Curr Oncol, Vol. 21, pp. e740-747; doi: http://dx.doi.org/10.3747/co.21.2029

ORIGINAL ARTICLE

BRAF mutation correlates with recurrent papillary thyroid carcinoma in Chinese patients

F.J. Huang MB,[a] W.Y. Fang PhD,*[a] L. Ye MD PhD,* X.F. Zhang PhD,* L.Y. Shen PhD,* R.L. Han PhD,* Q. Wei MD PhD,[†] X.C. Fei MB,[†] X. Chen MD PhD,[‡] W.Q. Wang MD PhD,* S. Wang MD PhD,*[§] and G. Ning MD PhD*[§]

ABSTRACT

Purpose

We investigated correlations of somatic *BRAF* V600E mutation and *RET/PTC1* rearrangement with recurrent disease in Chinese patients with papillary thyroid carcinoma (PTC).

Methods

This prospective study included 214 patients with PTC histologically confirmed between November 2009 and May 2011 at a single institute.

Results

We found somatic *BRAF* V600E mutation in 68.7% and *RET/PTC1* rearrangement in 25.7% of the patients. Although *BRAF* mutation was not significantly associated with clinicopathologic features such as patient sex or age, multicentric disease, thyroid capsule invasion, tumour stage, or nodal metastasis, it was significantly associated with recurrent disease. Multivariate analysis revealed that *BRAF* mutation and tumour size were independent risk factors associated with recurrent disease, with odds ratios of 9.072 and 2.387 respectively. The area under the receiver operating characteristic curve increased 8.3% when *BRAF* mutation was added to the traditional prognostic factors, but that effect was statistically nonsignificant (0.663 vs. 0.746, $p = 0.124$). *RET/PTC1* rearrangement and nodal metastasis were significantly associated in all patients ($p = 0.042$), marginally associated in PTC patients ($p = 0.051$), but not associated in microPTC patients ($p = 0.700$). *RET/PTC1* rearrangement was not significantly associated with recurrent disease.

Conclusions

BRAF positivity is an independent predictor of recurrent disease in PTC.

KEY WORDS

Papillary thyroid carcinoma, *BRAF, RET/PTC1,* recurrence

1. INTRODUCTION

The prevalence of thyroid cancer has been increasing worldwide[1], including in China[2]. The disease has become the fastest-increasing cancer, and it ranks 5th among the most common cancers in women[3]. Notably, the current increase is almost entirely attributable to papillary thyroid carcinoma (PTC)[4]. The prognosis of differentiated thyroid cancer is benign, with a survival rate of 96.4% after 30 years follow-up[5]. However, a significant percentage of PTC patients experience recurrent disease or distant metastasis, reducing the survival rate to 40%[6].

Several staging systems have been applied to stratify the risk of a poor outcome with PTC. However, all are based on histopathologic parameters after surgery[7]. They therefore cannot be used before an operation to determine the extent of surgery. With the emerging understanding of molecular genetics in thyroid cancer, several specific mutations in PTC have been determined[8]. The *BRAF* V600E mutation has been associated with worse prognostic features (such as extrathyroidal extension, lymph node metastasis or advanced tumour stage), poor clinical outcome, and mortality[9–13]. The *RET/PTC* oncogenes are believed to play an important role in radiation-induced PTC[14]. In contrast to *BRAF* mutation, *RET/PTC* rearrangement has been associated with better prognosis[15]. Yet despite those findings, controversy remains[16–21]. In addition, questions about the clinical significance of these mutations in the management of microPTC are ongoing[22].

[a] These authors contributed equally to the present work.

We investigated the associations of *BRAF* V600E mutation and *RET*/*PTC1* rearrangement with clinicopathologic features and outcomes in Chinese patients with PTC, including microPTC.

2. METHODS

2.1 Patients

The board of medical ethics of Ruijin Hospital, Shanghai Jiaotong University, School of Medicine approved the study, and all patients gave written informed consent. The study enrolled 214 patients with histologically confirmed PTC who underwent surgery in Ruijin Hospital between November 2009 and May 2011. We performed genotyping analysis using frozen PTC tissue. Because of sample limitations, only 101 patients were analyzed for *RET*/*PTC1* rearrangement. Patients were followed twice annually using, as necessary, tests for thyroid-stimulating hormone, thyroglobulin, thyroglobulin antibody; imaging by ultrasonography and computed tomography; and biopsy by fine-needle aspiration. Recurrent disease was defined by histologic or cytologic findings or by clinical appearance on imaging studies. Median follow-up was 36 months (range: 12–50 months), and the follow-up rate was 79.4%.

2.2 Detection of *BRAF* Mutation

Snap-frozen fresh tissues, including both cancerous and adjacent normal thyroid tissue, were collected by experienced pathologists and stored in liquid nitrogen until use. Evaluation of DNA extracted using a DNA purification kit (Omega Bio-Tek, Norcross, GA, U.S.A.) was performed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). The presence of *BRAF* exon 15 mutation was determined by Sanger sequencing, as previously reported[23]. Polymerase chain reactions were performed in 25 μL buffer containing 1.5 mmol/L $MgCl_2$, 200 mmol/L deoxynucleoside triphosphate, 50–100 ng genomic DNA, 0.5 mmol/L each primer, and 2.5 U TaKaRa Taq DNA polymerase (Takara Bio, Tokyo, Japan). Thirty-five cycles with annealing temperatures optimized at 60°C. Polymerase chain reaction product was purified and sequenced using an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, U.S.A.).

2.3 Detection of *RET*/*PTC1* Rearrangement

Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, U.S.A.) as described in the manufacturer's protocol. An aliquot containing 1 μg total RNA was reverse-transcribed using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI, U.S.A.), and *RET*/*PTC1* was amplified by nested polymerase chain reaction, separated on 1.2% agarose gel, and visualized under ultraviolet illumination as previously reported[24]. As a positive control, RNA from the TPC1 cell line was used.

2.4 Statistical Analysis

Statistical analyses were performed using SPSS Statistics (version 17.0: SPSS, Chicago, IL, U.S.A.) and MedCalc (version 13.0: MedCalc Software, Ostend, Belgium). The Fisher exact test was used for nominal variables, and the Mann–Whitney nonparametric test was used for continuous variables. Univariate and multivariate logistic regression were used to determine the risk factors associated with recurrent disease. Odds ratios (ORs) were calculated by exponentiation of logistic regression analysis and reported with 95% confidence intervals (CIs). To measure the discriminative improvement attributable to the risk score for recurrent disease, we plotted receiver operating characteristic curves for two logistic regression models: Model 1, which included traditional prognostic factors (that is, age, sex, thyroid capsule invasion, multicentricity, TNM stage), and Model 2, which included the traditional factors, plus *BRAF* mutation. All p values are two-tailed, and $p < 0.05$ was accepted as statistically significant.

3. RESULTS

3.1 Clinicopathologic Characteristics

As Table I shows, microPTC accounted for 30.4% of the tumours in the study group; most patients (65.0%) presented stage I or II disease. No distant metastases were found at first diagnosis; of the 170 patients who were subsequently followed, 15 (8.8%) experienced recurrent disease.

A somatic *BRAF* V600E mutation was carried by 68.7% of all study patients, and a somatic *RET*/*PTC1* rearrangement by 25.7% of the 101 whose samples could be tested. Dual mutations (*BRAF* and *RET*/*PTC1*) were harbored by 15 patients [14.9% (15 of 101)]. *BRAF* mutation was more prevalent in patients with PTC than with microPTC (73.2% vs. 58.5%, $p = 0.038$, Table II). No *BRAF* V601 mutation was found. No *BRAF* mutation or *RET*/*PTC1* rearrangement was found in the normal tissue adjacent to the PTC tumour.

3.2 Correlations Between *BRAF* Mutation, *RET*/*PTC1* Rearrangement, and Clinicopathological Characteristics of Patients with PTC

As shown in Table III, *BRAF* mutation was significantly associated with Hashimoto disease ($p = 0.000$), PTC ($p = 0.001$), and microPTC ($p = 0.015$). *BRAF* mutation was less prevalent in patients with Hashimoto disease (32.3%) than in those without (74.9%). We found no association between *BRAF* mutation and sex, age, multicentricity, thyroid capsule invasion,

tumour stage, or nodal metastasis. We observed a nonsignificant trend toward positive *BRAF* mutation status in patients with tumours of advanced TNM stage ($p = 0.176$).

In tested patients, *RET/PTC1* rearrangement was significantly associated with nodal metastasis ($p = 0.042$) and marginally with PTC ($p = 0.051$), but not with microPTC ($p = 0.700$). We found no significant association of *RET/PTC1* rearrangement with other clinicopathologic features (Table IV).

TABLE I Clinicopathologic features in 214 cases of papillary thyroid carcinoma (PTC)

Feature	*Value*
Sex (*n* men/women)	66/148
Mean age (years)	43±12
Hashimoto disease [*n* (%)]	31 (14.5)
MicroPTC [*n* (%)]	65 (30.4)
Multicentricity [*n* (%)]	49 (22.9)
Thyroid capsule invasion [*n* (%)]	76 (35.5)
Tumour size	
Mean (cm)	1.6±0.9
Category [*n* (%)]	
≤1 cm	65 (30.4)
≤2 cm	104 (48.6)
≤4 cm	43 (20.1)
>4 cm	2 (0.9)
Nodal metastases	106 (49.5)
Stage[a] [*n* (%)]	
I	135 (63.1)
II	4 (1.9)
III	38 (17.8)
IV	37 (17.3)
BRAF V600E [*n* (%)]	147 (68.7)
RET/PTC1[b] [*n* (%)]	26 (25.7)
Recurrence-free survival [*n* (%)]	155 (91.2[c])

[a] Using the American Joint Committee on Cancer's TNM classification system for differentiated thyroid carcinoma.
[b] Because of sample limitations, only 101 analyses for *RET/PTC1* rearrangement were performed.
[c] Only 170 patients were followed.

TABLE II *BRAF* mutation and *RET/PTC1* rearrangement in papillary thyroid carcinoma (PTC)

Genetic characteristic		*Disease type [*n *(%)]*		p *Value*
		PTC[a]	*MicroPTC*[b]	
BRAF	Yes	109 (73.2)	38 (58.5)	0.038
	No	40 (26.9)	27 (41.5)	
RET/PTC1	Yes	16 (22.5)	10 (33.3)	0.320
	No	55 (77.5)	20 (66.7)	

[a] Tumours greater than 1 cm in size.
[b] Tumours 1 cm or less in size.

3.3 Prognostic Factors Associated with Recurrent Disease

We found recurrent disease in 15 patients. Tumour size and *BRAF* mutation status were associated with recurrence. Recurrent tumours were larger (2.0 ± 0.2 cm vs. 1.5 ± 0.1 cm, $p = 0.017$) and more likely to harbour *BRAF* mutation (93.3% vs. 64.5%, $p = 0.022$, Table V). No association of recurrent disease with Hashimoto disease, TNM stage, type of surgery, or *RET/PTC1* rearrangement was found. Multivariate analysis confirmed that tumour size and *BRAF* mutation were independent prognostic factors associated with recurrent PTC (Table VI). Compared with patients whose tumours lacked the somatic *BRAF* V600E mutation, patients with tumours harbouring the mutation had a risk of recurrent disease that was increased by a factor of 9 (OR: 9.072; 95% CI: 1.072 to 76.739; $p = 0.043$). Table VII shows correlations between *BRAF* mutation and other clinicopathologic variables.

We further evaluated the incremental value of *BRAF* (with respect to traditional prognostic factors) to predict recurrent disease. The area under the receiver operating characteristic curve was calculated for two models: Model 1 included the traditional prognostic factors (age at diagnosis, sex, thyroid capsule invasion, multicentricity, and stage); Model 2 included the Model 1 variables, plus *BRAF* mutation. As Figure 1 and Table VIII show, the area under the curve was 0.663 (95% CI: 0.529 to 0.797) for Model 1 and 0.746 (95% CI: 0.631 to 0.860) for Model 2—achieving a nonsignificant increase of 8.3% with the addition of *BRAF* mutation ($p = 0.124$).

4. DISCUSSION

In the present study, we genotyped PTC tumours and investigated the associations of mutations with clinicopathologic features and clinical outcome. We found that *BRAF* mutation was an independent predictor of recurrent disease, although it not associated with advanced clinicopathologic features; on the other hand, *RET/PTC1* rearrangement was associated with nodal disease, but not with recurrent disease.

Although most PTC patients experience favourable outcomes, significant numbers of patients develop recurrent disease and experience poor outcomes. To improve clinical outcomes, disease staging systems have been established to stratify management strategies[25]. Large tumour size, older age, extrathyroidal invasion, male sex, multicentricity, distant metastasis, and lymph node metastasis

TABLE III Correlations between clinicopathologic features and *BRAF* mutation by papillary thyroid carcinoma (PTC) type

Feature	BRAF-*positive patients*					
	Overall		*With* PTC[a]		*With micro*PTC[b]	
	*[*n *(%)]*	p *Value*	*[*n *(%)]*	p *Value*	*[*n *(%)]*	p *Value*
Sex						
Men	49/66 (74.2)	0.267	41/51 (80.4)	0.176	8/15 (53.3)	0.767
Women	98/148 (66.2)		68/98 (69.4)		30/50 (60.0)	
Age						
<45 Years	68/103 (66.0)	0.462	55/79 (69.6)	0.356	13/24 (54.2)	0.612
≥45 Years	79/111 (71.2)		54/70 (77.1)		25/41 (61.0)	
Hashimoto disease						
Yes	10/31 (32.3)	0.000	6/17 (35.3)	0.001	4/14 (28.6)	0.015
No	137/183 (74.9)		103/132 (78.0)		34/51 (66.7)	
Multicentricity						
Yes	34/49 (69.4)	1.000	27/37 (73.0)	1.000	7/12 (58.3)	1.000
No	113/165 (68.5)		82/112 (73.2)		31/53 (58.5)	
Thyroid capsule invasion						
Yes	56/76 (73.7)	0.282	51/67 (76.1)	0.578	5/9 (55.6)	1.000
No	91/138 (65.9)		58/82 (70.7)		33/56 (58.9)	
T-Stage[c]						
T1–2	90/137 (65.7)	0.223	57/81 (70.4)	0.460	33/56 (58.9)	1.000
T3–4	57/77 (74.0)		52/68 (76.5)		5/9 (55.6)	
Nodal metastasis						
Yes	69/106 (65.1)	0.303	54/76 (71.1)	0.584	15/30 (50.0)	0.219
No	78/108 (72.2)		55/73 (75.3)		23/35 (65.7)	
Stage[c]						
I–II	94/139 (67.6)	0.758	68/98 (69.4)	0.176	26/41 (63.4)	0.310
III–IV	53/75 (70.7)		41/51 (80.4)		12/24 (50.0)	

[a] Tumours greater than 1 cm in size.
[b] Tumours 1 cm or less in size.
[c] Using the American Joint Committee on Cancer's TNM classification system for differentiated thyroid carcinoma.

are the main determinants of a poor outcome in PTC patients[6,25–27]. Currently, the need for preoperative risk stratification and for the enlarged "grey zone" of microPTC require an optimized risk scoring system. Genetic markers such as *BRAF* mutation and *RET*/*PTC* rearrangements seem to be able to fill the gap. Positivity for *BRAF* mutation has been associated with a negative prognosis and poor clinical outcome. However, conflicting data have also been reported. The clinical significance of *BRAF* analysis in PTC patients has been disputed because the association of positivity with poor outcome was believed to depend on the mutation's association with aggressive tumour behavior. Our data provide an important piece of evidence to help resolve the dispute. We did not find a significant association of *BRAF* mutation with negative prognostic indicators such as large tumour size, old age, extrathyroidal invasion, male sex, multicentricity, lymph node metastasis, or TNM stage. However, *BRAF* mutation and tumour size were both associated with recurrent disease (although not with tumour stage and extent of surgery). Compared with patients lacking a *BRAF* mutation, those with the mutation were more likely (by a factor of 9) to experience recurrent disease. *BRAF* analysis provided an 8.3% increment over traditional predictors for recurrent disease, but that increase was not statistically significant (p = 0.124). Our data confirm the clinical significance of *BRAF* analysis in PTC patients and suggest a need for more extensive surgery and for more aggressive postsurgical management such as radioactive iodine ablation, suppression of thyroid-stimulating hormone below 0.1 mU/L, and closer follow-up in somatic *BRAF* carriers. However, knowing a patient's *BRAF* status did not substantially improve prediction overall, probably because of the limited number of recurrence events.

The lack of any associations between *BRAF* mutation and advanced clinicopathologic features

TABLE IV Correlations between clinicopathologic features and *RET*/*PTC1* rearrangement by papillary thyroid carcinoma (PTC) type

Feature	RET/PTC1-*positive patients*					
	Overall		*With* PTC[a]		*With micro*PTC[b]	
	*[*n *(%)]*	p *Value*	*[*n *(%)]*	p *Value*	*[*n *(%)]*	p *Value*
Sex						
Men	7/30 (23.3)	0.807	5/22 (22.7)	1.000	2/8 (25.0)	0.682
women	19/71 (26.8)		11/49 (22.4)		8/22 (36.4)	
Age						
<45 Years	11/41 (26.8)	1.000	9/31 (29.0)	0.268	2/10 (20.0)	0.419
≥45 Years	15/60 (25.0)		7/40 (17.5)		8/20 (40.0)	
Hashimoto disease						
Yes	7/19 (36.8)	0.249	5/11 (45.5)	0.109	2/8 (25.0)	0.682
No	19/82 (23.2)		11/60 (18.3)		8/22 (36.4)	
Multicentricity						
Yes	6/19 (31.6)	0.565	4/16 (25.0)	0.746	2/3 (66.7)	0.251
No	20/82 (24.4)		12/55 (21.8)		8/27 (29.6)	
Thyroid capsule invasion						
Yes	9/31 (29.0)	0.629	8/28 (28.6)	0.389	1/3 (33.3)	1.000
No	17/70 (24.3)		8/43 (18.6)		9/27 (33.3)	
T-Stage[c]						
T1–2	17/69 (24.6)	0.808	8/42 (19.0)	0.406	9/27 (33.3)	1.000
T3–4	9/32 (28.1)		8/29 (27.6)		1/3 (33.3)	
Nodal metastases						
Yes	17/48 (35.4)	0.042	11/33 (33.3)	0.051	6/15 (40.0)	0.700
No	9/53 (17.0)		5/38 (13.2)		4/15 (26.7)	
Stage[c]						
I–II	14/63 (22.2)	0.350	9/43 (20.9)	0.774	5/20 (25.0)	0.231
III–IV	12/38 (31.6)		7/28 (25.0)		5/10 (50.0)	

[a] Tumours greater than 1 cm in size.
[b] Tumours 1 cm or less in size.
[c] Using the American Joint Committee on Cancer's TNM classification system for differentiated thyroid carcinoma.

might have several explanations. First, our cohort came from a single centre serving patients from Shanghai and its surrounding area, where iodine is sufficient. Guan *et al.*[28] reported that high iodine intake is a significant risk factor for *BRAF* mutation, and a meta-analysis showed that a high prevalence of the *BRAF* V600E mutation tended to have a smaller meta-risk of extrathyroidal invasion and lymph node metastasis[7]. Second, a significant number of our patients underwent subtotal thyroidectomy or lobectomy, which might lead to an underestimation of aggressive pathologic characteristics[21,29]. Consistent with an earlier study[7], *BRAF* mutation was confirmed as an independent predictor of recurrence in our cohort at 3 years of follow-up. Our data indicate the importance of performing follow-up studies.

Other important findings of our study include the observation that, in addition to *BRAF* mutation, tumour size is another independent predictor of recurrent PTC. Also, *BRAF* V600E was, in general, less prevalent in microPTC than in PTC, which is consistent with most earlier studies[7,13]. In addition, *BRAF* mutation was inversely associated with Hashimoto disease, which has also been reported in a Korean cohort[30]. This inverse correlation suggests that *BRAF* mutation and Hashimoto disease are exclusively involved in PTC initiation. Moreover, where dual mutation of *BRAF* and *RET*/*PTC1* occurred (15 patients), 16.7% experienced recurrent disease, compared with the 11.8% of patients harbouring solely a *BRAF* mutation (p = 0.664, Table IX). That observation is consistent with an earlier finding that patients with dual mutation are more susceptible to recurrent disease[31].

The limitations of our study include its lack of multicentre data and long-term follow-up. The limitation resulting from extent of surgery has already been discussed. Papillary thyroid carcinoma is slow-growing. Long-term follow-up, including data on both recurrence and mortality, is required to comprehensively elucidate the role of *BRAF* mutation

TABLE V Factors prognostic for survival in 170 patients with papillary thyroid carcinoma (PTC)

Factor	*Recurrence*		p *Value*
	No	*Yes*	
Mean tumour size (cm)	1.5±0.1	2.0±0.2	0.017
Mean age (years)	44±1	46±3	0.711
Age category (*n*)			
<45 Years	69	4	0.275
≥45 Years	86	11	
Sex (*n*)			
Men	49	7	0.258
Women	106	8	
Hashimoto disease (*n*)			
Yes	25	3	0.716
No	130	12	
MicroPTC (*n*)			
Yes	53	2	0.148
No	102	13	
Multicentricity (*n*)			
Yes	32	2	0.738
No	123	13	
Thyroid capsule invasion (*n*)			
Yes	53	5	1.000
No	102	10	
T-Stage[a] (*n*)			
T1–2	102	9	0.777
T3–4	53	6	
Nodal metastases (*n*)			
Yes	72	8	0.788
No	83	7	
Stage[a] (*n*)			
I–II	98	7	0.267
III–IV	57	8	
Thyroid surgery (*n*)			
Total thyroidectomy	69	8	0.408
Subtotal thyroidectomy	66	7	
Lobectomy	20	0	
Lymph node dissection (*n*)			
No	48	6	0.528
Central	75	5	
Central and lateral	32	4	
BRAF mutation (*n*)			
Yes	100	14	0.022
No	55	1	
RET/*PTC1* rearrangement (*n*)			
Yes	20	2	1.000
No	51	4	

[a] Using the American Joint Committee on Cancer's TNM classification system for differentiated thyroid carcinoma.

analysis in the management of this most frequent thyroid cancer.

5. CONCLUSIONS

We analyzed both *BRAF* mutation and *RET*/*PTC1* rearrangement in 214 Chinese PTC patients with a median follow-up of 36 months. We found that *BRAF* mutation was an independent predictor of recurrent disease despite a lack of association with advanced clinicopathologic features. Our data indicate that *BRAF* mutation is not just a marker of aggressiveness, but a true prognostic factor. Although the increase was nonsignificant, *BRAF* mutation analysis added prognostic value over that of traditional predictors. In accord with previous studies, we suggest that *BRAF* mutation analysis has a positive role to play in the presurgical assessment of PTC patients. However, larger series and longer-term follow-up data are required.

6. ACKNOWLEDGMENTS

This study was supported by the grants from Science and Technology Commission of Shanghai (13QH1402000) and Key Project of the Science and Technology Commission of Shanghai (10411951100). We thank Professor Min Xu from the Clinical Epidemiology Group in the Shanghai Institute of Endocrine and Metabolic Disease for statistical assistance.

7. CONFLICT OF INTEREST DISCLOSURES

All authors declare no competing financial interests with respect to the preparation of this work.

8. REFERENCES

1. Chen AY, Jemal A, Ward EM. Increasing incidence of differentiated thyroid cancer in the United States, 1988–2005. *Cancer* 2009;115:3801–7.
2. Wang Y, Wang W. Increasing incidence of thyroid cancer in Shanghai, China, 1983–2007. *Asia Pac J Public Health* 2012;:[Epub ahead of print].
3. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics 2010. *CA Cancer J Clin* 2010;60:277–300.
4. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973–2002. *JAMA* 2006;295:2164–7.
5. Elisei R, Molinaro E, Agate L, *et al.* Are the clinical and pathological features of differentiated thyroid carcinoma really changed over the last 35 years? Study on 4187 patients from a single Italian institution to answer this question. *J Clin Endocrinol Metab* 2010;95:1516–27.
6. Schlumberger MJ. Papillary and follicular thyroid carcinoma. *N Engl J Med* 1998;338:297–306.
7. Lupi C, Giannini R, Ugolini C, *et al.* Association of *BRAF* V600E mutation with poor clinicopathological outcomes in 500 consecutive cases of papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2007;92:4085–90.

TABLE VI Univariate and multivariate analysis of clinicopathologic features and recurrence-free survival in papillary thyroid carcinoma (PTC)

Feature	*Analysis*					
	Univariate			*Multivariate*		
	OR	*95% CI*	p *Value*	*OR*	*95% CI*	p *Value*
Male sex	1.893	0.650 to 5.515	0.242	1.471	0.459 to 4.715	0.516
Age	1.008	0.966 to 1.052	0.717	0.978	0.923 to 1.036	0.445
Multicentricity	0.591	0.127 to 2.755	0.503	0.256	0.037 to 1.785	0.169
Thyroid capsule invasion	0.962	0.313 to 2.960	0.947	0.267	0.058 to 1.226	0.089
Stage III–IV	1.965	0.677 to 5.703	0.214	5.216	0.989 to 27.518	0.052
Tumour size	1.639	1.012 to 2.656	0.045	2.387	1.197 to 4.761	0.013
BRAF mutation	7.700	0.986 to 60.128	0.052	9.072	1.072 to 76.739	0.043

TABLE VII Correlations between *BRAF* mutation and other variables in the multivariate model

Variable	*Presence of* BRAF *mutation*	
	Correlation coefficient[a]	p *Value*
Age	0.132	0.086
Sex	0.039	0.618
Multicentricity	–0.025	0.746
Capsule invasion	0.056	0.471
Stage	0.088	0.255
Tumour size	0.123	0.110

[a] Spearman rho.

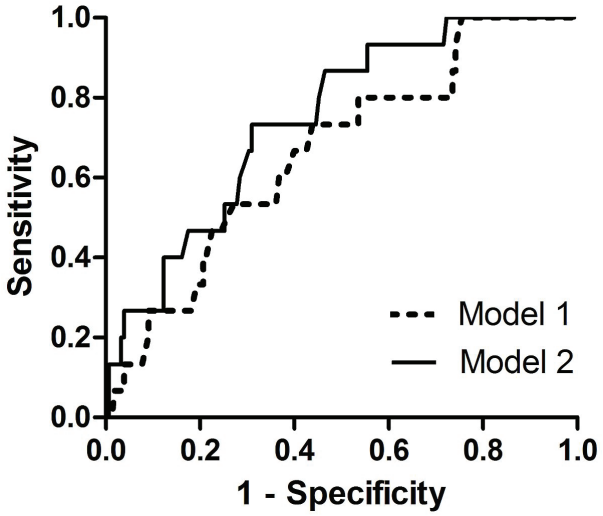

FIGURE 1 *Predictability of recurrent papillary thyroid carcinoma using combined prediction models. Model 1 included traditional prognostic factors (age, sex, thyroid capsule invasion, multicentricity, TNM stage), and Model 2 included traditional factors, plus* BRAF *mutation. The area under the curve was 0.663 (95% confidence interval: 0.529 to 0.797) for Model 1, and 0.746 (95% confidence interval: 0.631 to 0.860) for Model 2. The addition of* BRAF *mutation to traditional prognostic factors increased the predictability by 8.3%, but without statistical significance (*p = *0.124).*

TABLE VIII Predictability of recurrent papillary thyroid carcinoma, measured by area under the curve (AUC)

Model	*Prediction score*[a]		
	AUC	*95% CI*	p *Value*[b]
1[c]	0.663	0.529 to 0.797	0.124
2[d]	0.746	0.631 to 0.860	

[a] Calculated from logistic regression. Model 1 = –3.323 – 0.018(age in years) + 0.645(sex: male=1, female=0) – 0.667(multicentricity: yes=1, no=0) – 0.434(thyroid capsule invasion: yes=1, no=0) + 1.233(stage III–IV=1, I–II=0); Model 2 = –5.094 – 0.022(age in years) + 0.651(sex: male=1,; female=0) – 0.795(multicentricity: yes=1, no=0) – 0.715(thyroid capsule invasion: yes=1, no=0) + 1.443(stage III/IV=1, I–II=0) + 2.150(*BRAF* mutation: yes=1, no=0). A higher prediction score corresponds to a higher risk of recurrence.

[b] Model 1 versus model 2.

[c] Included traditional prognostic factors (that is, age, sex, thyroid capsule invasion, multicentricity, TNM stage).

[d] Included traditional factors plus *BRAF* mutation.

TABLE IX Prevalence of sole and dual *BRAF* mutations in recurrent and recurrence-free papillary thyroid carcinoma

Mutation status	*Recurrence [*n *(%)]*		p *Value*
	No	*Yes*	
BRAF-positive only	30 (88.2)	4 (11.8)	0.664
BRAF- and *RET/PTC1*-positive	10 (83.3)	2 (16.7)	

8. Kim TH, Park YJ, Lim JA, *et al.* The association of the *BRAF*(V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: a meta-analysis. *Cancer* 2012;118:1764–73.
9. Elisei R, Viola D, Torregrossa L, *et al.* The *BRAF*(V600E) mutation is an independent, poor prognostic factor for the outcome of patients with low-risk intrathyroid papillary thyroid carcinoma: single-institution results from a large cohort study. *J Clin Endocrinol Metab* 2012;97:4390–8.
10. Li C, Lee KC, Schneider EB, Zeiger MA. *BRAF* V600E mutation and its association with clinicopathological features of papillary thyroid cancer: a meta-analysis. *J Clin Endocrinol Metab* 2012;97:4559–70.
11. Xing M, Clark D, Guan H, *et al. BRAF* mutation testing of thyroid fine-needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer. *J Clin Oncol* 2009;27:2977–82.
12. Xing M, Alzahrani AS, Carson KA, *et al.* Association between *BRAF* V600E mutation and mortality in patients with papillary thyroid cancer. *JAMA* 2013;309:1493–501.
13. Walczyk A, Kowalska A, Kowalik A, *et al.* The *BRAF* V600E mutation in papillary thyroid microcarcinoma: does the mutation have an impact on clinical outcome? *Clin Endocrinol (Oxf)* 2014;80:899–904.
14. Nikiforov YE, Rowland JM, Bove KE, Monforte–Munoz H, Fagin JA. Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. *Cancer Res* 1997;57:1690–4.
15. Tallini G, Santoro M, Helie M, *et al. RET*/*PTC* oncogene activation defines a subset of papillary thyroid carcinomas lacking evidence of progression to poorly differentiated or undifferentiated tumor phenotypes. *Clin Cancer Res* 1998;4:287–94.
16. Ito Y, Yoshida H, Kihara M, Kobayashi K, Miya A, Miyauchi A. *BRAF*(V600E) mutation analysis in papillary thyroid carcinoma: is it useful for all patients? *World J Surg* 2014;38:679–87.
17. Gouveia C, Can NT, Bostrom A, Grenert JP, van Zante A, Orloff LA. Lack of association of *BRAF* mutation with negative prognostic indicators in papillary thyroid carcinoma: the University of California, San Francisco, experience. *JAMA Otolaryngol Head Neck Surg* 2013;139:1164–70.
18. Brzezianska E, Pastuszak–Lewandoska D, Wojciechowska K, *et al.* Investigation of V600E *BRAF* mutation in papillary thyroid carcinoma in the Polish population. *Neuro Endocrinol Lett* 2007;28:351–9.
19. Fugazzola L, Mannavola D, Cirello V, *et al. BRAF* mutations in an Italian cohort of thyroid cancers. *Clin Endocrinol (Oxf)* 2004;61:239–43.
20. Kim TY, Kim WB, Song JY, *et al.* The *BRAF* mutation is not associated with poor prognostic factors in Korean patients with conventional papillary thyroid microcarcinoma. *Clin Endocrinol (Oxf)* 2005;63:588–93.
21. Ito Y, Yoshida H, Maruo R, *et al. BRAF* mutation in papillary thyroid carcinoma in a Japanese population: its lack of correlation with high-risk clinicopathological features and disease-free survival of patients. *Endocr J* 2009;56:89–97.
22. Soares P, Sobrinho–Simões M. Cancer: small papillary thyroid cancers—is *BRAF* of prognostic value? *Nat Rev Endocrinol* 2011;7:9–10.
23. Qing W, Fang WY, Ye L, *et al.* Density of tumor-associated macrophages correlates with lymph node metastasis in papillary thyroid carcinoma. *Thyroid* 2012;22:905–10.
24. Wang YL, Wang JC, Wu Y, *et al.* Incidentally simultaneous occurrence of *RET*/*PTC*, *H4–PTEN* and *BRAF* mutation in papillary thyroid carcinoma. *Cancer Lett* 2008;263:44–52.
25. Sipos JA, Mazzaferri EL. Thyroid cancer epidemiology and prognostic variables. *Clin Oncol (R Coll Radiol)* 2010;22:395–404.
26. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. *Am J Med* 1994;97:418–28.
27. Durante C, Haddy N, Baudin E, *et al.* Long-term outcome of 444 patients with distant metastases from pappilary and follicular thyroid carcinoma: benefits and limits of radioiodine therapy. *J Clin Endocrinol Metab* 2006;91:2892–9.
28. Guan H, Ji M, Bao R, *et al.* Association of high iodine intake with the T1799A *BRAF* mutation in papillary thyroid cancer. *J Clin Endocrinol Metab* 2009;94:1612–17.
29. Xing M. [Comments on the Ito *et al.* article (*Endocr J* 2008 Oct 8 Epub ahead of print)] The lack of clinicopathological correlation of *BRAF* mutation in papillary thyroid cancer needs to be interpreted with caution. *Endocr J* 2009;56:305–6.
30. Kim SK, Song KH, Lim SD, *et al.* Clinical and pathological features and the *BRAF*(V600E) mutation in patients with papillary thyroid carcinoma with and without concurrent Hashimoto thyroiditis. *Thyroid* 2009;19:137–41.
31. Henderson YC, Shellenberger TD, Williams MD, *et al.* High rate of *BRAF* and *RET*/*PTC* dual mutations associated with recurrent papillary thyroid carcinoma. *Clin Cancer Res* 2009;15:485–91.

Correspondence to: Lei Ye, 197 Ruijin 2nd Road, Shanghai 200025 PR China.
E-mail: lei.yelei@gmail.com

* Shanghai Key Laboratory for Endocrine Tumors, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, and Shanghai e-Institute for Endocrinology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, PR China.
† Department of Pathology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, PR China.
‡ Department of General Surgery, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, PR China.
§ Laboratory for Endocrine and Metabolic Diseases of Institute of Health Science, Shanghai JiaoTong University School of Medicine, and Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, PR China.