

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: 0.97–1.80; 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 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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The authors have no conflicts of interest to disclose.

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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 metaanalysis 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 Braf" 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

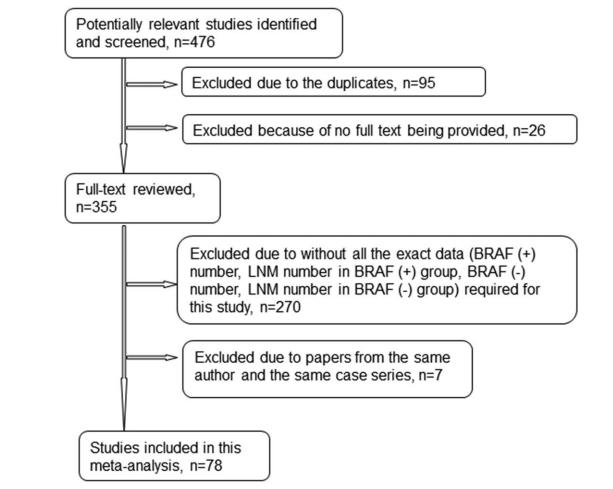
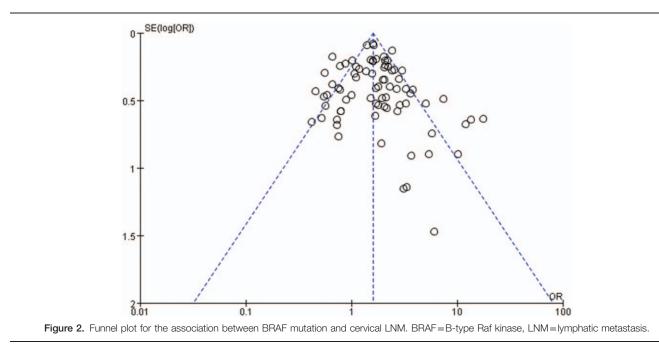


Figure 1. The PRISMA flow diagram of study selection for this meta-analysis.

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		у	•	191	92	34	7
2	Agnieszka Walczyk	2014	Poland				78	39	35	20
3	Ah Young Park	2014	South Korea		У		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		У		96	37	185	38
7 8	Alona Finke	2016 2012	Israel India				49 46	12 35	10 40	3 12
9	Avik Chakraborty Aylin Yazgan	2012	Turkey	у			40 75	11	21	4
10	Azliana Mohamad Yusof	2018	Malaysia	у			8	6	3	1
11	Bo Hyun Kim	2015	South Korea			у	49	36	23	14
12	Brian Hung-Hin Lang	2014	South Korea		У	,	628	235	217	50
13	Bülent Kurt	2012	Turkey				40	16	6	1
14	C. Eloy	2012	Portugal	у			19	6	46	17
15	C.L. Shi	2015	China		У		87	37	39	5
16	Carol Li	2013 2010	USA South Koroo	У			253	150	62 37	32
17 18	Chan-Kwon Jung Chiara Tuccilli	2010	South Korea Italy				110 38	45 15	37 38	19 20
19	Christine J. O'Neill	2018	Australia		у		60	20	30 41	16
20	Christopher Gouveia	2013	USA		y		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 1524	27 449
27 28	Fei Wang Gina M. Howell	2018 2013	USA USA	У	у		1094 72	437 36	84	22
29	Greta Gandolfi	2013	Italy		у		58	23	74	11
30	Guibin Zheng	2017	China				105	27	25	7
31	Hai-Jiang Qu	2018	China		У		209	103	204	63
32	Haixia Guan	2008	USA				19	5	19	3
33	Hee Jung Moon	2009	South Korea		У	У	42	14	42	14
34	Helmi Khadra	2018	USA				48	17	93	18
35	Hwa Young Ahn	2014	South Korea				70 32	45 18	15 28	6 12
36 37	Hye Sook Min Hyung Seok Park	2008 2012	South Korea South Korea				32 152	83	28 29	12
38	J. Lukas	2012	the Czech Republic				76	42	61	23
39	Jae Yun Lim	2013	South Korea	У			2219	914	728	219
40	Ji-Yong Joo	2012	South Korea	,	У		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		У		44	29	19	9
45 46	Kuai-Lu Lin Le Zhao	2010 2016	China China	N.	У	У	21 55	11 34	40 25	14 3
40 47	Li-Bo Yang	2010	China	У	У	У	170	115	373	206
48	Linwah Yip	2009	USA		у		106	77	100	63
49	M. Li	2017	China		y		115	36	158	44
50	Meiling Huang	2018	China		5		1444	943	264	116
51	Min-Hee Kim	2014	nm (TCGA)	у		у	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 R.C. da Silva	2012	Turkey				43 74	12 37	66	7
55 56	Rui-chao Zeng	2015 2016	Brasil China		у		465	343	42 154	15 100
57	Salvatore Ulisse	2010	Italy		у		403	15	47	25
58	Sara Watutantrige-Fernando	2018	Italy		У	у	14	11	10	5
59	Seo Ki Kim	2016	South Korea		y	,	2530	1111	577	207
60	Si–Yang Dong	2017	China		ý		171	125	116	64
61	Soo Young Chung	2013	South Korea				86	43	25	8
62	Su-jin Kim	2012	South Korea		У	у	381	133	166	42
63	Sun Y	2013	South Korea		У	у	72	11	29	7
64	Tung-Sun Huang Uiju Cho	2014	China South Korea				31	23	9	2
65 66	Vito Rodolico	2017 2007	Italy		У	У	12 88	2 23	140 126	5 19
67	Vivian Y. Park	2007	South Korea		у	у	214	103	44	20
68	Weibin Wang	2016	China		y	у	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		У	У	241	110	86	37
74 75	Yongbo Huang	2013	China South Koroo	N.			33	19	36	17
75 76	Yoon Kyoung So Yoon Yang Jung	2011 2015	South Korea South Korea	У			44 393	22 239	27 74	7 32
76 77	Young Jung Chai	2015	nm (TCGA)	у			393 156	239 93	74 128	32 84
78	Zhanna Mussazhanova	2010	Japan	y y			20	11	16	10
-							-		-	



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 Asian's 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).

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Publication bias through Harbo Number of studies=78		Root MST = 1.767								
Z/sqrt(V)	Coef.	Std. Err.	Т	P > t	[95% Conf. Interv	al]				
Sqrt(V) bias	0.3979862	0.0944981	4.21	.000	0.2097769	0.5861956				
Test of HO: no small-study effects	0.3074126 <i>P</i> =.391	0.3565126	0.86	.391	-0.4026438	1.017469				

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

tudy	BRAI		BRAF		Odde Potio	00	05	0/_01	Mainh
tudy	Events	iotal	Events	iotal	Odds Ratio	OR	95	%-UI	Weigh
ongbo Huang 2013	19	33	17	36		1.52	[0.59;		1.0%
lelmi Khadra 2018	17	48	18	93		2.28			1.3%
hristine J. O'Neill 2010	20	60	16	41		0.78	[0.34;	1.78]	1.2%
.C. da Silva 2015	37	74	15	42		1.80	[0.83;	3.92]	1.3%
DEGPTXJX IE CHEN 2018	92	191	7	34		3.58	[1.49;		1.1%
.L. Shi 2015	37	87	5	39		5.03	[1.80; 1		0.9%
an Chen 2018	13	34	1	6		3.10	[0.32; 2		0.3%
uibin Zheng 2017	27	105	7	25		0.89	[0.34;		1.0%
lai-Jiang Qu 2018	103	209	63	204		2.17	[1.45;	3.25]	2.1%
unliang Lu 2015	81	121	3	29		17.55	[5.01; 6	51.48]	0.7%
uai-Lu Lin 2010	11	21	14	40		2.04	[0.70;	5.99]	0.9%
e Zhao 2016	34	55	3	25	<u> </u>	11.87	[3.16; 4	14.59]	0.7%
i-Bo Yang 2015	115	170	206	373	- the second sec	1.70	[1.16;	2.48]	2.1%
1. LI 2017	36	115	44	158	- <u>deni</u> -	1.18	[0.70;	2.00]	1.8%
leiling Huang 2018	943	1444	116	264		2.40	[1.84;	3.13]	2.4%
ui-chao Zeng 2016	343	465	100	154	10000 -	1.52	[1.03;	2.24]	2.1%
I?YANG DONG 2017	125	171	64	116		2.21	[1.34;		1.8%
ung-Sun Huang 2014	23	31	2	9		10.06	[1.72; 5		0.4%
Veibin Wang 2016	128	312	59	145		1.01	[0.68;		2.1%
i Wei 2014	170	254	32	72	1	2.53	[1.48;		1.8%
iaolei Guan 2017	16	28	3	16		5.78	[1.34; 2		0.6%
vik Chakraborty 2012	35	46	12	40		7.42	[2.85; 1		1.0%
	26	56	12	40 53		2.67			1.2%
lelson George, 2018		56 49	13	53 10			[1.18;	6.04]	0.5%
Iona Finke 2016	12					0.76	[0.17;	3.40]	
hiara Tuccilli 2018	15	38	20	38	and a second	0.59		1.46]	1.1%
Frasca 2008	40	125	27	198		2.98	[1.71;	5.18]	1.7%
Freta Gandolfi 2013	23	58	11	74		3.76			1.2%
alvatore Ulisse 2012	15	44	25	47		0.46			1.2%
ara Watutantrige-Fernando 2018	11	14	5	10		3.67	[0.62; 2		0.4%
ito Rodolico 2007	23	88	19	126	100	1.99	[1.01;		1.5%
asuhiro Ito 2014	58	281	138	485		0.65	[0.46;	0.93]	2.2%
hanna Mussazhanova 2013	11	20	10	16		0.73	[0.19;	2.81]	0.6%
zliana Mohamad Yusof 2018	6	8	1	3		6.00	[0.34; 10	07.42]	0.2%
lin-Hee Kim 2014	41	83	16	25		0.55	[0.22;	1.38]	1.1%
oung Jun Chai 2016	93	156	84	128		0.77	[0.48;	1.26]	1.9%
gnieszka Walczyk 2014	39	78	20	35		0.75	[0.34;	1.67]	1.2%
Idona Kowalska 2017	60	475	35	248	-	0.88	[0.56;	1.38]	2.0%
. Eloy 2012	6	19	17	46		0.79	[0.25;	2.46]	0.8%
lexander Abrosimov 2006	10	23	11	17		0.42	[0.12;	1.53]	0.7%
TAKACSOVA 2017	58	103	67	96		0.56	[0.31;	1.00]	1.7%
h Young Park 2014	148	476	38	212		2.07	[1.38;	3.09]	2.1%
o Hyun Kim 2015	36	49	14	23		1.78	[0.62;	5.09]	0.9%
rian Hung-Hin Lang 2014	235	628	50	217	ingen	2.00	[1.40;	2.85]	2.2%
	235 45	110		37					
han-Kwon Jung 2010			19		100	0.66	[0.31;	1.39]	1.3%
longbin Ahn 2012	16	85	5	22		0.79	[0.25;	2.45]	0.8%
longjun Jeong 2013	108	159	3	22		13.41	[3.80; 4		0.7%
un Sook Kim 2012	110	224	26	55		1.08		1.94]	1.6%
lee Jung Moon 2009	14	42	14	42		1.00	[0.40;	2.48]	1.1%
lwa Young Ahn 2014	45	70	6	15	<u>† </u>	2.70	[0.86;	8.47]	0.8%
lye Sook Min 2008	18	32	12	28		1.71	[0.62;	4.77]	0.9%
lyung Seok Park 2012	83	152	12	29		1.70	[0.76;	3.81]	1.2%
ae Yun Lim, 2013	914	2219	219	728		1.63	[1.36;	1.95]	2.5%
i-Yong Joo 2012	28	79	10	69	÷	3.24	[1.44;	7.31]	1.2%
ong-kyu Kim 2018	265	581	40	116	1000	1.59	[1.05;	2.42]	2.0%
ung-Soo Pyo 2013	60	110	5	12	- <u>i</u>	1.68	[0.50;	5.62]	0.7%
lin-Kyung Yeo 2017	48	85	10	14		0.52	[0.15;	1.79]	0.7%
eo Ki Kim 2016	1111	2530	207	577		1.40	[1.16;	1.69]	2.5%
oo Young Chung 2013	43	86	8	25		2.12	[0.83;	5.44]	1.0%
u–jin Kim 2012	133	381	42	166	and an	1.58		2.38]	2.0%
iju Cho 2017	2	12	5	140	and the second s		[0.93; 3		0.4%
oon Yang Jung 2015	239	393	32	74		2.04	[1.23;		1.8%
un Y 2013	11	72	7	29		0.57	[0.20;	1.65]	0.9%
ivian Y. Park 2016	103	214	20	44		1.11	[0.20,		1.5%
VON SEO PARK 2013	50	214	20	23					1.0%
					I	1.95	[0.76; [0.68;		
ong-Seok Kim 2013	110	241	37	86	1000	1.11		1.83]	1.8%
oon Kyoung So 2011	22	44	7	27		2.86	[1.01;		0.9%
Lukas 2014	42	76	23	61		2.04	[1.03;		1.4%
ylin Yazgan 2016	11	75	4	21		0.73	[0.21;		0.7%
ülent Kurt 2012	16	40	1	6		3.33	[0.36; 3		0.3%
leslihan Kurtulmus 2012	12	43	7	66		3.26	[1.17;		0.9%
li S Alzahran 2013	37	96	38	185		2.43	[1.41;	4.18]	1.7%
arol Li 2013	150	253	32	62		1.37	[0.78;	2.38]	1.7%
hristopher Gouveia 2013	136	314	31	115		2.07	[1.30;	3.31]	1.9%
ei Wang 2018	437	1094		1524	rin .	1.59		1.88]	2.5%
ina M. Howell 2013	36	72	22	84	-	2.82	[1.44;		1.5%
laixia Guan 2008	5	19	3	19		1.90	[0.38;		0.5%
athleen C. Lee 2012	29	44	9	19		2.15	[0.72;		0.9%
inwah Yip 2009	77	106	63	100	1000	1.56	[0.87;		1.6%
nwan np 2009	11	100	03	100	100m 1	1.00	[0.07,	2.01]	1.0%
		17196		8710	•	1.63	[1.44;	1 8/1	100 00
andom effects model									

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

	Otrada.	BRA		BRAF		Odda Datia	00	05% 01	Main h4
	Study	Events	Iotal	Events	lotal	Odds Ratio	OR	95%-CI	weight
	ADEGPTXJX IE CHEN 2018	92	191	7	34	 	3 58	[1.49; 8.63]	1.8%
	Ah Young Park 2014	148	476	38	212			[1.38; 3.09]	3.4%
	Bo Hyun Kim 2015	36	49	14	23			[0.62; 5.09]	1.4%
	Brian Hung-Hin Lang 2014	235	628	50	217			[1.40; 2.85]	3.6%
	C.L. Shi 2015	37	87	5	39			[1.80; 14.10]	1.5%
	Chan-Kwon Jung 2010	45	110	19	37			[0.31; 1.39]	2.2%
	Dan Chen 2018	13	34	1	6			[0.32; 29.53]	0.4%
	Dongbin Ahn 2012	16	85	5	22			[0.25; 2.45]	1.3%
	Dongjun Jeong 2013	108	159	3	22	H		[3.80; 47.39]	1.1%
	Eun Sook Kim 2012	110	224	26	55		1.08	[0.60; 1.94]	2.7%
	Guibin Zheng 2017	27	105	7	25		0.89	[0.34; 2.36]	1.6%
	Hai-Jiang Qu 2018	103	209	63	204		2.17	[1.45; 3.25]	3.4%
	Hee Jung Moon 2009	14	42	14	42		1.00	[0.40; 2.48]	1.7%
	Hwa Young Ahn 2014	45	70	6	15		2.70	[0.86; 8.47]	1.3%
	Hye Sook Min 2008	18	32	12	28		1.71	[0.62; 4.77]	1.5%
	Hyung Seok Park 2012	83	152	12	29				2.0%
	Jae Yun Lim, 2013	914	2219	219	728			[1.36; 1.95]	4.2%
	Ji-Yong Joo 2012	28	79	10	69	<u>+</u>		[1.44; 7.31]	2.0%
	Jong-kyu Kim 2018	265	581	40	116			[1.05; 2.42]	3.4%
	Jung-Soo Pyo 2013	60	110	5	12			[0.50; 5.62]	1.2%
	Junliang Lu 2015	81	121	3	29			[5.01; 61.48]	1.1%
	Kuai-Lu Lin 2010	11	21	14	40			[0.70; 5.99]	1.4%
	Le Zhao 2016	34	55	3	25			[3.16; 44.59]	1.0%
	Li-Bo Yang 2015	115	170	206	373			[1.16; 2.48]	3.5%
	M. LI 2017	36	115	44	158			[0.70; 2.00]	2.9%
	Meiling Huang 2018	943	1444	116	264			[1.84; 3.13]	3.9%
	Min-Kyung Yeo 2017	48 343	85 465	10 100	14 154			[0.15; 1.79] [1.03; 2.24]	1.1% 3.5%
	Rui−chao Zeng 2016 Seo Ki Kim 2016	1111	2530	207	577			[1.16; 1.69]	4.2%
	SI?YANG DONG 2017	125	171	64	116			[1.34; 3.63]	3.0%
	Soo Young Chung 2013	43	86	8	25			[0.83; 5.44]	1.7%
	Su-jin Kim 2012	133	381	42	166	<u> </u>		[1.05; 2.38]	3.4%
	Sun Y 2013	11	72	7	29			[0.20; 1.65]	1.4%
	Tung-Sun Huang 2014	23	31	2	9	-		[1.72; 58.81]	0.6%
	Uiju Cho 2017	2	12	5	140			[0.93; 31.42]	0.6%
	Vivian Y. Park 2016	103	214	20	44			[0.58; 2.14]	2.5%
	Weibin Wang 2016	128	312	59	145	-		[0.68; 1.51]	3.4%
	WON SEO PARK 2013	50	98	8	23			[0.76; 5.03]	1.6%
	Xi Wei 2014	170	254	32	72			[1.48; 4.31]	2.9%
	Xiaolei Guan 2017	16	28	3	16		5.78	[1.34; 24.92]	0.9%
	Yasuhiro Ito 2014	58	281	138	485	-	0.65	[0.46; 0.93]	3.6%
	Yong-Seok Kim 2013	110	241	37	86		1.11	[0.68; 1.83]	3.0%
	Yongbo Huang 2013	19	33	17	36		1.52	[0.59; 3.93]	1.6%
	Yoon Kyoung So 2011	22	44	7	27			[1.01; 8.12]	1.4%
	Yoon Yang Jung 2015	239	393	32	74			[1.23; 3.37]	3.0%
	Zhanna Mussazhanova 2013	11	20	10	16		0.73	[0.19; 2.81]	1.0%
	Random effects model		13319		5078	•	1.73	[1.49; 2.02]	100.0%
	Heterogeneity: $I^2 = 66\%$, $\tau^2 = 0$.					r ti i		[,]	
A	Test for overall effect: $z = 13.28$					0.1 0.51 2 10			

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 metaanalysis, 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

Study	BRAF Events		BRAF Events		Odds Ratio	OR	95%-CI	Weight
Agnieszka Walczyk 2014	39	78	20	35		0.75	[0.34; 1.67]	3.5%
Aldona Kowalska 2017	60	475	35	248	-	0.88	[0.56; 1.38]	5.2%
Alexander Abrosimov 2006	10	23	11	17		0.42	[0.12; 1.53]	2.0%
Ali S Alzahran 2013	37	96	38	185	÷	2.43	[1.41; 4.18]	4.7%
Alona Finke 2016	12	49	3	10		0.76	[0.17; 3.40]	1.6%
Avik Chakraborty 2012	35	46	12	40		- 7.42		2.9%
Aylin Yazgan 2016	11	75	4	21		0.73	[0.21; 2.58]	2.1%
Azliana Mohamad Yusof 2018	6	8	1	3		6.00	[0.34; 107.42]	0.5%
Bülent Kurt 2012	16	40	1	6		3.33	[0.36; 31.26]	0.8%
C. Eloy 2012	6	19	17	46	<u> </u>	0.79	[0.25; 2.46]	2.4%
Carol Li 2013	150	253	32	62		1.37	[0.78; 2.38]	4.7%
Chiara Tuccilli 2018	15	38	20	38	- 12	0.59	[0.24; 1.46]	3.1%
Christine J. O'Neill 2010	20	60	16	41		0.78	[0.34; 1.78]	3.4%
Christopher Gouveia 2013	136	314	31	115	- Marine	2.07	[1.30; 3.31]	5.1%
E. TAKACSOVA 2017	58	103	67	96		0.56	[0.31; 1.00]	4.5%
F Frasca 2008	40	125	27	198		2.98	[1.71; 5.18]	4.7%
Fei Wang 2018	437	1094	449	1524	+	1.59	[1.35; 1.88]	6.4%
Gina M. Howell 2013	36	72	22	84	-	2.82	[1.44; 5.51]	4.1%
Greta Gandolfi 2013	23	58	11	74		3.76	[1.64; 8.62]	3.4%
Haixia Guan 2008	5	19	3	19	a	1.90	[0.38; 9.44]	1.5%
Helmi Khadra 2018	17	48	18	93		2.28	[1.04; 5.00]	3.6%
J. Lukas 2014	42	76	23	61		2.04	[1.03; 4.06]	4.0%
Kathleen C. Lee 2012	29	44	9	19		2.15	[0.72; 6.42]	2.5%
Linwah Yip 2009	77	106	63	100		1.56	[0.87; 2.81]	4.5%
Nelson George, 2018	26	56	13	53		2.67	[1.18; 6.04]	3.5%
Neslihan Kurtulmus 2012	12	43	7	66		3.26	[1.17; 9.13]	2.7%
R.C. da Silva 2015	37	74	15	42		1.80	[0.83; 3.92]	3.6%
Salvatore Ulisse 2012	15	44	25	47	- <u>-</u>	0.46	[0.20; 1.06]	3.4%
Sara Watutantrige-Fernando 2018		14	5	10		— 3.67	[0.62; 21.73]	1.2%
Vito Rodolico 2007	23	88	19	126		1.99	[1.01; 3.94]	4.1%
Random effects model		3638		3479		1.57	[1.26; 1.96]	100.0%
Heterogeneity: $I^2 = 64\%$, $\tau^2 = 0.1886$,				1				
B Test for overall effect: $z = 8.41$ ($p < 0.1$	01)			0.0	01 0.1 1 1	0 100		
			Figure	4. (Conti	inued).			

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 metaanalysis^[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

Study	BRAF Events		BRAF Events		Odds Ratio	OR	∋5%−C I	Weight
ADEGPTXJX IE CHEN 2018	92	191	7	34	-:	- 3.58 [1.49	9; 8.63]	2.2%
Ah Young Park 2014	148	476	38	212	<u>-is</u>	2.07 [1.38	3; 3.09]	7.3%
Ali S Alzahran 2013	37	96	38	185		2.43 [1.4]	1; 4.18]	4.8%
Brian Hung-Hin Lang 2014	235	628	50	217	- <u>im</u> -	2.00 [1.40); 2.85]	8.3%
C.L. Shi 2015	37	87	5	39		- 5.03 [1.80	; 14.10]	1.7%
Christine J. O'Neill 2010	12	60	6	41		1.46 [0.50); 4.26]	1.6%
Gina M. Howell 2013	36	72	22	84		2.82 [1.44		3.5%
Hai-Jiang Qu 2018	103	209	63	204		2.17 [1.4	5; 3.25]	7.2%
Hee Jung Moon 2009	14	42	14	42		1.00 [0.40		2.1%
Ji-Yong Joo 2012	28	79	10	69		3.24 [1.44		2.5%
Kathleen C. Lee 2012	29	44	9	19		2.15 [0.72	2; 6.42]	1.5%
Kuai-Lu Lin 2010	11	21	14	40		2.04 [0.70); 5.99]	1.6%
Le Zhao 2016	17	55	3	25	÷.	— 3.28 [0.86	; 12.47]	1.0%
Linwah Yip 2009	71	106	57	100	-=-	1.53 [0.8]		4.6%
M. LI 2017	36	115	44	158		1.18 [0.70); 2.00]	5.1%
Rui-chao Zeng 2016	334	465	96	154		1.54 [1.0	5; 2.26]	7.6%
Sara Watutantrige-Fernando 2018		14	3	10		- 2.33 [0.42		0.6%
Seo Ki Kim 2016	1111	2530	207	577		1.40 [1.10		13.5%
SI?YANG DONG 2017	125	171	64	116		2.21 [1.34	1; 3.63]	5.5%
Su-jin Kim 2012	126	381	39	166			5; 2.44]	6.9%
Sun Y 2013	8	72	3	29		1.08 [0.2]		0.9%
Uiju Cho 2017	2	12	5	140	+ + +	5.40 [0.93		0.6%
Vivian Y. Park 2016	103	214	20	44		1.11 [0.58		3.7%
Yong-Seok Kim 2013	110	241	35	86		1.22 [0.74	ł; 2.02]	5.5%
Random effects model		6381		2791	↓ ↓	1.80 [1.56	; 2.07]	100.0%
Heterogeneity: $I^2 = 28\%$, $\tau^2 = 0.0288$, C Test for overall effect: $z = 10.22$ ($p < 0$						10		
C Test for overall effect: $z = 10.22$ ($p < 0$	0.01)				0.1 0.5 1 2	10		
			Figure 4	. (Conti	nued).			

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.

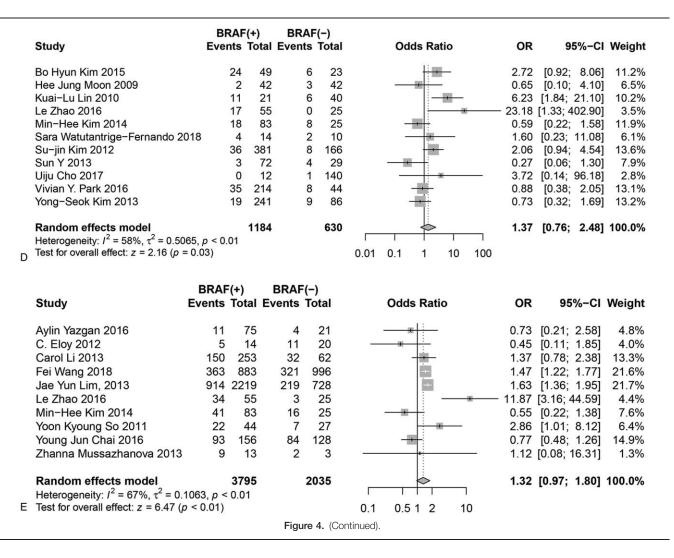
Before we did meta-analysis, we did the quality assessment through CochraneROB quality assessment scale. Because the included studies had no selection bias, performance bias, detection bias, attrition bias or reporting bias, the results gotten from these studies were more credible.

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 metaanalysis. 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



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.

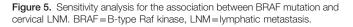
Only 10 articles provide the information of CPTC and <10 papers had mentioned the data of non-CPTC subtype (follicular PTC), so we tried to analyze the analysis with the data in CPTC. The outcome in CPTC (OR = 1.37; 95% CI: 0.76–2.48; P=.29 > .05) was different from the main results (OR = 1.63; 95% CI: 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

Study	Odds Ratio	OR	95%-0	Study	Odds	Ratio	OR	95%-CI
Omitting Yongbo Huang 2013	1 -	1.63	[1.44; 1.85	Adding Alexander Abrosimov 2006 (k=1)	<u> </u>	E-1		[0.12; 1.53]
Omitting Helmi Khadra 2018			[1.43; 1.84	Adding Vito Rodolico 2007 (k=2) Adding F Frasca 2008 (k=3)		100		[0.22; 4.60] [0.68; 3.91]
Omitting Christine J. O'Neill 2010			[1.45; 1.86	Adding Hye Sook Min 2008 (k=4)	-			[0.88; 3.31]
Omitting R.C. da Silva 2015 Omitting ADEGPTXJX IE CHEN 2018			[1.44; 1.85 [1.43; 1.83	Adding Haixia Guan 2008 (k=5)		- 101		[1.01; 3.13]
Omitting C.L. Shi 2015			[1.43; 1.82	Adding Hee Jung Moon 2009 (k=6)				[0.96; 2.70]
Omitting Dan Chen 2018			[1.44; 1.84	Adding Linwah Yip 2009 (k=7)				[1.09; 2.46]
Omitting Guibin Zheng 2017			[1.45; 1.86	Adding Christine J. O'Neill 2010 (k=8) Adding Kuai-Lu Lin 2010 (k=9)		1000 mm		[0.97; 2.21] [1.05; 2.21]
Omitting Hai-Jiang Qu 2018	-		[1.43; 1.84	Adding Chan-Kwon Jung 2010 (k=10)		- <u>111</u> -		[0.93; 2.00]
Omitting Junliang Lu 2015		1.60		Adding Yoon Kyoung So 2011 (k=11)		-		[1.00; 2.08]
Omitting Kuai-Lu Lin 2010		1.63		Adding Avik Chakraborty 2012 (k=12)				[1.08; 2.46]
Omitting Le Zhao 2016			[1.42; 1.82	Adding Salvatore Ulisse 2012 (k=13)				[0.95; 2.25]
Omitting Li–Bo Yang 2015 Omitting M. LI 2017		1.63		Adding C. Eloy 2012 (k=14) Adding Dongbin Ahn 2012 (k=15)				[0.94; 2.13] [0.92; 2.02]
Omitting Meiling Huang 2018		1.62		Adding Eun Sook Kim 2012 (k=16)		1000		[0.92; 2.02]
Omitting Rui-chao Zeng 2016		1.63		Adding Hyung Seok Park 2012 (k=17)		- 110-		[0.97; 1.91]
Omitting SI?YANG DONG 2017		1.62		Adding Ji-Yong Joo 2012 (k=18)				[1.03; 2.00]
Omitting Tung-Sun Huang 2014		1.62		Adding Su-jin Kim 2012 (k=19)				[1.08; 1.95]
Omitting Weibin Wang 2016	-	1.65		Adding Bülent Kurt 2012 (k=20)		100		[1.10; 1.97]
Omitting Xi Wei 2014			[1.43; 1.83	Adding Neslihan Kurtulmus 2012 (k=21) Adding Kathleen C. Lee 2012 (k=22)				[1.14; 2.02] [1.17; 2.03]
Omitting Xiaolei Guan 2017		1.62		Adding Yongbo Huang 2013 (k=23)				[1.18; 2.01]
Omitting Avik Chakraborty 2012	_		[1.42; 1.81	Adding Greta Gandolfi 2013 (k=24)				[1.23; 2.09]
Omitting Nelson George, 2018 Omitting Alona Finke 2016		1.62		Adding Zhanna Mussazhanova 2013 (k=25)			1.57	[1.21; 2.04]
Omitting Chiara Tuccilli 2018		1.65		Adding Dongjun Jeong 2013 (k=26)				[1.26; 2.20]
Omitting F Frasca 2008			[1.43; 1.83	Adding Jae Yun Lim, 2013 (k=27)		अ张张张张张张张梁浩给给老老老老老老老老老老老老老老老老老老老老老老老老老老老老老		[1.31; 2.10]
Omitting Greta Gandolfi 2013		1.61		Adding Jung-Soo Pyo 2013 (k=28) Adding Soo Young Chung 2013 (k=29)				[1.32; 2.09] [1.34; 2.10]
Omitting Salvatore Ulisse 2012	-	1.65	[1.46; 1.87	Adding Sun Y 2013 (k=30)				[1.30; 2.03]
Omitting Sara Watutantrige-Fernando 2018		1.63		Adding WON SEO PARK 2013 (k=31)		*		[1.31; 2.03]
Omitting Vito Rodolico 2007	-	1.63		Adding Yong-Seok Kim 2013 (k=32)		100		[1.30; 1.98]
Omitting Yasuhiro Ito 2014			[1.47; 1.87	Adding Ali S Alzahran 2013 (k=33)				[1.33; 2.00]
Omitting Zhanna Mussazhanova 2013		1.64		Adding Carol Li 2013 (k=34) Adding Christopher Gouveia 2013 (k=35)		100		[1.33; 1.97] [1.36; 1.98]
Omitting Azliana Mohamad Yusof 2018 Omitting Min-Hee Kim 2014		1.63		Adding Gina M. Howell 2013 (k=36)		100		[1.39; 2.01]
Omitting Young Jun Chai 2016		1.65		Adding Tung-Sun Huang 2014 (k=37)				[1.41; 2.05]
Omitting Agnieszka Walczyk 2014	-	1.65		Adding Xi Wei 2014 (k=38)		tion to the second seco	1.72	[1.44; 2.07]
Omitting Aldona Kowalska 2017		1.65		Adding Yasuhiro Ito 2014 (k=39)		100		[1.36; 2.02]
Omitting C. Eloy 2012		1.64	[1.45; 1.86	Adding Min-Hee Kim 2014 (k=40)		*		[1.33; 1.97]
Omitting Alexander Abrosimov 2006		1.65		Adding Agnieszka Walczyk 2014 (k=41) Adding Ah Young Park 2014 (k=42)				[1.31; 1.93] [1.33; 1.93]
Omitting E. TAKACSOVA 2017		1.66		Adding Brian Hung-Hin Lang 2014 (k=43)				[1.35; 1.93]
Omitting Ah Young Park 2014		1.62		Adding Hwa Young Ahn 2014 (k=44)		*		[1.36; 1.94]
Omitting Bo Hyun Kim 2015		1.63		Adding J. Lukas 2014 (k=45)				[1.37; 1.95]
Omitting Brian Hung-Hin Lang 2014 Omitting Chan-Kwon Jung 2010		1.62		Adding R.C. da Silva 2015 (k=46)				[1.38; 1.94]
Omitting Dongbin Ahn 2012		1.64		Adding C.L. Shi 2015 (k=47)		畫		[1.41; 1.98]
Omitting Dongjun Jeong 2013		1.61		Adding Junliang Lu 2015 (k=48) Adding Li-Bo Yang 2015 (k=49)		100		[1.44; 2.06] [1.45; 2.04]
Omitting Eun Sook Kim 2012	-		[1.45; 1.86	Adding Bo Hyun Kim 2015 (k=50)				[1.45; 2.04]
Omitting Hee Jung Moon 2009	S=	1.64		Adding Yoon Yang Jung 2015 (k=51)				[1.47; 2.04]
Omitting Hwa Young Ahn 2014		1.62		Adding Le Zhao 2016 (k=52)		王		[1.50; 2.09]
Omitting Hye Sook Min 2008	-	1.63		Adding Rui-chao Zeng 2016 (k=53)				[1.50; 2.07]
Omitting Hyung Seok Park 2012			[1.44; 1.85	Adding Weibin Wang 2016 (k=54) Adding Alona Finke 2016 (k=55)		100		[1.48; 2.03] [1.47; 2.02]
Omitting Jae Yun Lim, 2013	-	1.64		Adding Young Jun Chai 2016 (k=56)		100		[1.44; 1.98]
Omitting Ji-Yong Joo 2012 Omitting Jong-kyu Kim 2018		1.62	[1.43; 1.83	Adding Seo Ki Kim 2016 (k=57)		- E		[1.44; 1.94]
Omitting Jung-Soo Pyo 2013			[1.44; 1.85	Adding Vivian Y. Park 2016 (k=58)		÷	1.66	[1.43; 1.92]
Omitting Min-Kyung Yeo 2017	-	1.64		Adding Aylin Yazgan 2016 (k=59)		*		[1.42; 1.90]
Omitting Seo Ki Kim 2016	-		[1.44; 1.87	Adding Guibin Zheng 2017 (k=60)		100		[1.41; 1.88]
Omitting Soo Young Chung 2013	-		[1.44; 1.84	Adding M. LI 2017 (k=61) Adding SI?YANG DONG 2017 (k=62)		南		[1.40; 1.86] [1.41; 1.87]
Omitting Su-jin Kim 2012	-		[1.44; 1.85	Adding Xiaolei Guan 2017 (k=63)				[1.43; 1.89]
Omitting Uiju Cho 2017	-		[1.44; 1.84	Adding Aldona Kowalska 2017 (k=64)		min .		[1.41; 1.86]
Omitting Yoon Yang Jung 2015	-		[1.43; 1.84	Adding E. TAKACSOVA 2017 (k=65)				[1.38; 1.83]
Omitting Sun Y 2013	-		[1.46; 1.86	Adding Min-Kyung Yeo 2017 (k=66)				[1.37; 1.81]
Omitting Vivian Y. Park 2016			[1.45; 1.86	Adding Uiju Cho 2017 (k=67)			1.58	[1.38; 1.82]
Omitting WON SEO PARK 2013 Omitting Yong-Seok Kim 2013			[1.44; 1.84 [1.45; 1.86	Adding Helmi Khadra 2018 (k=68) Adding ADEGPTXJX IE CHEN 2018 (k=69)		憲憲		[1.39; 1.83] [1.40; 1.85]
Omitting Yoon Kyoung So 2011	_		[1.43; 1.84	Adding Dan Chen 2018 (k=70)		-		[1.40; 1.85]
Omitting J. Lukas 2014	-		[1.44; 1.84	Adding Hai-Jiang Qu 2018 (k=71)		100		[1.42; 1.86]
Omitting Aylin Yazgan 2016		1.64		Adding Meiling Huang 2018 (k=72)		#	1.64	[1.43; 1.87]
Omitting Bülent Kurt 2012		1.63	[1.44; 1.84	Adding Nelson George, 2018 (k=73)				[1.44; 1.88]
Omitting Neslihan Kurtulmus 2012	-	1.62	[1.43; 1.83	Adding Chiara Tuccilli 2018 (k=74) Adding Sara Watutantrige-Fernando 2018 (k=75)		*		[1.43; 1.86] [1.43; 1.87]
Omitting Ali S Alzahran 2013	-	1.62		Adding Azliana Mohamad Yusof 2018 (k=76)				[1.43; 1.87]
Omitting Carol Li 2013		1.64		Adding Jong-kyu Kim 2018 (k=77)		1011 1011		[1.44; 1.86]
Omitting Christopher Gouveia 2013	-	1.62		Adding Fei Wang 2018 (k=78)		ana ana		[1.44; 1.84]
Omitting Fei Wang 2018 Omitting Gina M. Howell 2013		1.64						
Omitting Haixia Guan 2008		1.62		Random effects model			1.63	[1.44; 1.84]
Omitting Kathleen C. Lee 2012		1.63			0.2 0.5	1 2 5		
Omitting Linwah Yip 2009		1.63			0.2 0.5	2 3		
				Figure 6. Metacum analysis for the as	sociation be	tween BRAF	F mu	tation an
Random effects model	-	- 1.63	[1.44; 1.84	cervical LNM. BRAF=B-type Raf kina				



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 metaanalysis. 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.

Author contributions

Conceptualization: Hongzhi Ma.

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Visualization: Ling Feng.

Writing - original draft: Hongzhi Ma.

Writing - review & editing: Hongzhi Ma.

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