary carcinoma” OR “Papillary thyroid cancer”) AND (“B Raf kinase” OR “BRAF Kinases” OR “B-Raf protein, human”) AND (“Lymphatic Metastasis” OR “lymph node metastasis”);

Embase: “thyroid papillary carcinoma” AND “lymph node metastasis” AND “B Raf kinase”

Cochranelibrary: (“Thyroid neoplasms” OR “Thyroid Cancers; Thyroid Carcinomas”) AND (“Lymphatic Metastasis” OR “Lymphatic Metastases”) AND (“Proto-Oncogene Proteins B-raf” OR “B-raf Kinases; BRAF Kinases”).

Additionally, if the same author published >1 studies based on the same case series, we will select the study of most recent publication or with the largest sample size. Any disagreement was settled by discussion and subsequently consensus with the authors.

2.3. Quality assessment

The quality assessment of each study was carried out independently by 2 authors. Quality appraisal of quantitative and qualitative studies was carried out using CochraneROB quality assessment scale. The checklists from the CochraneROB were used to assess and assign a quality score. Any disagreement regarding the quality of the study were resolved after discussion, and referred to a third author, if necessary.

2.4. Data extraction

We extracted the following information from each study: the first author, year of publication, patient sex, association of BRAF (+) with cervical LNM in each paper, the number of BRAF (+) cases, the number of LNM cases in BRAF (+) group, the number of BRAF (–) cases, the number of LNM cases in BRAF (–) group, and the details of subgroups (including ethnicity, metastatic site, and subtype of PTC if provided).

2.5. Statistical analysis

The Review Manager (RevMan) 5.3, stata14, and R 3.6.1 statistical software were employed to deal with quantitative data. Firstly, funnel plot and Harbord were constructed to evaluate whether publication bias might influence the validity of the estimates. A fixed or random effect model was then used to measure the risk of cervical LNM (odds ratio: OR) and its 95% confidence intervals (CIs) through RevMan5.3. The significance of the pooled estimate was made using the Z-test, and if $P < .05$, is considered to be statistical significant. Cochran Q-statistic was applied to estimate the degree of heterogeneity among studies. The I^2 test was also used to quantify the heterogeneity (range from 0% to 100%). Random-effect model was used when a significant Q-test with $P < .05$ or $I^2 > 50\%$. Fixed-effects model was adopted when there was no statistical heterogeneity. In order to explore the potential sources of heterogeneity, subgroup analyses were performed based on ethnicity (East Asians, Non East Asians), metastatic site (central lymph node metastasis: CLNM, lateral lymph node metastasis: LLNM), and subtype of PTC (classic/conventional PTC: CPTC). To evaluate the influence of individual study on overall estimate, we performed a sensitivity analysis by omitting each study in turn. Finally, we use Meta cum to assess the stability of the results. All tests were 2-sided and a P value of $<.05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics of included studies

Figure 1 shows the PRISMA diagram of how the studies were identified and screened. A total of 476 articles associated with the searched keywords were identified at first (PubMed: 126 papers; Embase: 290 papers; Web of Science: 59 papers, and Cochranelibrary: 1 paper). Of these articles, 95 were excluded due to the duplicates; 26 studies were removed because we could not get the full text. The full text of the left 355 papers was obtained. Another 270 papers were eliminated after reviewing the full text, and also 7 papers were removed for they were from the same author with the same case series. Eventually, 78 studies met our inclusion criteria were enrolled in our qualitative research after removing all unqualified records and the review papers.[11–16,22–93]

Table 1 lists the studies that were included in this meta-analysis and shows the baseline characteristics of all eligible studies. These 78 retrospective cohort studies included 25,906 PTC patients, among which 17,196 cases are BRAF mutation positive while 8710 are BRAF mutation negative. Overall, 46 studies were conducted among East Asians (China, South Korea, and Japan). Real-time PCR was performed to detect the BRAF mutation in 75 studies. Twenty four articles had provided the data of CLNM, 11

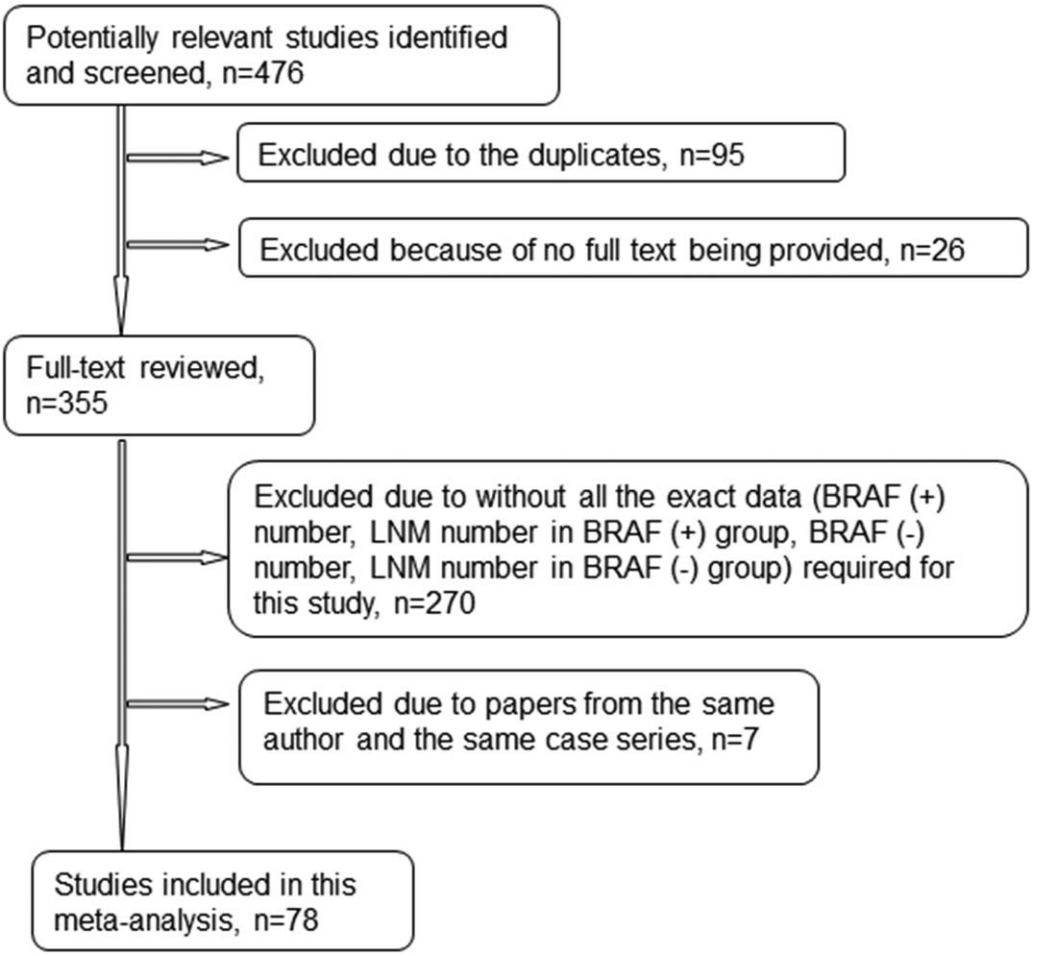

Figure 1. The PRISMA flow diagram of study selection for this meta-analysis.

Table 1

The summary of the 78 studies included in the meta-analysis.

No	First author	Year	Country	CPTC analysis	CLNM analysis	LLNM analysis	BRAF+	LNM in BRAF+	BRAF–	LNM in BRAF–
1	Adegptxjx le Chen	2018	China		y		191	92	34	7
2	Agnieszka Walczyk	2014	Poland				78	39	35	20
3	Ah Young Park	2014	South Korea		y		476	148	212	38
4	Aldona Kowalska	2017	Poland				475	60	248	35
5	Alexander Abrosimov	2006	Russian Federation				23	10	17	11
6	Ali S. Alzahran	2013	USA		y		96	37	185	38
7	Alona Finke	2016	Israel				49	12	10	3
8	Avik Chakraborty	2012	India				46	35	40	12
9	Aylin Yazgan	2016	Turkey	y			75	11	21	4
10	Azliana Mohamad Yusof	2018	Malaysia				8	6	3	1
11	Bo Hyun Kim	2015	South Korea			y	49	36	23	14
12	Brian Hung-Hin Lang	2014	South Korea		y		628	235	217	50
13	Bülent Kurt	2012	Turkey				40	16	6	1
14	C. Eloy	2012	Portugal	y			19	6	46	17
15	C.L. Shi	2015	China		y		87	37	39	5
16	Carol Li	2013	USA	y			253	150	62	32
17	Chan-Kwon Jung	2010	South Korea				110	45	37	19
18	Chiara Tuccilli	2018	Italy				38	15	38	20
19	Christine J. O'Neill	2010	Australia		y		60	20	41	16
20	Christopher Gouveia	2013	USA				314	136	115	31
21	Dan Chen	2018	China				34	13	6	1
22	Dongbin Ahn	2012	South Korea				85	16	22	5
23	Dongjun Jeong	2013	South Korea				159	108	22	3
24	E. Takacsova	2017	Slovakia				103	58	96	67
25	Eun Sook Kim	2012	South Korea				224	110	55	26
26	F Frasca	2008	Italy				125	40	198	27
27	Fei Wang	2018	USA	y			1094	437	1524	449
28	Gina M. Howell	2013	USA		y		72	36	84	22
29	Greta Gandolfi	2013	Italy				58	23	74	11
30	Guibin Zheng	2017	China				105	27	25	7
31	Hai-Jiang Qu	2018	China		y		209	103	204	63
32	Haixia Guan	2008	USA				19	5	19	3
33	Hee Jung Moon	2009	South Korea		y	y	42	14	42	14
34	Helmi Khadra	2018	USA				48	17	93	18
35	Hwa Young Ahn	2014	South Korea				70	45	15	6
36	Hye Sook Min	2008	South Korea				32	18	28	12
37	Hyung Seok Park	2012	South Korea				152	83	29	12
38	J. Lukas	2014	the Czech Republic				76	42	61	23
39	Jae Yun Lim	2013	South Korea	y			2219	914	728	219
40	Ji-Yong Joo	2012	South Korea		y		79	28	69	10
41	Jong-kyu Kim	2018	South Korea				581	265	116	40
42	Jung-Soo Pyo	2013	South Korea				110	60	12	5
43	Junliang Lu	2015	China				121	81	29	3
44	Kathleen C. Lee	2012	USA		y		44	29	19	9
45	Kuai-Lu Lin	2010	China		y	y	21	11	40	14
46	Le Zhao	2016	China	y	y	y	55	34	25	3
47	Li-Bo Yang	2015	China				170	115	373	206
48	Linwah Yip	2009	USA		y		106	77	100	63
49	M. Li	2017	China		y		115	36	158	44
50	Meiling Huang	2018	China				1444	943	264	116
51	Min-Hee Kim	2014	nm (TCGA)	y		y	83	41	25	16
52	Min-Kyung Yeo	2017	South Korea				85	48	14	10
53	Nelson George	2018	India				56	26	53	13
54	Neslihan Kurtulmus	2012	Turkey				43	12	66	7
55	R.C. da Silva	2015	Brasil				74	37	42	15
56	Rui-chao Zeng	2016	China		y		465	343	154	100
57	Salvatore Ulisse	2012	Italy				44	15	47	25
58	Sara Watutantrige-Fernando	2018	Italy		y	y	14	11	10	5
59	Seo Ki Kim	2016	South Korea		y		2530	1111	577	207
60	Si–Yang Dong	2017	China		y		171	125	116	64
61	Soo Young Chung	2013	South Korea				86	43	25	8
62	Su-jin Kim	2012	South Korea		y	y	381	133	166	42
63	Sun Y	2013	South Korea		y	y	72	11	29	7
64	Tung-Sun Huang	2014	China				31	23	9	2
65	Uiju Cho	2017	South Korea		y	y	12	2	140	5
66	Vito Rodolico	2007	Italy				88	23	126	19
67	Vivian Y. Park	2016	South Korea		y	y	214	103	44	20
68	Weibin Wang	2016	China				312	128	145	59
69	Won Seo Park	2013	South Korea				98	50	23	8
70	Xi Wei	2014	China				254	170	72	32
71	Xiaolei Guan	2017	China				28	16	16	3
72	Yasuhiro Ito	2014	Japan				281	58	485	138
73	Yong-Seok Kim	2013	South Korea		y	y	241	110	86	37
74	Yongbo Huang	2013	China				33	19	36	17
75	Yoon Kyoung So	2011	South Korea	y			44	22	27	7
76	Yoon Yang Jung	2015	South Korea				393	239	74	32
77	Young Jun Chai	2016	nm (TCGA)	y			156	93	128	84
78	Zhanna Mussazhanova	2013	Japan	y			20	11	16	10

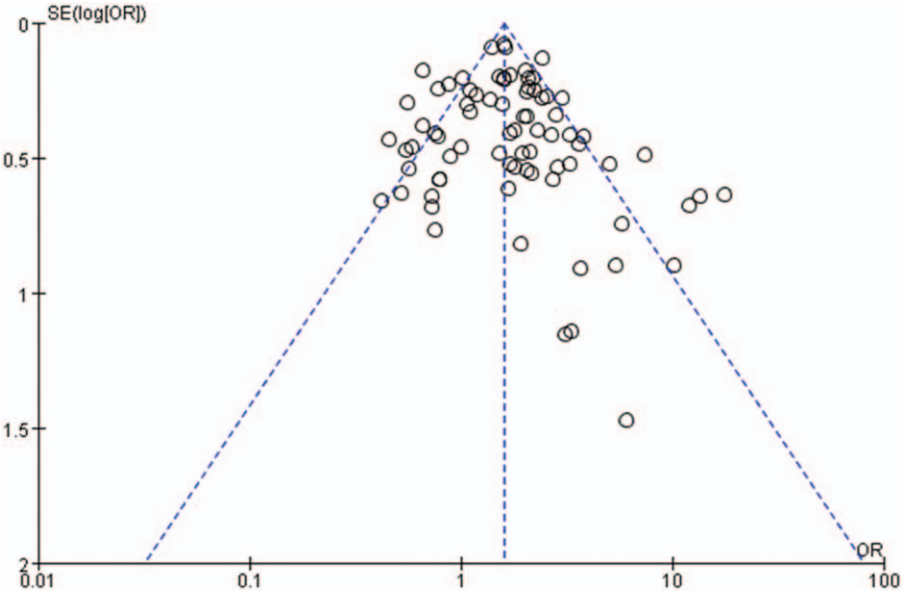

Figure 2. Funnel plot for the association between BRAF mutation and cervical LNM. BRAF = B-type Raf kinase, LNM = lymphatic metastasis.

articles with the data of LLNM, and CPTC was analyzed in 10 studies.

The quality assessment of all eligible studies was done through CochraneROB quality assessment scale. All our 78 studies had no selection bias, performance bias, detection bias, attrition bias, reporting bias, or any other bias.

3.2. The results of publication bias

The funnel graph looks symmetrical (Fig. 2). And in Harbord evaluation, P value is .391 > .05, which indicates that there is no publication bias in our study. Table 2 showed the results of Harbord evaluation.

3.3. The main results of this meta-analysis

A total of 78 studies consisting of 25,906 patients were analyzed to evaluate the relationship between BRAF mutation and cervical LNM. LNM was detected in 7957 (46.27%) out of 17,196 patients with BRAF (+) mutation, and in 2867 (32.91%) out of 8710 patients without BRAF (+) mutation. A fixed effect model showed that there was a moderate heterogeneity of the data (I^2 = 66%, P < .1). Thereby the random effects model was carried out. A significant association existed between BRAF mutation and cervical LNM (OR = 1.63; 95% CI: 1.44–1.84; P < .05) (Fig. 3).

3.4. The results of subgroup meta-analysis

3.4.1. Ethnicity: East Asians and non-East Asians. Forty-six studies were conducted among East Asians, which included South Korea (26 studies), China (18 studies), and Japan (2 studies). Thirty studies were from USA, Italy, Portland, India, Australia, Russian, and are presented in Table 1. Data of study 51(66) and 77(92) was from TCGA. Random effects model showed significant association between BRAF (+) mutation and cervical LNM both in East Asians (OR = 1.73; 95% CI: 1.49–2.02; P < .05) (Fig. 4A) and in non-East Asians (OR = 1.57; 95% CI: 1.26–1.96; P < .05) (Fig. 4B).

3.4.2. Metastatic site: CLNM and LLNM. The data of CLNM were provided in 24 studies, and LLNM were provided in 11 studies, which were shown in Table 1. In CLNM, random effects model showed significant association between BRAF (+) mutation and cervical LNM (OR = 1.80; 95% CI: 1.56–2.07; P < .05) (Fig. 4C), while no significant association (OR = 1.37; 95% CI: 0.76–2.48; P = .29 > .05) (Fig. 4D) was found in LLNM.

3.4.3. Subtype of PTC: CPTC. The data of CPTC were provided in 10 studies. Random effects model showed no significant association between BRAF mutation and cervical LNM in CPTC (OR = 1.32; 95% CI: 0.97–1.80; P = 0.08 > 0.05) (Fig. 4E).

Table 2

Publication bias through Harbord evaluation.

Number of studies = 78			Root MST = 1.767			
Z/sqrt(*V*)	Coef.	Std. Err.	*T*	*P*>|*t*|	[95% Conf. Interval]	
Sqrt(V) bias	0.3979862	0.0944981	4.21	.000	0.2097769	0.5861956
	0.3074126	0.3565126	0.86	.391	–0.4026438	1.017469
Test of H0: no small-study effects	P = .391					

Note: Regress *Z*/sqrt(*V*) where *Z* is efficient score and *V* is score variance.

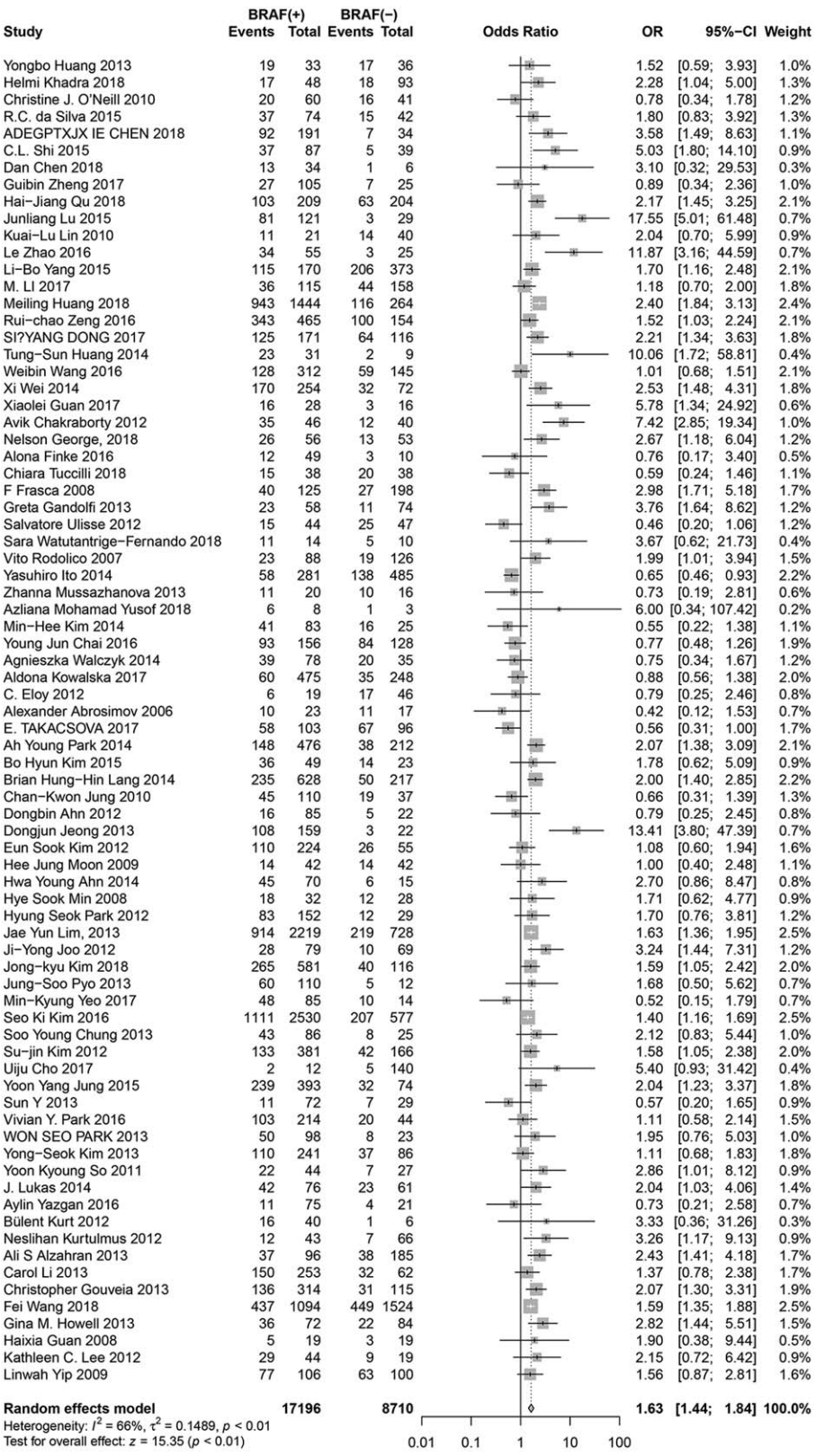

Figure 3. Forest plot of the association between BRAF mutation and Cervical LNM. BRAF = B-type Raf kinase, LNM = lymphatic metastasis.

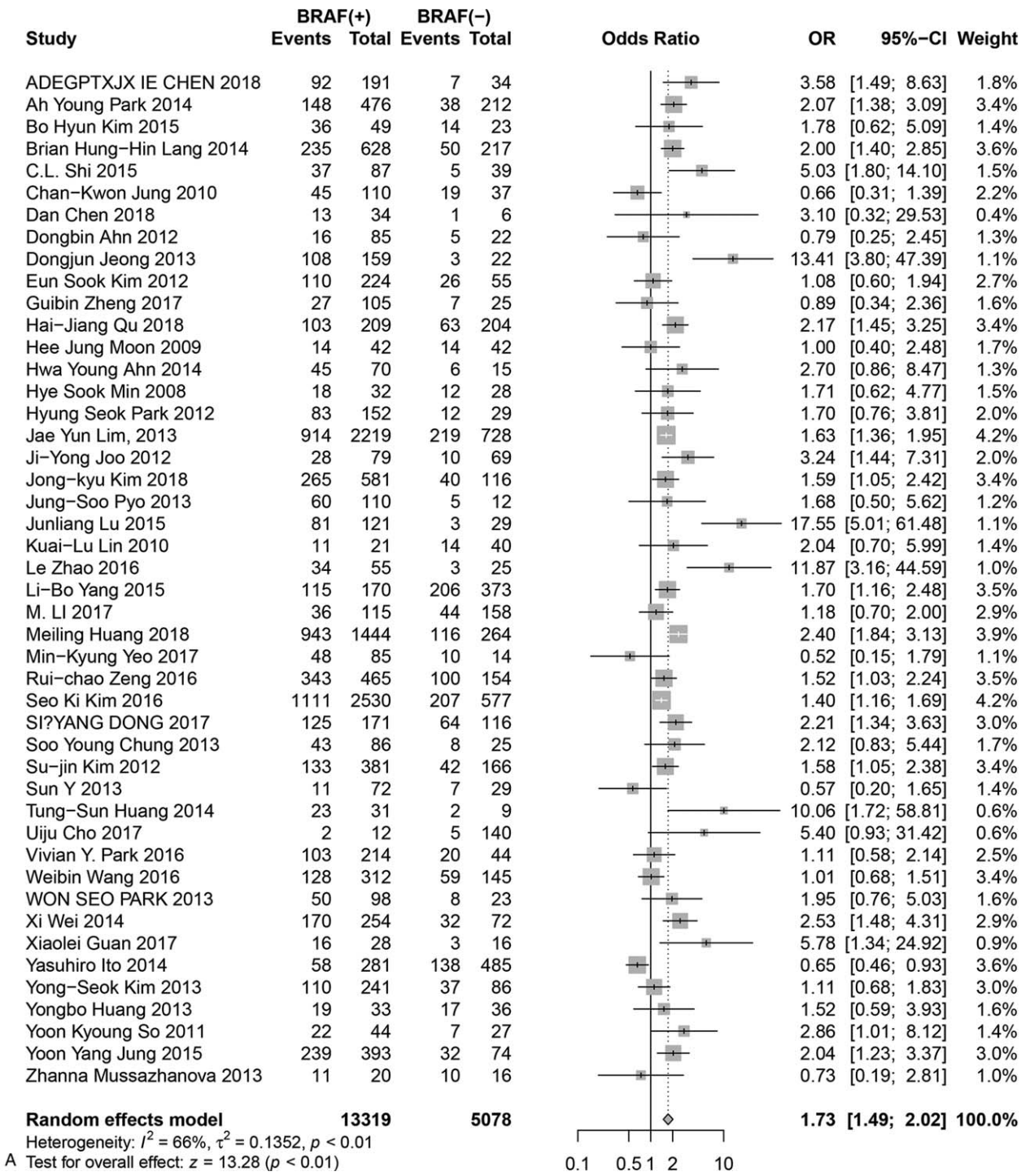

Figure 4. Stratified analysis for the association between BRAF mutation and cervical LNM. A. Ethnicity: east Asians; B. Ethnicity: non-east Asians; C. Metastatic site: CLNM; D. Metastatic site: LLNM; E. Subtype of PTC: CPTC. BRAF = B-type Raf kinase, CLNM = central lymph node metastasis, CPTC = classic/conventional PTC, LLNM = lateral lymph node metastasis, LNM = lymphatic metastasis, PTC = papillary thyroid cancer.

3.5. The results of sensitivity analysis

Sensitivity analysis was performed and we did not find any other major changes when sensitivity analysis was performed. There was no any study that had a major contribution to the heterogeneity. Within this sensitivity meta-analysis, a significant association still existed between BRAF mutation and cervical LNM after exclusion of any 1 study (Fig. 5).

3.6. The results of metacum analysis

The metacum analysis is done sorted by year. No significant association existed before 2012. A significant association began

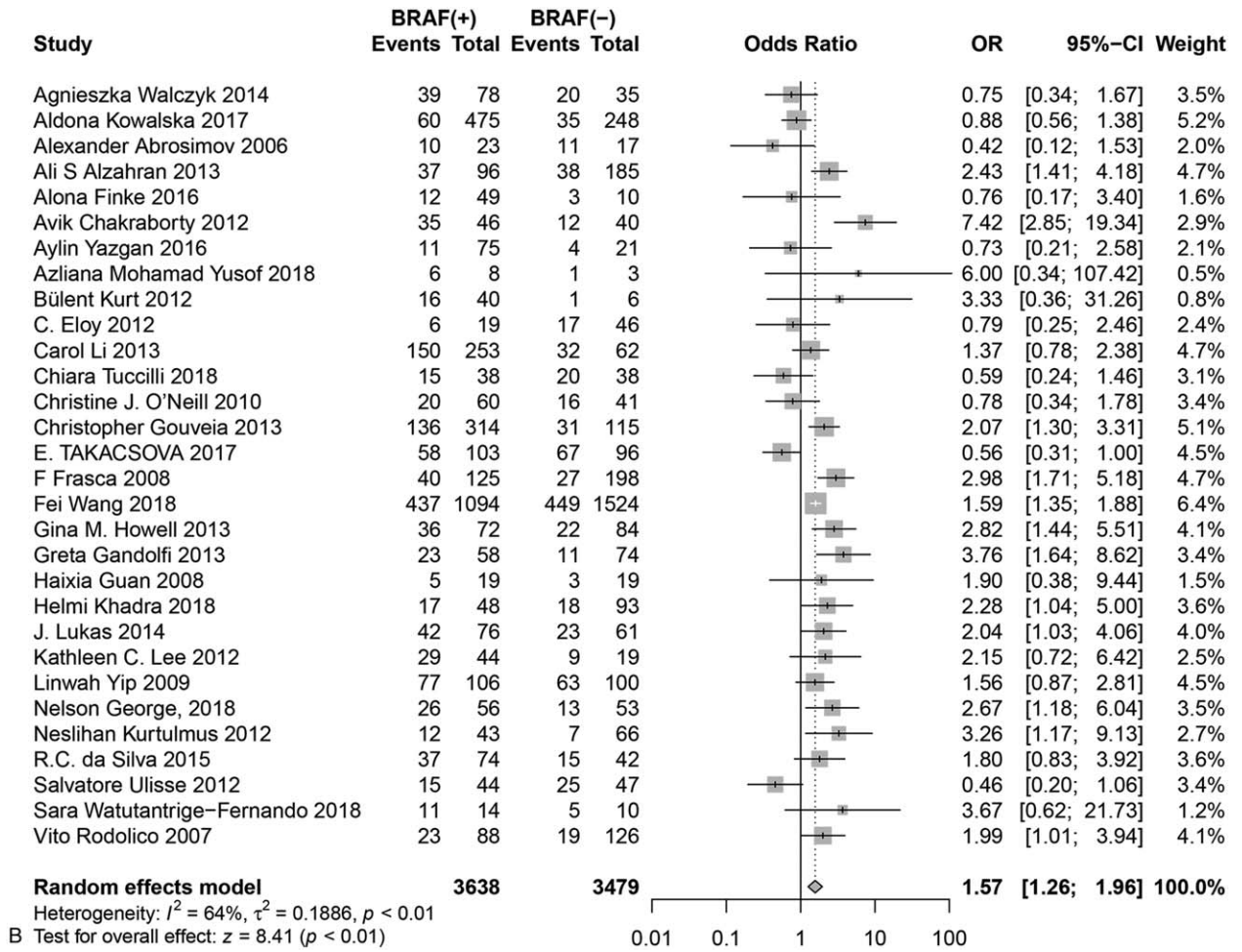

Figure 4. (Continued).

to exist between BRAF mutation and LNM from 2012, and this association became stable from 2017 (Fig. 6).

4. Discussion

BRAF gene is considered as the key factor in the occurrence and development of PTC, and it has been proved that BRAF (+) mutation had a close relationship with the aggressiveness of PTC. However, the results of the researches on the association between BRAF (+) and cervical LNM are not the same. Even among the articles published in 2018, there still were 7 papers which indicate a positive association versus 4 papers with negative results. Many studies have discussed this question, and several meta-analysis have done on the association between BRAF (+) and cervical LNM. No agreed conclusion was obtained. And the statistic method was not strict or normative, which might lead to the wrong results.

We find 10 meta-analyses on this topic. Although most meta-analysis[94–102] showed that there were a statistical significance between BRAF (+) and cervical LNM, Lee study[103] based on 12 primary studies indicated that the risk of cervical LNM in BRAF (+) mutation group was not higher than that in BRAF (−) mutation group (OR = 1.500; 95% CI: 0.992–2.268; $P = 0.055$) in 2007. In the above 10 meta-analysis, the study on the relationship between BRAF (+) mutation and LNM was only presented as a small part of the research on the relationship of BRAF (+) mutation and clinical characteristics in thyroid cancer, and subgroup analysis seldom was done or done in a nonstandard way. Almost all of them have some flaws on statistic methods. Heterogeneity was not evaluated regularly in most of the 10 meta-analysis. For example, a fixed-effects model was used despite the I^2 was 79% in one meta-analysis written by Jing-yong Song (m5) in 2018. And 3 of the 10 have an $I^2 > 75\%$, which were not suitable for meta-analysis.

We learned from the above meta-analyses papers and improved our study to effectively avoid the bias through obeying the rules of the statistical methods, which made our results more accurate and comprehensive.

Firstly, we search articles in a thorough way. Loss of articles can result in bias which will draw the wrong conclusion. In order to make a further study on the relationship between BRAF (+) and LNM, we searched articles from the 4 official electronic documents database, and found 476 articles. After removing the duplicates with the same title and authors in the same journal at the time, we got 381 articles. Additionally, we check the duplicated papers with a strict and reasonable way. Papers published with the same author and use the repeated cases are also removed as duplicates, and there were 9 excluded papers in

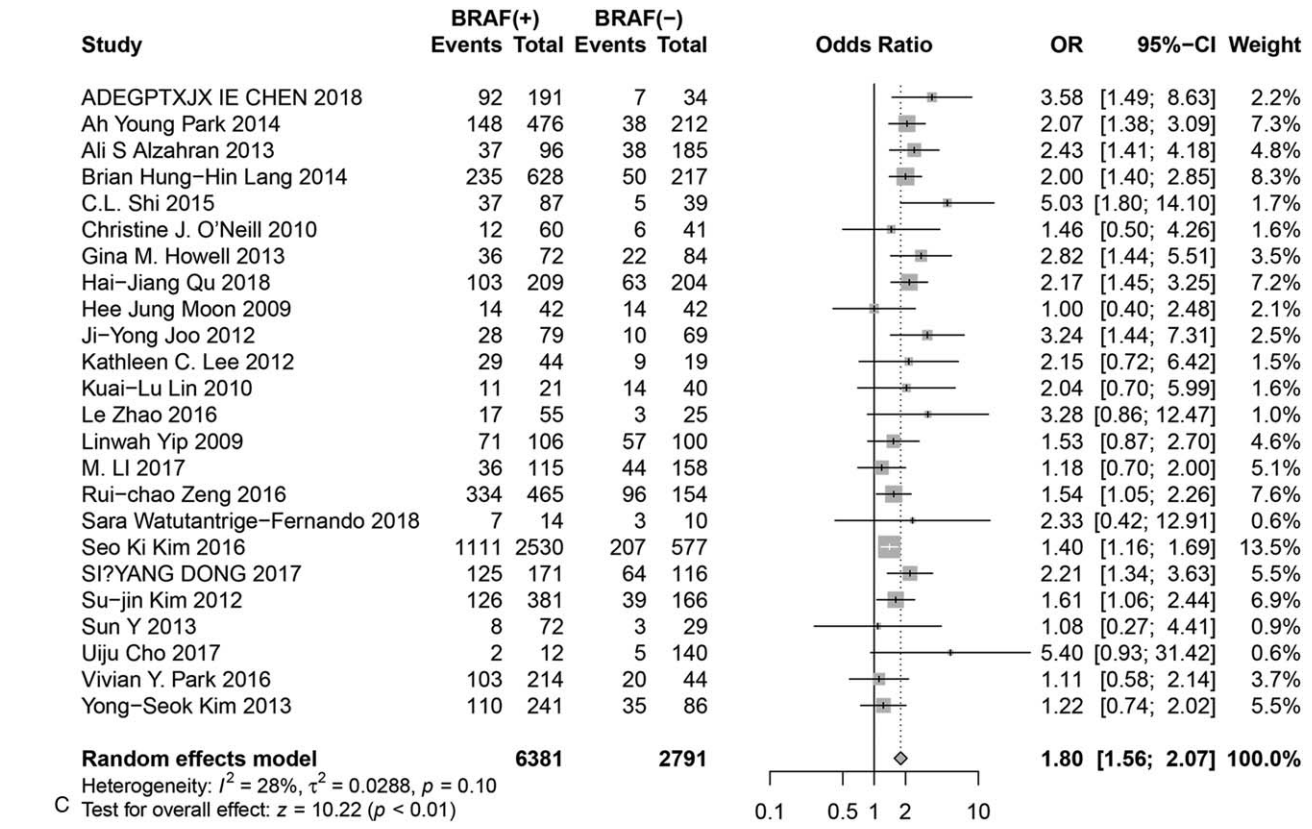

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Figure 4. (Continued).

our study for this reason. And in this situation, we chose the latest paper or the paper with the largest cases as our included materials. Finally, 78 articles met our criteria and are selected into our meta-analysis which has the largest studies up to now. Papers analyzed in the past meta-analysis were almost papers published before 2017. In our study, 19 of the 78 papers are published in 2017 and 2018. So it is likely to give a different result from the meta-analyses before.

Before we did meta-analysis, we did the quality assessment through CochraneROB quality assessment scale. Because the included studies had no selection bias, performance bias, detection bias, attrition bias or reporting bias, the results gotten from these studies were more credible.

Secondly, we tried both Funnel and Harbord assessment to evaluate the publication bias. Funnel graph is more simple and popular. If the graph looks symmetrical, it is considered as no publication bias. But it is an evaluation with more subjective disturbance, and can be influenced by heterogeneity. Harbord is a quantitative analysis, and it is applied to binary variable. Our P value in Harbord was .391 (>.05) which showed that no publication bias existed in our study. After confirming the comprehensiveness and credibility of our selected 78 materials by evaluation of publication bias and Cochran Q-statistic, we then continue our meta-analysis.

Thirdly, we analyzed all the papers and did a further analysis on heterogeneity. Before we analyze our materials, we should make clear what kind of studies they are. It may be a puzzle to most researchers. In most of our papers, they called it a retrospective cohort study, which sound like a cohort study, and RR is better for cohort study in meta-analysis than OR. In fact, they are cross-sectional studies because the data are obtained at one time point, so OR should be calculated in this kind of meta-analysis. There was a moderate heterogeneity ($I^2 = 66\%$, $P < .1$) in our study, and Random-effect model is carried out. The results of our study showed $P < .05$; OR = 1.63; 95% CI: 1.44 to 1.84, which indicated that an association existed between the BRAF (+) mutation and LNM.

Heterogeneity is classified into low, moderate, and high heterogeneity respectively according to the I^2 value. If the I^2 value is > 75%, it indicates the heterogeneity is too big to accept and meta-analysis may be not suitable to apply. If the I^2 value is <25%, it indicates there is no heterogeneity. If the I^2 value is between 25% and 50%, the heterogeneity is low. And the I^2 value between 50% and 75% means the heterogeneity is moderate and further study should be done to explore the heterogeneity. So subgroup study should be carried out in our study. How to determinate the subgroup? We cannot decide a subgroup randomly. Subgroup should be sorted by the factor that may affect both BRAF and LNM. Combined with the information provided by the 78 papers, we chose 3 subgroups: ethnicity (East Asians and non-East Asians), metastatic site (CLNM and LLNM), and the subtype of PTC (CPTC).

In the first subgroup, the relationship between BRAF mutation and cervical LNM may be different in different ethnic groups. We found more than half of the studies (46 papers) were got from East Asians; the other 30 were from the other regions other than East Asia. We did a subgroup meta-analysis, and P values were <.05 both in East Asians (OR 1.71; 95% CI, 1.47–1.99) and non-East Asian (OR 1.52; 95% CI, 1.22–1.90). So races (East Asians and non-East Asians) did not have any contribution to the

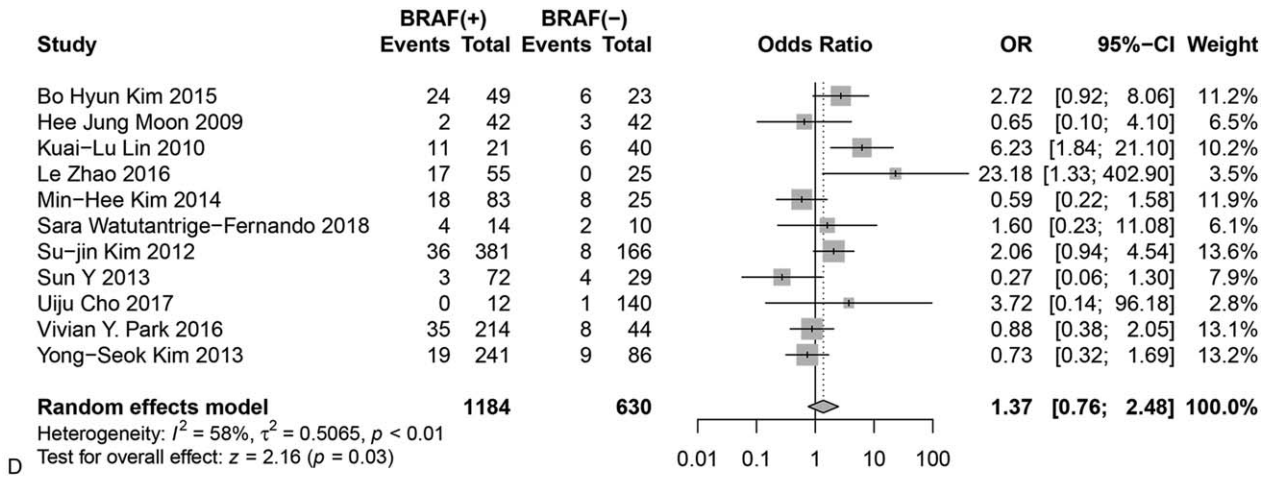

Figure 4. (Continued).

heterogeneity of our study. Subgroup meta-analysis also should be done on other ethnic groups, such as African/Caucasians, if the data of them were provided.

In the analysis of metastatic site subgroup, not all the 78 studies provided the information of CLNM and LLNM. We only got data of CLNM in 24 studies and data of LLNM in 11 studies. The outcomes were different in CLNM (OR = 1.80; 95% CI: 1.56–2.07; $P < .05$) and LLNM (OR = 1.37; 95% CI: 0.76–2.48, $P = .29 > .05$), so metastatic site may be a reason of the heterogeneity in our study. In addition, the results showed that there was no significant association between BRAF (+) and LLNM. We do not recommend BRAF (+) as the biomarker for lateral neck dissection in PTC although BRAF (+) mutation was considered as a predictor in aggressive PTC.

Only 10 articles provide the information of CPTC and <10 papers had mentioned the data of non-CPTC subtype (follicular PTC), so we tried to analyze the analysis with the data in CPTC. The outcome in CPTC (OR = 1.37; 95% CI: 0.76–2.48; $P = .29 > .05$) was different from the main results (OR = 1.63; 95% CI: 1.44–1.84; $P < .05$), which indicated that the subtype may be a source of heterogeneity. Similar to the recommendation above, we do not recommend BRAF (+) as the biomarker for neck dissection in CPTC.

Tumor size and invasion of extra capsule also may be factors influence both to BRAF mutation and LNM, but the data were not provided in almost all of these articles. So we could not make subgroup meta-analysis on these factors.

Finally, we do sensitivity analysis and metacum analysis to evaluate the stability of our outcomes. Sensitivity analysis showed all the estimate values were between the 95% CI: 1.44 to 1.84. So the main results of our study were stable. Further metacum analysis sorted by year indicated that no significant association was found in the early year of this kind of study. From 2012, significant association began to exist, and the OR value became stable from 2017.

Our comprehensively collected articles have no significant bias, and further meta-analysis showed the outcome has become stable. All these indicated that our results were credible. And our results showed a significant association existed between BRAF mutation and cervical LNM (OR = 1.63; 95% CI: 1.44–1.84; $P < .05$). However, this result will make us confused, if we the look into the details in our study. Thirty four out of our 78 papers have the positive results that agreed that there was association between BRAF (+) mutation and LNM in PTC, while another 43 papers showed negative outcomes. And 1 article based on the data of 766 patients in 2014 by ITO indicated that BRAF (+) mutation is a protective factor from LNM for PTC patients. The 46 papers with negative results were distributed evenly in the past years: 4 papers published in 2018, 5 in 2017, 6 in 2016, 2 in 2015, 5 in 2014, 8 in 2013, 7 in 2012, and 9 before 2012. And the

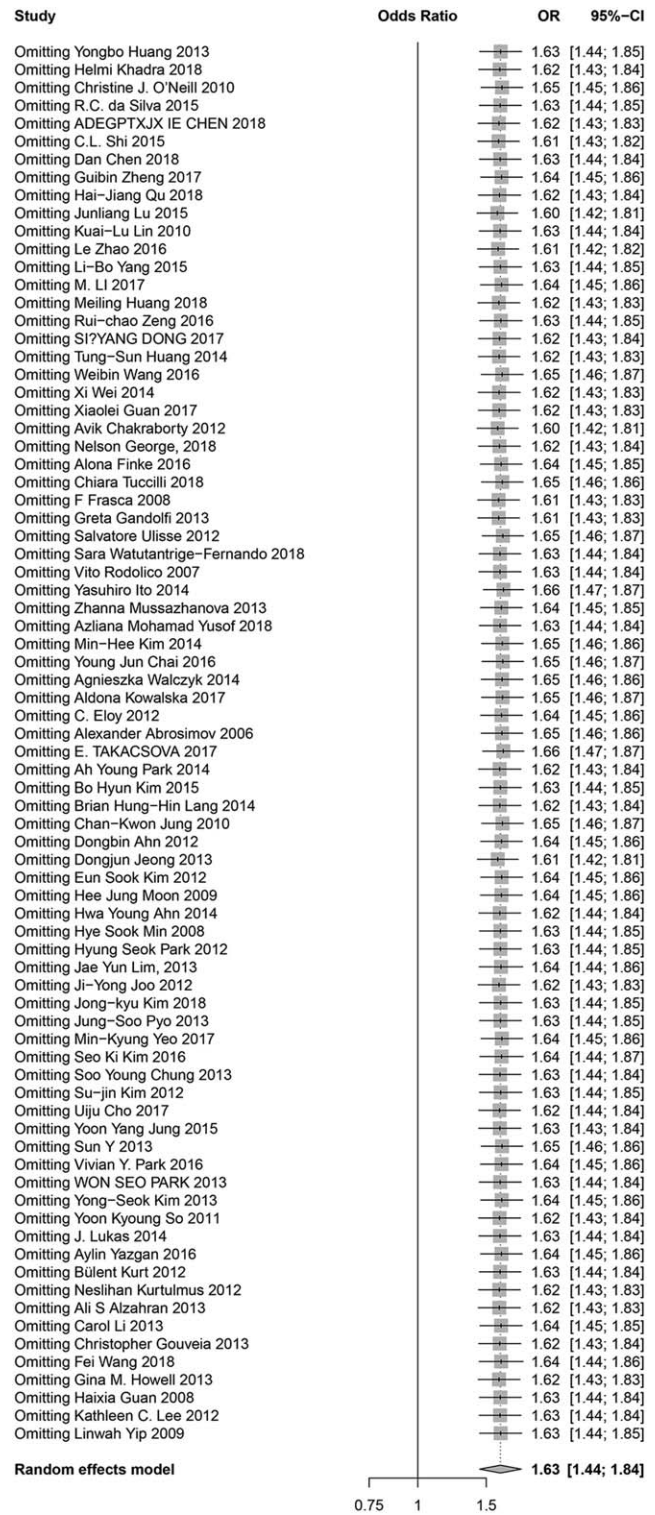

Figure 5. Sensitivity analysis for the association between BRAF mutation and cervical LNM. BRAF = B-type Raf kinase, LNM = lymphatic metastasis.

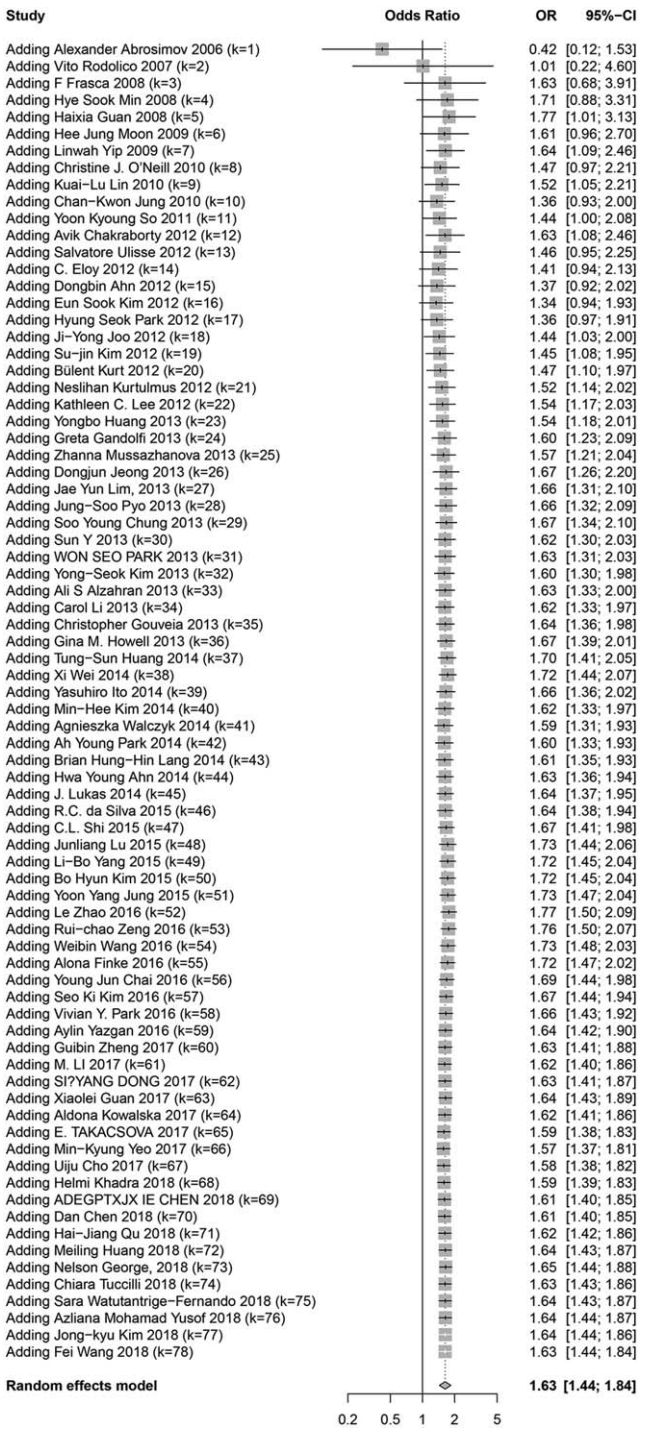

Figure 6. Metacum analysis for the association between BRAF mutation and cervical LNM. BRAF = B-type Raf kinase, LNM = lymphatic metastasis.

above distribution of the negative papers indicated that the negative results in our study were not individual or accidental issues. More than half of our articles have a negative result, which seems opposite to our main result. Why? One reason is that the papers with positive results have a higher weight in the meta-analysis. Another reason is the heterogeneity. Heterogeneity is very popular in the meta-analyses of this topic. Our study has a moderate heterogeneity (66%). And further subgroup analysis indicated metastatic site (CLNM, LLNM) and subtype of PTC (CPTC) were the reasons of heterogeneity. Further studies on heterogeneity may reveal some valuable factors between BRAF gene mutation and LNM. Subgroups such as: tumor size and invasion of extra capsule, age and sex, etc, should be considered into the research of this kind of study.

In conclusion, we consider that a significant association exists between BRAF mutation and cervical LNM. However, because of the high heterogeneity, subgroup meta-analysis may reveal more valuable outcomes. And we do not recommend that BRAF (+) as the biomarker for LNM in PTC.

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Methodology: Hongzhi Ma.
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Resources: Jugao Fang, Lizhen Hou, Zhigang Huang.
Software: Xiao Chen.
Validation: Xiao Chen, Zhigang Huang.
Visualization: Ling Feng.
Writing – original draft: Hongzhi Ma.
Writing – review & editing: Hongzhi Ma.

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