Chronic lymphocytic thyroiditis and BRAF V600E in papillary thyroid carcinoma

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Abstract

It has been reported that papillary thyroid carcinoma (PTC) with chronic lymphocytic thyroiditis (CLT) is less associated with extrathyroidal extension (ETE), advanced tumor stage and lymph node (LN) metastasis. Other studies have suggested that concurrent CLT could antagonize PTC progression, even in BRAF-positive patients. Since the clinical significance of the BRAF mutation has been particularly associated with conventional PTC, the purpose of this study was to determine the clinical significance of CLT according to BRAF mutation status in conventional PTC patients. We retrospectively reviewed the medical records of 3332 conventional PTC patients who underwent total thyroidectomy with bilateral central neck dissection at the Thyroid Cancer Center of Samsung Medical Center between January 2008 and June 2015. In this study, the prevalence of BRAF mutation was significantly less frequent in conventional PTC patients with CLT (76.9% vs 86.6%). CLT was an independent predictor for low prevalence of ETE in both BRAF-negative (OR=0.662, $P=0.023$) and BRAF-positive (OR=0.817, $P=0.027$) conventional PTC patients. In addition, CLT was an independent predictor for low prevalence of CLNM in both BRAF-negative (OR=0.675, $P=0.044$) and BRAF-positive (OR=0.817, $P=0.030$) conventional PTC patients. In conclusion, BRAF mutation was significantly less frequent in conventional PTC patients with CLT. However, CLT was an independent predictor for less aggressiveness in conventional PTC patients regardless of BRAF mutation status.

Key Words

- ▶ BRAF mutation
- ▶ papillary thyroid carcinoma
- ▶ chronic lymphocytic thyroiditis
- ▶ central lymph node metastasis
- ▶ extrathyroidal extension

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Introduction

With a rapidly increasing incidence worldwide, papillary thyroid carcinoma (PTC) represents the majority of thyroid cancer cases (Ahn & Park 2009, Siegel *et al.* 2014). The recently discovered BRAF V600E mutation (hereafter referred to as the BRAF mutation) is the most potent activator of the mitogen-activated protein kinase pathway, which plays a central role in the regulation of

cell growth, division and proliferation (Xing 2007). The BRAF mutation is found predominantly in PTC, particularly in the conventional and tall cell variants (Cohen *et al.* 2003). Numerous studies have demonstrated a strong association between the BRAF mutation and aggressive clinicopathological characteristics of PTC such as advanced stage, extrathyroidal extension (ETE), lymph

node (LN) metastasis and tumor recurrence (Kebebew *et al.* 2007, Lupi *et al.* 2007, Xing 2007). However, there were other studies that did not demonstrate the association between the BRAF mutation and aggressive clinicopathological characteristics of PTC (Lee *et al.* 2012a,b, Gouveia *et al.* 2013, Kim *et al.* 2015).

Chronic lymphocytic thyroiditis/Hashimoto's thyroiditis (hereafter referred to as CLT) is a female-predominant autoimmune disease (Monaco 2012). Since the association between CLT and PTC was reported in 1955 (Dailey *et al.* 1955), many authors have reported that there is an increased risk of developing PTC in those with preexisting CLT (Zhang *et al.* 2012, Lee *et al.* 2013a). On the other hand, some investigators have reported that PTC with the coexistence of CLT is less associated with ETE, advanced stage, LN metastasis and recurrence (Loh *et al.* 1999, Kim *et al.* 2009a, Lang *et al.* 2014). Interestingly, in two previous studies focused on BRAF mutation status in patients with PTC with and without CLT in a Korean population, the BRAF mutation was found less frequently in subjects with CLT than without CLT (64% vs 90% and 72.9% vs 95.3% respectively) (Kim *et al.* 2005a, Kim *et al.* 2009b). Moreover, there was a study that suggested that concurrent CLT could antagonize PTC progression, even in BRAF-positive patients (Marotta *et al.* 2013).

On the basis of these results, we tried to determine the clinical significance of CLT according to BRAF mutation status. Since a previous study showed no association between the BRAF mutation and aggressive clinicopathological characteristics in the follicular variant of PTC (FV-PTC) (Proietti *et al.* 2010), we focused on a large-scale group of conventional PTC patients in particular, excluding other variants such as FV-PTC. Because a previous study suggested that concurrent CLT could antagonize PTC progression, even in BRAF-positive patients under a univariate analysis (Marotta *et al.* 2013), we designed a multivariate analysis regarding the relationship between CLT and BRAF mutation status in conventional PTC patients.

Materials and methods

Patient selection

This study was approved by the institutional review board at Samsung Medical Center. We retrospectively reviewed the medical records of 3332 pathologically-proven, conventional PTC patients who had undergone total thyroidectomy with bilateral central neck dissection (CND) at the Thyroid Cancer Center of the Samsung

Medical Center between January 2008 and June 2015. In accordance with the American Thyroid Association (ATA) management guidelines (American Thyroid Association Guidelines Taskforce on Thyroid N, Differentiated Thyroid C *et al.* 2009), CND was defined as a level VI dissection, including pre- and paratracheal nodes, the precricoid (delphian) node, the perithyroidal nodes and the LN along the recurrent laryngeal nerves. Patients with the following conditions were excluded from this study: age younger than 20 years or older than 80 years; PTC variants other than the conventional type; or the presence of lateral LN or distant metastasis.

Histopathological examination with surgical specimens

Surgical specimens were microscopically examined by two or more experienced pathologists and the following histopathologic factors were assessed: cell type of the main lesion, primary tumor size (measured as the longest diameter of the largest lesion), location, multiplicity, bilaterality, ETE, lymphovascular invasion, margin involvement, LN metastasis and the underlying conditions of the thyroid, such as CLT. The diagnosis of CLT was made on the presence of diffuse parenchymal infiltration by lymphocytes (particularly plasma B-cells), a germinal center formation, follicular destruction, Hurthle cell change and variable amounts of stromal fibrosis throughout the thyroid gland (Lloyd *et al.* 2002, Pearce *et al.* 2003, Cheng & Bostwick 2011, Kakudo *et al.* 2011, Nikiforov *et al.* 2012). To distinguish bilaterality from multiplicity, we defined multiplicity as more than two lesions of conventional PTC in one lobe, regardless of the presence of bilaterality.

BRAF mutation analysis

BRAF mutation analysis was performed at the Molecular Diagnostics Laboratory of the Samsung Medical Center. DNA samples for molecular analysis were extracted from preoperative fine needle aspiration biopsy (FNAB) specimens or postoperative surgical specimens using QIAamp DNA mini-kits (Qiagen). Three distinct molecular methods were used for molecular analysis of the BRAF mutation. Direct sequencing after conventional PCR was performed in an ABI PRISM 3100 sequencer using BigDye Terminator cycle sequencing ready reaction kits (Applied Biosystems, Foster City, CA, USA). Dual Priming Oligonucleotide (DPO)-based allele-specific PCR (AS-PCR) using the Seeplex BRAF ACE detection system (Seegene, Seoul, Korea) with amplified products was analyzed using the

ScreenTape system (Lab901 Ltd, Edinburgh, Scotland, UK). Mutant enrichment with 3'-modified oligonucleotides (MEMO)-based real-time PCR (RT-PCR) using Real-Q BRAF V600E detection kits (BioSewoom, Seoul, Korea) with amplified products was analyzed by BigDye Terminator Cycle Sequencing Kits v.3.1 (Applied Biosystems). DNA sequences from all three methods were compared with the normal BRAF gene exon 15 in the GenBank Database using sequence assembly software (Gene Codes Corp, Ann Arbor, MI, USA). When a thyroid nodule was evaluated using two or more molecular methods and yielded discordant results, the positive result was chosen for analysis.

Statistical analysis

Statistical analysis was performed using SPSS version 22.0 Software and statistically significant differences were defined as those with *P* values of less than 0.05. Continuous variables are presented as mean $\pm$ s.d. and categorical variables are presented as the number of cases, with percentage (%). For comparing the clinicopathological characteristics of conventional PTC patients, the χ^2 test was used for categorical variables and Student's *t*-test was used for continuous variables. Univariate logistic regression analysis was used for calculating odds ratios for ETE and central LN metastasis (CLNM) according to BRAF mutation status and variables with a *P* value <0.05 were included in the multivariate logistic regression analysis. Multivariate logistic regression analysis was conducted to determine the association between CLT and ETE and CLNM according to BRAF mutation status.

Results

Association between CLT and clinicopathological characteristics of conventional PTC

Among the 3332 pathologically proven conventional PTC patients, CLT was found in 1006 (30.2%) patients (Table 1). Conventional PTC patients with CLT were significantly younger than patients without CLT (46.0 years vs 47.6 years, $P<0.001$), and tumor size was significantly smaller in patients with CLT than without CLT (1.1 cm vs 1.2 cm, $P=0.001$). Female gender was significantly more frequent in patients with CLT (90.7% vs 72.5%, $P<0.001$). However, ETE (59.6% vs 67.1%, $P<0.001$), CLNM (53.1% vs 61.3%, $P<0.001$) and positive BRAF mutation (76.9% vs 86.6%, $P<0.001$) were significantly less frequent in patients with CLT.

Table 1 Association between CLT and clinicopathological characteristics of conventional PTC

Clinicopathological characteristics	All conventional PTC (n=3332)		P value
	CLT (–) (n=2326)	CLT (+) (n=1006)	
	No. (%)	No. (%)	
Age (years)			
Mean $\pm$ s.d.	47.6 $\pm$ 11.9	46.0 $\pm$ 11.4	<0.001
<45	991 (42.6)	482 (47.9)	
≥ 45	1335 (57.4)	524 (52.1)	0.005
Sex			
Male	639 (27.5)	94 (9.3)	
Female	1687 (72.5)	912 (90.7)	<0.001
Tumor size (cm)			
Mean $\pm$ s.d.	1.2 $\pm$ 0.8	1.1 $\pm$ 0.7	0.001
≤ 0.5	332 (14.3)	155 (15.4)	
0.5–1.0	852 (36.6)	400 (39.8)	
1.0–2.0	868 (37.3)	362 (36.0)	
2.0–4.0	255 (11.0)	83 (8.3)	
> 4.0	19 (0.8)	6 (0.6)	0.011
Multiplicity			
Absent	1648 (70.9)	692 (68.8)	
Present	678 (29.1)	314 (31.2)	0.232
Bilaterality			
Absent	1645 (70.7)	698 (69.4)	
Present	681 (29.3)	308 (30.6)	0.437
ETE			
Absent	765 (32.9)	406 (40.4)	
Present	1561 (67.1)	600 (59.6)	<0.001
CLNM			
Absent	899 (38.7)	472 (46.9)	
Present	1427 (61.3)	534 (53.1)	<0.001
BRAF			
Negative	311 (13.4)	232 (23.1)	
Positive	2015 (86.6)	774 (76.9)	<0.001

CLT, chronic lymphocytic thyroiditis; PTC, papillary thyroid carcinoma; ETE, extrathyroidal extension; CLNM, central lymph node metastasis.

Multiplicity and bilaterality were not significantly associated with CLT.

Association between CLT and clinicopathological characteristics of conventional PTC according to BRAF mutation status

BRAF mutations were found in 2789 (83.7%) of the 3332 conventional PTC patients in this study (Table 2). Among the 543 BRAF-negative patients, CLT was seen in 232 (42.7%) patients and among the 2789 BRAF-positive patients, CLT was seen in 774 (27.8%) patients. The age of BRAF-negative patients was similar irrespective of the presence/absence of CLT (45.7 years vs 46.0 years, $P=0.822$). However, BRAF-positive patients with CLT were significantly younger than those without CLT (46.1 years vs 47.8 years, $P<0.001$). Female gender was

Table 2 Association between CLT and clinicopathological characteristics of conventional PTC according to BRAF mutation status

Clinicopathological characteristics	BRAF (–) conventional PTC (n=543)			BRAF (+) conventional PTC (n=2789)		
	CLT (–) (n=311)	CLT (+) (n=232)	P value	CLT (–) (n=2015)	CLT (+) (n=774)	P value
	No. (%)	No. (%)		No. (%)	No. (%)	
Age (years)						
Mean $\pm$ s.d.	46.0 $\pm$ 11.7	45.7 $\pm$ 11.9	0.822	47.8 $\pm$ 11.9	46.1 $\pm$ 11.2	<0.001
<45	144 (46.3)	111 (47.8)		847 (42.0)	371 (47.9)	
$\geq$ 45	167 (53.7)	121 (52.2)	0.722	1168 (58.0)	403 (52.1)	0.005
Sex						
Male	75 (24.1)	16 (6.9)		564 (28.0)	78 (10.1)	
Female	236 (75.9)	216 (93.1)	<0.001	1451 (72.0)	696 (89.9)	<0.001
Tumor size (cm)						
Mean $\pm$ s.d.	1.3 $\pm$ 1.0	1.1 $\pm$ 0.7	0.021	1.2 $\pm$ 0.8	1.1 $\pm$ 0.7	0.015
$\leq$ 0.5	54 (17.4)	46 (19.8)		278 (13.8)	109 (14.1)	
0.5–1.0	108 (34.7)	84 (36.2)		744 (36.9)	316 (40.8)	
1.0–2.0	105 (33.8)	80 (34.5)		763 (37.9)	282 (36.4)	
2.0–4.0	40 (12.9)	21 (9.1)		215 (10.7)	62 (8.0)	
>4.0	4 (1.3)	1 (0.4)	0.145	15 (0.7)	5 (0.6)	0.048
Multiplicity						
Absent	223 (71.7)	173 (74.6)		1425 (70.7)	519 (67.1)	
Present	88 (28.3)	59 (25.4)	0.457	590 (29.3)	255 (32.9)	0.06
Bilaterality						
Absent	240 (77.2)	178 (76.7)		1405 (69.7)	520 (67.2)	
Present	71 (22.8)	54 (23.3)	0.903	610 (30.3)	254 (32.8)	0.193
ETE						
Absent	120 (38.6)	116 (50.0)		645 (32.0)	290 (37.5)	
Present	191 (61.4)	116 (50.0)	0.008	1370 (68.0)	484 (62.5)	0.006
CLNM						
Absent	118 (37.9)	121 (52.2)		781 (38.8)	351 (45.3)	
Present	193 (62.1)	111 (47.8)	0.001	1234 (61.2)	423 (54.7)	0.002

CLT, chronic lymphocytic thyroiditis; PTC, papillary thyroid carcinoma; ETE, extrathyroidal extension; CLNM, central lymph node metastasis.

significantly more frequent in both BRAF-negative (93.1% vs 75.9%, $P<0.001$) and BRAF-positive (89.9% vs 72.0%, $P<0.001$) patients with CLT. Tumor size was significantly smaller in patients with CLT than without CLT for both BRAF-negative (1.1 cm vs 1.3 cm, $P=0.021$) and BRAF-positive (1.1 cm vs 1.2 cm, $P=0.015$) mutation status. ETE (BRAF-negative, 50.0% vs 61.4%, $P=0.008$; BRAF-positive, 62.5% vs 68.0%, $P=0.006$) and CLNM (BRAF-negative, 47.8% vs 62.1%, $P=0.001$; BRAF-positive, 54.7% vs 61.2%, $P=0.002$) were consistently less frequent in patients with

CLT regardless of BRAF mutation status. Multiplicity and bilaterality were not associated with CLT in either BRAF-negative or BRAF-positive patients.

CLT was an independent predictor for low prevalence of ETE in both BRAF-negative and BRAF-positive conventional PTC patients

As seen in Table 2, ETE was significantly less frequent in conventional PTC patients with CLT (Tables 3 and 4).

Table 3 Association between ETE and clinicopathological characteristics of BRAF-negative conventional PTC

Clinicopathological characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI) for ETE	P value	OR (95% CI) for ETE	P value
Age (per 10 years)	0.822 (0.714–0.947)	0.007	0.875 (0.753–1.017)	0.081
Female	0.659 (0.413–1.053)	0.080	NA	NA
Tumor size (per 0.1 cm)	1.961 (1.513–2.543)	<0.001	1.818 (1.394–2.371)	<0.001
Multiplicity	1.586 (1.072–2.346)	0.021	1.509 (1.005–2.256)	0.048
Bilaterality	1.311 (0.871–1.973)	0.193	NA	NA
CLT	0.628 (0.445–0.886)	0.008	0.662 (0.464–0.944)	0.023

ETE, extrathyroidal extension; PTC, papillary thyroid carcinoma; OR, odds ratio; CLT, chronic lymphocytic thyroiditis.

Table 4 Association between ETE and clinicopathological characteristics of BRAF-positive conventional PTC

Clinicopathological characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI) for ETE	P value	OR (95% CI) for ETE	P value
Age (per 10 years)	0.885 (0.829–0.945)	<0.001	0.865 (0.807–0.928)	<0.001
Female	1.025 (0.851–1.235)	0.792	NA	NA
Tumor size (per 0.1 cm)	2.088 (1.809–2.410)	<0.001	2.162 (1.870–2.501)	<0.001
Multiplicity	1.681 (1.404–2.011)	<0.001	1.695 (1.398–2.053)	<0.001
Bilaterality	1.357 (1.140–1.615)	0.001	1.146 (0.950–1.383)	0.155
CLT	0.786 (0.661–0.934)	0.006	0.817 (0.683–0.977)	0.027

ETE, extrathyroidal extension; PTC, papillary thyroid carcinoma; OR, odds ratio; CLT, chronic lymphocytic thyroiditis.

Therefore, we analyzed whether CLT could be an independent predictor for low prevalence of ETE. As seen in Tables 3 and 4, CLT was an independent predictor for low prevalence of ETE in both BRAF-negative (OR=0.662, $P=0.023$) and BRAF-positive (OR=0.817, $P=0.027$) patients. In BRAF-negative patients, tumor size per 0.1 cm (OR=1.818, $P<0.001$) and multiplicity (OR=1.509, $P=0.048$) were independent predictors for high prevalence of ETE. In BRAF-positive conventional PTC patients, age per 10 years (OR=0.865, $P<0.001$) was an independent predictor for low prevalence of ETE. Tumor size per 0.1 cm (OR=2.088, $P<0.001$) and multiplicity (OR=1.695, $P<0.001$) were independent predictors for high prevalence of ETE.

CLT was an independent predictor for low prevalence of CLNM in both BRAF-negative and BRAF-positive conventional PTC patients

As seen in Table 2, CLNM was also significantly less frequent in conventional PTC patients with CLT (Tables 5 and 6). We investigated whether CLT could be an independent predictor for low prevalence of CLNM. As seen in Tables 5 and 6, CLT was an independent predictor for low prevalence of CLNM in both BRAF-negative (OR=0.675, $P=0.044$) and BRAF-positive (OR=0.817, $P=0.030$)

patients. In BRAF-negative patients, age per 10 years (OR=0.757, $P=0.001$) and female gender (OR=0.481, $P=0.009$) were independent predictors for low prevalence of CLNM. Tumor size per 0.1 cm (OR=1.680, $P<0.001$), multiplicity (OR=2.336, $P<0.001$) and ETE (OR=2.401, $P<0.001$) were independent predictors for high prevalence of CLNM. In BRAF-positive conventional PTC patients, age per 10 years (OR=0.652, $P<0.001$) and female gender (OR=0.482, $P<0.001$) were independent predictors for low prevalence of CLNM. Tumor size per 0.1 cm (OR=1.965, $P<0.001$), multiplicity (OR=1.280, $P=0.006$) and ETE (OR=1.478, $P<0.001$) were independent predictors for high prevalence of CLNM.

Discussion

The purpose of this study was to determine whether CLT could be an independent predictor for aggressiveness in conventional PTC patients according to BRAF mutation status. Although a previous study suggested that concurrent CLT could antagonize PTC progression even in BRAF-positive patients (Marotta et al. 2013), the analysis was univariate and evaluated only a small number of exclusively BRAF-positive patients. In this study, we performed a multivariate analysis and revealed that CLT was an independent predictor for the low prevalence of

Table 5 Association between CLNM and clinicopathological characteristics of BRAF-negative conventional PTC

Clinicopathological characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI) for CLNM	P value	OR (95% CI) for CLNM	P value
Age (per 10 years)	0.725 (0.627–0.838)	<0.001	0.757 (0.642–0.891)	0.001
Female	0.395 (0.239–0.652)	<0.001	0.481 (0.278–0.831)	0.009
Tumor size (per 0.1 cm)	2.165 (1.651–2.840)	<0.001	1.680 (1.266–2.229)	<0.001
Multiplicity	2.475 (1.645–3.724)	<0.001	2.336 (1.499–3.642)	<0.001
Bilaterality	0.846 (0.567–1.263)	0.414	NA	NA
ETE	3.165 (2.222–4.509)	<0.001	2.401 (1.642–3.512)	<0.001
CLT	0.561 (0.397–0.792)	0.001	0.675 (0.460–0.989)	0.044

CLNM, central lymph node metastasis; PTC, papillary thyroid carcinoma; OR, odds ratio; CLT, chronic lymphocytic thyroiditis.

Table 6 Association between CLNM and clinicopathological characteristics of BRAF-positive conventional PTC

Clinicopathological characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI) for CLNM	P value	OR (95% CI) for CLNM	P value
Age (per 10 years)	0.674 (0.631–0.721)	<0.001	0.652 (0.607–0.700)	<0.001
Female	0.435 (0.358–0.529)	<0.001	0.482 (0.391–0.594)	<0.001
Tumor size (per 0.1 cm)	2.134 (1.866–2.440)	<0.001	1.965 (1.709–2.260)	<0.001
Multiplicity	1.211 (1.026–1.430)	0.024	1.280 (1.072–1.530)	0.006
Bilaterality	1.139 (0.967–1.343)	0.119	NA	NA
ETE	1.626 (1.387–1.907)	<0.001	1.478 (1.240–1.761)	<0.001
CLT	0.763 (0.645–0.902)	0.002	0.817 (0.681–0.981)	0.030

CLNM, central lymph node metastasis; PTC, papillary thyroid carcinoma; OR, odds ratio; CLT, chronic lymphocytic thyroiditis.

ETE and CLNM in both BRAF-positive and BRAF-negative conventional PTC patients.

Classically, CLT frequently occurs in females (F:M=10:1) (Monaco 2012), and we consistently found a strong association of CLT with female gender, regardless of BRAF mutation status. It has been suggested that concurrent CLT is significantly associated with a lower frequency of BRAF mutations (Kim *et al.* 2005a, Kim *et al.* 2009b), and we also observed that the BRAF mutation was significantly less frequent in conventional PTC patients with CLT (76.9% vs 86.6%, $P<0.001$). A recent study proposed that the prevalence of the BRAF mutation in patients with CLT has been underestimated by a dilution effect on the mutated alleles of infiltrating lymphocytes, which carry WT BRAF (Guerra *et al.* 2012). Further investigations are needed to elucidate the association between CLT and the BRAF mutation.

In all conventional PTC patients regardless of BRAF mutation status, CLT was significantly associated with a low prevalence of aggressive clinicopathological characteristics, including tumor size, ETE and CLNM (Tables 1 and 2). Furthermore, from the multivariate analysis (Tables 3 and 4), CLT was an independent predictor for the low prevalence of conventional PTC in both BRAF-negative and BRAF-positive patients. These results support the hypothesis that the coexistence of CLT counteracts tumor progression and recurrence in PTC (Loh *et al.* 1999, Kim *et al.* 2009a, Lang *et al.* 2014). Furthermore, there were previous studies that did not demonstrate the association between BRAF mutation and aggressive clinicopathological characteristics of PTC (Lee *et al.* 2012a,b, Gouveia *et al.* 2013, Kim *et al.* 2015). These studies could explain why the impact of CLT was not different according to BRAF mutation status.

Particularly, CLT can be detected in three ways preoperatively. First, FNAB is a sensitive and specific tool

for the detection of CLT (Carson *et al.* 1996, Kini & Grossl 1997, MacDonald & Yazdi 2011). Secondly, diffuse parenchymal heteroechogenicity in US is commonly regarded as a strong indicator for CLT (Gutekunst *et al.* 1989, Marcocci *et al.* 1991, Rago *et al.* 2001). Thirdly, the presence of thyroid autoantibodies, typically anti-thyroid peroxidase antibodies in the serum, however, seronegative CLT is also possible (Grani *et al.* 2015). Based on results from previous work (Loh *et al.* 1999, Kim *et al.* 2009a, Lang *et al.* 2014) and this study, preoperative recognition of CLT by careful reviewing of FNAB, US and serology can be valuable for risk stratification in conventional PTC patients.

In agreement with previous studies, we found that young age (Roh *et al.* 2011, Kim *et al.* 2015), male gender (Lang *et al.* 2014, Kim *et al.* 2015), large tumor size (Roh *et al.* 2011, Joo *et al.* 2012, Lang *et al.* 2014, Kim *et al.* 2015), multiplicity (Lang *et al.* 2014, Kim *et al.* 2015) and ETE (Roh *et al.* 2011, Joo *et al.* 2012, Lang *et al.* 2014, Kim *et al.* 2015) were independent predictors for high prevalence of CLNM regardless of BRAF mutation status, and bilaterality (Lang *et al.* 2014, Kim *et al.* 2015) was not. Primarily, for clear analysis, we excluded conventional PTC patients with tumor size >4.0 cm. Because there were only 25 patients who met the inclusion criteria of this study and the size variation of the 25 patients was very wide, from 4.1 to 8.0 cm. However, our results and conclusion were not changed whether 25 conventional PTC patients with tumor size >4.0 cm were included or excluded. Therefore, we included 25 conventional PTC patients with tumor size >4.0 cm

This study had several limitations. First, since the study had a retrospective design, patient information was not fully collected. Further prospective studies might be required to clarify the relationship between PTC and CLT. Secondly, since this study was conducted in a

BRAF-prevalent area (Kim *et al.* 2004, Kim *et al.* 2005b), the results may not be applicable to other populations. Thirdly, since we enrolled only conventional PTC patients, the results of this study cannot be applied to other types of thyroid cancers or other variants of PTC. Fourthly, this study included conventional PTC patients who underwent total thyroidectomy with bilateral CND. Total thyroidectomy was performed according to ATA management guidelines (American Thyroid Association Guidelines Taskforce on Thyroid *et al.* 2009) when primary tumor size >1 cm, multifocality, bilaterality, ETE, or abnormal lymphadenopathy was detected during preoperative or intraoperative examination. Furthermore, therapeutic CND was performed when abnormal lymphadenopathy was detected during preoperative or intraoperative examination, and prophylactic CND was performed on PTC patients with clinically uninvolved central neck LNs, in particular for advanced primary tumors (T₃/T₄). Therefore, the selection of cases was possibly biased toward a high-risk group. Finally, three distinct types of molecular methods and two distinct types of specimens were used for BRAF mutation analysis. We did not perform BRAF mutation analysis as a routine preoperative examination, and molecular methods have evolved over the years since March 2008. This variability may have introduced inconsistencies in the results of BRAF mutation testing. Although we performed BRAF mutation analysis with both FNAB specimens and surgical specimens, numerous studies have demonstrated that BRAF mutation analysis can be performed readily and reliably using solely FNAB specimens preoperatively (Chung *et al.* 2006, Jin *et al.* 2006).

In conclusion, BRAF mutation was significantly less frequent in conventional PTC patients with CLT. However, CLT was an independent predictor for less aggressiveness in conventional PTC patients regardless of BRAF mutation status. Since there have been only a few studies regarding this topic, further investigations are needed to elucidate the relationship between BRAF mutations and CLT.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

S K Kim, J-W Woo, J H Lee and I Park were involved in the review of literature, acquisition of data and drafting and completing the manuscript. S K Kim, J-H Choe, J-H Kim and J S Kim conceived the study, participated in the co-ordination and the acquisition of data and helped to draft the manuscript. All authors read and approved the final manuscript.

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