

Comprehensive evaluation of risk factors for lymph node metastasis with papillary thyroid carcinoma in Southwest China patients

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Research

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Abstract

Background: With the increasing incidences of papillary thyroid cancer(PTC), it is important to risk-stratify patients who may have more aggressive tumor biology. This study aimed to evaluate the risk factors for lymph node metastasis with PTC in Southwest China Patients which may provide a substantial reference for clinical diagnosis and treatment.

Methods: 1045 PTCs (313 PTMC and 732 non-PTMC) between August 2016 and August 2019 were examined totally (including one Tibetan). BRAF V600E mutation was tested in all samples. The clinical data (gender, age, tumor location, sample source and pathological features) were retrospectively analyzed. Logistic regression analysis was performed to evaluate independent risk factors for LNM.

Results: 181 out of 313 PTMC cases (57.8%), 145 out of 732 non-PTMC cases (19.8%) had BRAF V600E mutation, the Tibetan had a double mutation of BRAF L597Q and V600E in two separate lesions. In PTMC, significant difference in gender and sample source was found (BRAF V600E mutation vs. wild-type). In non-PTMC, significant difference in gender was found (BRAF V600E mutation vs. wild-type). The female (OR=1.952; 95% CI= 1.373-2.774; $P=0.00$), age (31-59 years) and diameter of tumor ≤ 1 cm (OR=3.273; 95% CI= 2.417-4.432; $P=0.000$) were significant independent predictors of LNM in all PTCs. In PTMC, the female (OR= 3.002; 95% CI= 1.654-5.446; $P=0.00$) was a significant independent predictor of LNM. The tumor in left and right lobes simultaneously was an independent protective factor of LNM in each group (PTCs: OR=0.287; PTMC: OR=0.170; non-PTMC: OR=0.441, respectively). The BRAF V600E mutation rate of US-FNAC was much higher than FFPE in PTMC ($P=0.018$).

Conclusions: Unlike previous research, our findings suggested that the female patients and diameter of tumor ≤ 1 cm were risk factors for LNM and the BRAF V600E wild-type of PTMC might be more aggressive than others. Interestingly, the position of tumor in bilateral thyroid simultaneously was an independent protective factor for LNM. The US-FNA should be recommended for gene analysis (BRAF V600E) in PTMC. The BRAF L597Q mutation may be an independent aggressive factor in the Chinese Tibetan population. Hence, clinicians should consider an individualized treatment according to gene mutation, gender, age, tumor size and location of tumor in order to achieve a better therapeutic efficacy.

Background

Papillary thyroid carcinoma (PTC), is the most common histological subtype of thyroid cancer, which occurs in more than 90% of all thyroid malignancies, has increased incidence at an alarming pace in recent years^[1-4]. This recent dramatic change is primarily attributable to the increased using of the fine-needle aspiration (FNA) or ultrasonography-guided biopsy as the early diagnosis methods in patients without palpable thyroid nodes^[5, 6]. Although the mortality rate of PTC is relatively low, 20-50% of patients accompany with the risk of the worst clinical outcomes (e.g. distant metastases^[7], the high rate of long-term persistence of the disease and possibility of recurrence^[8]). Papillary thyroid microcarcinoma (PTMC, diameter of tumor ≤ 1 cm), accounts in more than 50% of all new-onset thyroid cancers, has been

increasing rapidly during the last several decades all around the world^[9,10]. Clinically, many cases have demonstrated that PTMC had a good prognosis in most instances following surgical interventions. What's more, PTMC tumor growth was usually very slow and some patients developed clinically problematic tumor growth after many years of observation^[11,12]. In addition, most PTMC also have a very indolent nature and excellent outcomes, expert consensus recommended that PTMC should be identified and managed separately^[13, 14].

The B-type Raf kinase (BRAF) mutation has frequently been the subject of intensive research to investigate the tumorigenic role and clinical implications in thyroid cancers, particularly PTCs. Approximately, 90% of BRAF mutations was T1799A transverse point mutation, resulting in a valineto glutamic acid switch at codon 600 (V600E)^[15, 16]. The kinase activity of BRAF V600E was 460-fold higher than the wild-type BRAF, and this active conformation can constitutively activate its downstream effects to transform normal cells or induce cancer proliferation without the need of RAS for activation^[17], which suggests that the mutation is a early event during PTCs development, and there is a complex process that might effect tumorigenesis and aggressiveness. The rare BRAF L597Q (c.T1790A) point mutation, which is regarded as an oncogene, has been previously reported and described in childhood acute lymphoblastic leukemia^[18].

As in PTC in general, lymph node metastasis has been reported to be a risk factor for increased tumor recurrence rates and also be closely in connection with reduced survival rate^[19]. In addition, in 2018, Lutz. et al^[20] reported that an imbalance in DNA repair gene expression is associated with aggressive clinicopathological features in PTCs. Given the controversies above, a total of 1045 PTCs patients were enrolled in this study, including 313 patients with PTMC and 732 patients in non-PTMC (diameter of tumor \leq 1 cm). The aim of this retrospective observational study was to verify the associations of BRAF V600E mutations with clinicopathologic features and next to identify the risk factors for LNM of PTC patients. One case of solitary brain metastasis from occult papillary thyroid carcinoma in the Chinese Tibetan population was reported firstly in detail. This information may bring benefits for clinicians to make correct clinical decisions for PTC patients in future.

Methods

Patient population

In this study, the clinical data of 1045 Southwest China patients (including one Tibetan) with PTCs were collected for analysis from August 2016 to August 2019. According to tumor diameter, patients were diagnosed with PTMC or non-PTMC. Among these patients, 313 were diagnosed with PTMC, 732 were diagnosed with non-PTMC. All participants of this study were Chinese without blood relationship with each other and have signed informed consent.

All patients met the inclusion criteria, which were the following: (1) underwent either a resection or a diagnostic procedure (biopsy or cytological specimen); (2) confirmed as PTC by intraoperative rapid

pathology or postoperative pathology detection; (3) analysis of gene mutation. Different locations of thyroid tumor were divided into seven regions: left lobe, right lobe, both left and right lobes, isthmus, left lobe and isthmus, right lobe and isthmus, bilateral lobes and isthmus according to US imaging results. Gender, age and diagnostic date, sample source were available for 1045 patients.

Pathological examination

PTC tissues were embedded in paraffin and were sectioned at 4 μ m according to standard procedures. The sections were processed for HE staining and were used for observation by light microscopy. Different type of PTCs and presence of lymph node metastasis were reviewed by two pathologists independently in a blinded manner. The inconsistent diagnostic cases have been discussed with a third pathologist.

Preoperative skull CT/MRI scan and molecular pathology diagnosis

The CT, MRI, color ultrasound diagnosis, ultrasound-guided fine-needle aspiration (US-FNA) and HE staining were used for morphological detection. The specific expression of thyroid cancer-related proteins (cytokeratin, CK; thyroglobulin, Tg and thyroid transforming factor-1, TTF-1) were detected by the immunohistochemical (IHC) analysis.

Sample collection, DNA extraction and Mutation Screening

DNA were extracted from formalin-fixed paraffin-embedded (FFPE) or FNAC by TRIzol reagent (Invitrogen U.S. Cat. No.15596-026) according to the manufacturer's protocols. DNA concentrations of all samples were determined by the NanoDrop ND-1000 spectrophotometer at 280 nm (Thermo Scientific, Waltham, and Mass). The gene mutation was detected by three different technology platforms (ARMS-PCR, NGS and Sanger sequencing). The ARMS-PCR reagents were provided by Amoy Diagnostics Co., Ltd (P215101901X, Xiamen, China). The NGS and Sanger sequencing were performed by laboratory developed tests (LDTs), and some high-frequency mutation and targeted drug-related genes of thyroid cancer have been detected by NGS (Table 7), the forward primer of BRAF in Sanger sequencing: 5'-GCTTGCTCTGATAGGAAAATGAG-3', the reverse primer of BRAF in Sanger sequencing: 5'-GGGCCAAAATTTAATCAGTGG-3' and the primers were synthesized by Invitrogen Bio-Tech Co., Ltd. (Shanghai, China).

Statistical analysis

Statistical analysis was performed using IBM SPSS 22.0 software (IBM Corp., Version 22.0, Armonk, NY, USA). Quantitative data were expressed as mean \pm SD. Qualitative data were represented as a percentage or frequency. The Chi square test or Fisher's exact test was used to evaluate the difference in clinical features between two different groups. The univariate and multivariate logistic regression analysis were performed to assess independent risk factors for presence of LNM in PTCs, results are reported as odds ratios (OR) with 95% confidence intervals (CI). A p-value less than 0.05 was considered statistically significant.

Results

Clinicopathological Characteristics of 1045 Patients with PTMC or non-PTMC

A retrospective study of 732 patients with non-PTMC and 313 patients with PTMC during the period of 2016.08 - 2019.08 was performed to assess the clinicopathological characteristics at diagnosis, including gender, age at diagnosis, sample source (FFPE tissues or FNAC), lymph node metastasis, different locations of thyroid tumor (left lobe, right lobe, both left and right lobes, isthmus, left lobe and isthmus, right lobe and isthmus, bilateral lobes and isthmus) and BRAF V600E mutation status, etc (Table 1). The BRAF V600E mutation rates of PTCs have been increasing during 2016.08 - 2019.08 (Figure 5), in addition, the mutation rate of PTMC was significant higher than non-PTMC ($P<0.05$).

298 male and 747 female patients have been analyzed in this study. The mean age was 41.97 ± 12.94 . The patients were divided into three subgroups according to age: young subgroup (<30 years, $n = 206$), middle subgroup (31-59 years, $n = 735$), and old subgroup (≥ 60 years, $n = 104$). Sample source was consist of FFPE ($n = 742$) and FNAC ($n = 303$). Lymph node metastasis was present in 181 cases (57.8%) of PTMC and 145 cases (19.8%) of non-PTMC. The lymph node metastasis was present in 31.2% patients. For location of thyroid tumor: 382 PTC patients in left lobe, 480 in right lobe, 6 in isthmus, 150 in both left and right lobes, 7 in left lobe and Isthmus, 15 in right lobe and Isthmus, 5 in bilateral lobes and isthmus. The BRAF V600E mutation occurred in 273 cases (87.2%) of PTMC and 566 cases (77.3%) of non-PTMC. The total mutation rate of BRAF V600E was 80.3%. The clinicopathological characteristics and sample source between PTMC and non-PTMC was compared in this study (Table 1). The BRAF V600E mutation rate in PTMC group was much higher than the non-PTMC group ($P=0.00$). The frequency of lymph node metastasis in PTMC group was also significantly higher than the non-PTMC group ($P=0.00$). Other clinical parameters showed no significant differences between the two groups.

BRAF V600E mutational status and clinical characteristics in patients with PTMC or non-PTMC

The relationship of BRAF mutation status and clinical characteristics of 313 PTMC patients were analyzed in this study. The BRAF V600E mutation showed significant association with male gender ($P=0.026$) and sample source from FFPE tissues ($P=0.018$) compared with the BRAF V600E wild-type in PTMC patients. However, there was no difference in lymph node metastases, age and location of thyroid tumor between the BRAF V600E mutation and the BRAF V600E wild-type (Table 2).

The BRAF V600E mutation showed significant association with male gender ($P=0.003$) compared with the BRAF V600E wild-type in non-PTMC patients. However, there was no difference in other clinical features between the BRAF V600E mutation and the BRAF V600E wild-type (Table 2).

The clinicopathological characteristics and sample source in all BRAF V600E mutation patients were compared additionally (Table 3). A lower rate of lymph node metastasis ($P=0.00$, $\chi^2=42.369$) was presented in PTMC than in non-PTMC. The number of middle subgroup (31-59, $P=0.004$, $\chi^2=11.306$) had

statistical significance between PTMC and non-PTMC. However, there was no difference in other clinical features between PTMC and non-PTMC.

Univariate and multivariate analysis of risk factors for LNM in PTCs, PTMC and non-PTMC

In PTCs (Table 4), the female (OR = 1.952; 95% CI= 1.373-2.774; $P= 0.00$), middle subgroup (31-59) (OR = 1.560; 95% CI= 1.050-2.318; $P= 0.028$), PTMC (OR = 3.273; 95% CI= 2.417-4.432; $P= 0.000$) were characterized as independent risky factors for LNM. Moreover, the tumor in left and right lobes simultaneously (OR= 0.287; 95% CI= 0.166-0.497; $P= 0.000$) was characterized as a protective factor for LNM. However, there was no difference between BRAF V600E mutation and LNM ($P> 0.05$).

In PTMC (Table 5), the female (OR = 3.002; 95% CI= 1.654-5.446; $P= 0.00$) was characterized as independent risky factor, the tumor in left and right lobes simultaneously (OR =0.170; 95% CI= 0.071-0.405; $P= 0.00$) was characterized as a protective factor. The age, BRAF V600E mutation did not show statistical differences with LNM ($P> 0.05$).

In non-PTMC (Table 6), the tumor in left and right lobes simultaneously (OR =0.441; 95% CI= 0.220-0.882; $P= 0.00$) was characterized as a protective factor for LNM. The gender, age and BRAF V600E mutation did not show statistical differences with LNM ($P> 0.05$).

The BRAF L597Q mutation of PTC in one Tibetan

A 57-year-old Tibetan male patient (with a protruding mass on the left forehead) came to hospital with numbness in the right limb for three weeks. The preoperative skull CT and MRI scan showed a 9.3 cm × 8.1 cm mass in the left frontal (Fig. 1A and B). The skin on the surface of the mass was normal and hard in texture. The preoperative conventional color ultrasound diagnosis showed that the size of the bilateral thyroid was normal, and the echo in the right lobe was uneven. A mass (1.2 cm × 0.7 cm) was visible, with oval shape and clearly defined border. The “left frontal lobe occupancy and skull tumor resection” surgical plan was suggested for implementation by the multi-disciplinary team (MDT). Intraoperative display showed that the scalp tissue and the left frontal skull had obvious adhesion, and the protruding bone tissue surface was uneven and loose. The hyperplasia of the inner and outer plates of the skull was obvious (Fig. 1C). The tumor tissue was white and solid, and there was no adhesion with the surrounding brain tissue (Fig. 1D). The preoperative conventional color ultrasound diagnosis showed that the size of the bilateral thyroid was normal, and the echo in the right lobe was uneven. A mass (0.7 cm × 0.5 cm) was visible, with oval shape and clearly defined border (Fig. 2A). The US-FNA was used to biopsy the thyroid nodules, the tumor cells were relatively uniform in size, with round nuclei, small nucleoli visible in part (Fig. 2B).

Some nucleus of cancer cells showed Ground Glass Opacity (GGO) by HE staining. The nuclear grooves and pseudoinclusions in nucleus were observed clearly, and psammoma bodies was seen in interstitial tissue (Fig. 3A). The cytokeratin (CK), thyroglobulin (Tg) and thyroid transforming factor-1 (TTF-1) were immunoreactive (Fig. 3B, C and D) by IHC. The ARMS-PCR, NGS and Sanger sequencing analysis showed

that the patient had a double mutation of BRAF L597Q and V600E in two separate lesions (Fig. 4). The BRAF V600E (chr7:140453136 c.1799T>A) mutation was located in situ (Fig. 4A and C), but the BRAF L597Q (chr7:140453145 c.1790T>A) mutation was located in the intracranial metastases (Fig. 4B and D). The abundances of BRAF L597Q and V600E were 36.9% and 8.1% respectively, as determined by NGS, and which were successfully verified by Sanger sequencing (the gold standard of gene sequencing).

Discussion

With the increasing incidences of non-PTMC and PTMC, it is important to risk-stratify patients who may have more aggressive tumor biology in what is traditionally thought to be a more indolent disease, which will have management implications including whether or not to observe, the extent of surgical resection, the use of RIA therapy and the frequency of follow-up^[21]. Li^[22] have reported that the PTMC had an indolent course and excellent prognosis, while our results demonstrated that the incidence of LNM was more frequent in PTMC than in non-PTMC. The correlation analysis revealed that the incidence of BRAF V600E mutation in male PTMC was much higher than in female. Lee^[23] have recommended that the male could be as an independent prognostic factor for recurrence in non-PTMC, but it was not a prognostic factor in PTMC. In our study, the incidence of BRAF V600E mutation in FNAC was much higher than in FFPE which was consistent with some previous studies^[24, 25], however, the LNM was related to BRAF V600E wild-type in PTMC which was inconsistent with other study^[26]. Thus, our findings demonstrated that the BRAF V600E mutation was more likely to manifest among male patients and more easily to be detected in FNAC of PTMC.

To further analyze the difference between PTMC and non-PTMC in the biological behavior, the clinicopathological characteristics and sample source in BRAF V600E mutation patients were also compared. Although several studies have reported that BRAF V600E mutation in PTCs was associated with aggressive pathological features, negative influence on ¹³¹I avidity, reduced thyroperoxidase, the increased risk of lymph node metastasis and recurrence after treatment^[27-29], the clinical implications and clear mechanisms in PTMC and non-PTMC was contradictory. Zheng^[30] have reported that tumor diameter (>0.5 cm) was an independent risk factor correlated with LNM in PTCs. Interestingly, our findings definitely demonstrated that the LNM rate was much higher and was correlated with BRAF V600E wild-type in PTMC, which possibly indicated that BRAF V600E mutation in PTMC was less aggressive which was differ from a previous meta-analysis^[22]. A study^[31] have reported that most PTMC with BRAF V600E mutation did not display BRAF V600E protein expression.

Correct preoperative diagnosis is of importance. It is generally agreed that better knowledge about predictive risk factors for LNM may guide clinical decisions, while the greater risk of LNM remains debatable. After adjusting for other significant preoperative clinical factors, univariate and multivariate analysis was performed to identify the risk factors for LNM. Gender is a prominent patient background parameter for PTC. In recent years, the association between gender and recurrence or survival of PTC has been debated. A previous study reported that the male was an independent clinical prognostic factor of

poor outcome in PTC^[32], but not in PTMC^[23]. Recently, Roh^[33] have reported that there was no association between gender and LNM. In our study, although the female had a lower BRAF V600E mutation than the male, multivariate analysis demonstrated that the female was a risk factor of LNM in PTMC and non-PTMC, which was different from above studies. A previous study^[34] also have explored that the female was an independent predictive risk factor of CLNM in PTC. Controversies in different studies could be related to different sample source, sample size and detection techniques. It has been reported that the female had an earlier age of onset, but the male had a higher mortality^[35, 36]. It is recommended that the molecular mechanisms between LNM and gender in PTC patients should be explored in future.

In our study, the middle age (31-59) group was about 1.56 times the young age (≤ 30) group for LNM in PTCs, which was inconsistent with other study^[37]. Our results have showed that the location of tumor in bilateral thyroid simultaneously was a protective factor for LNM in PTMC and non-PTMC, which was also inconsistent with a retrospective cohort study^[38].

The question is to clarify whether or not, after controlling the clinical importance of BRAF V600E mutation, to take into account that some other BRAF mutations deserve thoughtful analysis. The BRAF L597Q mutation incidence was less than 1%^[39] such as in childhood acute lymphoblastic leukemia^[18], which might be associated with aggressive clinicopathologic features, however, the potential role of the peculiar BRAF L597Q mutation of PTC was unclear. To the best of our knowledge, we have reported the first case of solitary brain metastasis from occult papillary thyroid carcinoma in the Chinese Tibetan population in detail, as all known, the common metastatic sites of thyroid cancer are lung and bone, skull metastases are extremely rare^[40]. Our results have demonstrated that the rare BRAF L597Q point mutation might play a specific role in inducing the solitary intracranial metastasis of occult papillary thyroid carcinoma in the Chinese Tibetan population. In addition, in this case, the biopsy or traditional gene sequencing technologies (such as the ARMS-PCR, solely for detecting the BRAF V600E mutation) had certain limitations, the combination of NGS and Sanger sequencing should help in detecting the rare gene mutation (e.g BRAF L597Q mutation), which was greatly recommended to improve the diagnostic accuracy and molecular mechanism analysis in rare PTC cases. It is concluded that the multilevel gene analysis may be a great substitute for the traditional gene testing^[41, 42].

Nonetheless, our retrospective study had several potential limitations. Firstly, the number of the rare cases (BRAF L597Q mutation with skull metastases) was few. Secondly, the detailed molecular mechanism should be confirmed by a large number of functional experiments and clinical researches which might help to manifest that the BRAF L597Q mutation might play a specific role in inducing the solitary intracranial metastasis of occult PTC. Thirdly, our study dealt with a group of patients with PTC only in Southwest of China.

Conclusions

In conclusion, we have demonstrated that the female, middle age (31-59 years) and PTMC were independently correlated with LNM in PTCs, while the tumor in left and right lobes simultaneously was a protective role in LNM. Meanwhile, our study demonstrated a negative result between BRAF V600E and LNM. Also, FNAC from tumor samples had a higher rate of BRAF V600E mutation than FFPE in PTMC, which confirmed that FNAC might be a reliable intervention to detect BRAF V600E mutation. Moreover, to our knowledge, a possible association between the rare BRAF L597Q mutation and intracranial metastasis of occult PTC in the Chinese Tibetan population was reported firstly which may be used as a therapeutic target in future. Therefore, we suggest that clinicians should make a comprehensively consideration of clinical features: sample source, BRAF mutation, tumor size, gender, location of thyroid tumor and multilevel gene sequencing technologies and therapeutic schedule in order to achieve a relatively good prognosis.

Abbreviations

PTC: papillary thyroid carcinoma; PTMC: papillary thyroid microcarcinoma; NGS: next generation sequencing; US-FNA: ultrasound-guided fine-needle aspiration; FFPE: formalin-fixed paraffin-embedded; FNAC: fine-needle aspiration cytology; HE: hematoxylin-eosin staining; IHC: immunohisto-chemical (IHC) analysis; PCR: polymerase chain reaction; LNM: lymph node metastasis; OR: odds ratios.

Declarations

Disclosure of Potential Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Conception and design: Yan Dong, Dan Wang and Xiaosong Li. Development of methodology: Huili Bai, Yifan Shen, Yangli Zhang, Xueping Chen and Yajing Zhao. Acquisition of data (acquired and managed patients, provided facilities, etc.): Xinliang Su, Jinqiu Zhao, Huandong Liu, Jungao Lu, Zuoyi Yao and Yajing Zhao. Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Ling Chen, Dan Wang and Yisheng Luo

Writing, review, and/or revision of the manuscript: Yan Dong, Dan Wang and Xiaosong Li. Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Ling Chen, Dan Wang and Xiaosong Li. Study supervision: Xiaosong Li. All authors contributed to this manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University Ethics Review Board.

Consent for publication

Not applicable.

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Tables

Table 1. Characteristics of patients

Characteristics	Total (N=1045)	PTMC (N=313)	non-PTMC (N=732)	<i>P</i> value
Gender				0.524
Male/Female	298 / 747	85 / 228	213 / 519	
Age (years)	41.97±12.94	42.57±11.40	41.60±13.78	0.130
≤30	206	58 (18.5%)	148 (20.2%)	
31-59	735	236 (75.4%)	499 (68.2%)	
≥60	104	19 (6.1%)	85 (11.6%)	
Lymph node metastasis	326	181 (57.8%)	145 (19.8%)	0.000
BRAF V600E mutation	839	273 (87.2%)	566 (77.3%)	0.000
Different locations of thyroid tumor				0.254 [▲]
Left lobe	382	104 (33.2%)	278 (38.0%)	
Right lobe	480	147 (47.0%)	333 (45.5%)	
Isthmus	6	3 (1.0%)	3 (0.4%)	
Left and right lobes	150	52 (16.6%)	98 (13.4%)	
Left lobe and isthmus	7	1 (0.3%)	6 (0.8%)	
Right lobe and isthmus	15	6 (1.9%)	9 (1.2%)	
Bilateral lobes and isthmus	5	0 (0.0%)	5 (0.7%)	
Sample source				0.058
FNAC	303	78 (24.9%)	225 (30.7%)	
FFPE	742	235 (75.1%)	507 (69.3%)	

Note: Quantitative data were showed as Mean±SD or N (%); *P* value of 0.05 or less was considered significant

[▲]Fisher exact test. FNAC: fine-needle aspiration cytology; FFPE: formalin-fixed, paraffin-embedded

Table 2. Correlation between clinicopathological characteristics and BRAF V600E mutation

Note:[▲]Fisher exact test

Characteristics	PTMC		<i>P</i> value	non-PTMC		<i>P</i> value
	BRAF V600E mutation	BRAF V600E wild-type		BRAF V600E mutation	BRAF V600E wild-type	
Gender			0.026			0.003
Male/Female	80/193	5/35		180/386	33/133	
Age (years)	42.02±11.31	43.07±11.48	0.478	43.32±14.06	36.83±11.74	0.180
≤30	52	6		105	43	
31-59	206	30		387	112	
≥60	15	4		74	11	
Lymph node metastasis			0.964			0.280
Yes (+)	158	23		449	138	
No (-)	115	17		117	28	
Different locations of thyroid tumor			0.475▲			0.448▲
Left lobe	88	16		211	67	
Right lobe	127	20		259	74	
Isthmus	3	0		3	0	
Left and right lobes	49	3		79	19	
Left lobe and isthmus	1	0		3	3	
Right lobe and isthmus	5	1		8	1	
Bilateral lobes and isthmus	0	0		3	2	
Sample source			0.018			0.699
FNAC	75	4		176	49	
FFPE	198	36		390	117	

Table 3. Comparison of progression between PTMC and non-PTMC with BRAF V600E mutation

Characteristics	BRAF V600E mutation		χ^2	P
	PTMC	non-PTMC		
Gender			0.537	0.464
Male/Female	80/193	180/386		
Age (years)	42.02±11.31	43.32±14.06	11.306	0.004
≤30	52	105		
31-59	206	387		
≥60	15	74		
Lymph node metastasis			42.369	0.000
Yes (+)	158	449		
No (-)	115	117		
Different locations of thyroid tumor			5.644	0.445▲
Left lobe	88	211		
Right lobe	127	259		
Isthmus	3	3		
Left and right lobes	49	79		
Left lobe and isthmus	1	3		
Right lobe and isthmus	5	8		
Bilateral lobes and isthmus	0	3		
Sample source			1.153	0.283
FNAC	75	176		
FFPE	198	390		

Note: ▲Fisher exact test

Table 4. Univariate and multivariate analysis of risk factors for LNM in PTC (n=1045)

Variable	Univariate analysis		Multivariate analysis	
	OR[95 % CI]	P	OR[95 % CI]	P
Gender (Male as reference)				
Female	1.868(1.340-2.604)	0.000	1.95 (1.373-2.774)	0.000
Age (≤30 as reference)				
31-59	1.48 (1.024-2.157)	0.037	1.56 (1.050-2.318)	0.028
≥60	1.32 (0.765-2.309)	0.313	1.53 (0.853-2.74)	0.154
BRAF V600E (Yes as reference)				
No (-)	0.72 (0.505-1.043)	0.084	-	-
Tumor type (non-PTMC as reference)				
PTMC	2.93 (2.198-3.910)	0.000	3.27 (2.417-4.432)	0.000
Different locations of thyroid tumor (Left lobe as reference)				
Right lobe	0.82 (0.611-1.103)	0.191	0.77 (0.564-1.050)	0.099
Left and right lobes		0.000		0.000
Isthmus	0.32 (0.189-0.543)	0.909	0.28 (0.166-0.497)	0.836
Bilateral lobes and isthmus	1.11 (0.200-6.117)	0.597	0.83 (0.146-4.740)	0.441
Right lobe and isthmus		0.858		0.796
Left lobe and isthmus	0.55 (0.061-4.996)	0.358	0.40 (0.041-4.024)	0.411
	1.11 (0.370-3.304)		0.85 (0.270-2.725)	
	0.37 (0.044-3.094)		0.40 (0.048-3.462)	

Note: LNM:lymph node metastasis; OR: odds ratio; CI: confidence interval

Table 5. Univariate and multivariate analysis of risk factors for LNM in PTMC

Variable	Univariate analysis		Multivariate analysis	
	OR[95 % CI]	<i>P</i>	OR[95 % CI]	<i>P</i>
Gender (Male as reference)				
Female	2.91 (1.671-5.077)	0.000	3.00 (1.654-5.446)	0.000
Age (≤30 as reference)				
31-59	1.38 (0.764-2.494)	0.285	1.76 (0.941-3.327)	0.077
≥60	1.48 (0.529-4.145)	0.455	1.45 (0.496-4.257)	0.496
BRAF V600E (Yes as reference)				
No (-)	0.93 (0.481-1.805)	0.835	0.72 (0.356-1.454)	0.360
Different locations of thyroid tumor (Left lobe as reference)				
Right lobe	0.70 (0.427-1.169)	0.176	0.71 (0.425-1.203)	0.207
Left and right lobes		0.000		0.000
Isthmus	0.15 (0.068-0.369)	0.641	0.17 (0.071-0.405)	0.426
Bilateral lobes and isthmus	1.78(0.157-20.263)	0.514	2.78(0.223-34.698)	0.556
Right lobe and isthmus		0.362		0.322
Left lobe and isthmus	0.44 (0.039-5.066)	1.000	0.47 (0.038-5.791)	1.00
	0.44 (0.078-2.539)		0.40 (0.068-2.417)	
	0.00		0.00	

Note: LNM:lymph node metastasis; OR: odds ratio; CI: confidence interval

Table 6. Univariate and multivariate analysis of risk factors for LNM in non-PTMC

Variable	Univariate analysis		Multivariate analysis	
	OR[95 % CI]	P	OR[95 % CI]	P
Gender (Male as reference)				
Female	1.42 (0.929-2.165)	0.105	1.52 (0.980-2.346)	0.061
Age (≤30 as reference)				
31-59	1.50 (0.910-2.484)	0.111	1.46 (0.873-2.437)	0.149
≥60	1.55 (0.777-3.091)	0.214	1.48 (0.729-3.002)	0.278
BRAF V600E (Yes as reference)				
No (-)	0.79 (0.500-1.241)	0.304	0.77 (0.482-1.224)	0.268
Different locations of thyroid tumor (Left lobe as reference)				
Right lobe	0.81 (0.550-1.197)	0.291	0.79 (0.533-1.175)	0.246
Left and right lobes		0.015		0.021
Isthmus	0.43 (0.215-0.850)	0.999	0.44 (0.220-0.882)	0.999
Bilateral lobes and isthmus	0.0	0.999	0.0	0.999
Right lobe and isthmus	0.0	0.476	0.0	0.567
Left lobe and isthmus	1.67 (0.407-6.874)	0.716	1.52 (0.361-6.419)	0.689
	0.67 (0.077-5.829)		0.64 (0.72-5.707)	

Note: LNM:lymph node metastasis; OR: odds ratio; CI: confidence interval

Table 7. The NGS panel for thyroid cancer

List	Genes
1	AKT1 (NM_001014432.1)
2	ALK (NM_004304.4)
3	BRAF (NM_004333.4)
4	CTNNB1 (NM_001904.3)
5	EIF1AX (NM_001412.3)
6	ETV6 (NM_001987.4)
7	GNAS (NM_080425.3)
8	HRAS (NM_005343.3)
9	KRAS (NM_033360.3)
10	NRAS (NM_002524.4)
11	NTRK1 (NM_001007792.1)
12	PIK3CA (NM_006218.3)
13	PPARG (NM_015869.4)
14	PTEN (NM_000314.6)
15	RET (NM_020975.4)
16	TERT (NM_198253.2)
17	TP53 (NM_000546.5)
18	TSHR (NM_000369.2)

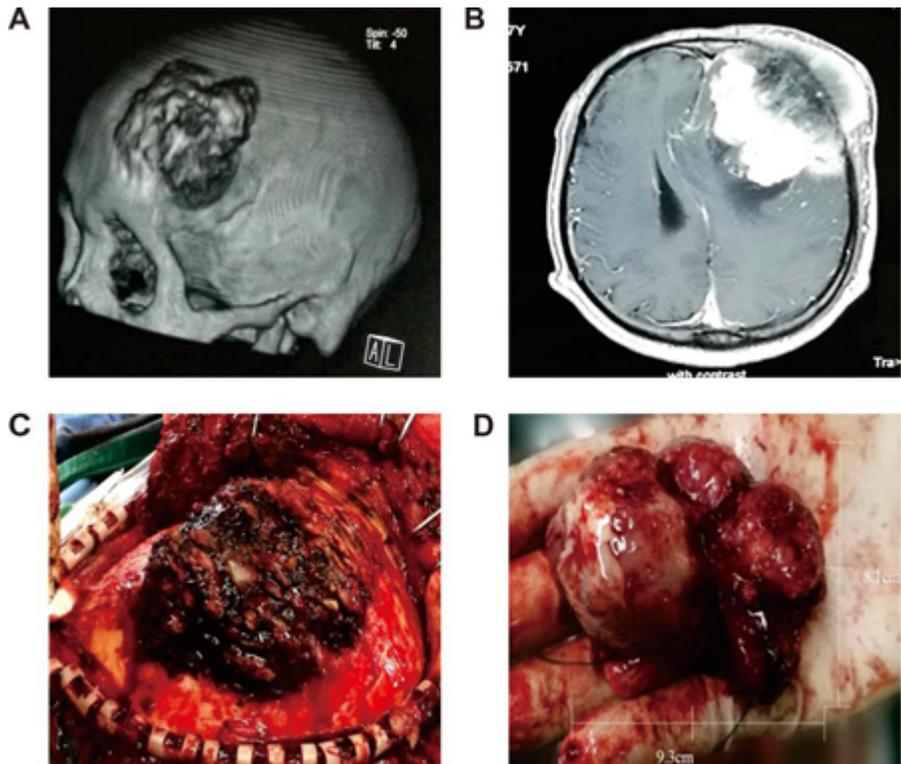


Figure 1

Preoperative skull CT/MRI scan and intraoperative photographs during resection and debulking. (A: Irregular bone destruction of the left frontal bone, the range is about 5.7 cm×6.3 cm. B: Irregular masses are seen in the left frontal cranial and subscalp, the size is about 4.9 cm×5.4 cm, it is uneven and obviously strengthened, and the center line structure is skewed to the right. C: The outer plate of skull, raised bone (4.8 cm×5.2 cm) surface of the skull's outer plate was uneven, showing a state of cancellous bone. D: The tumor isolated from the operation was hard in texture, about 5.7 cm×6.3 cm in size.)

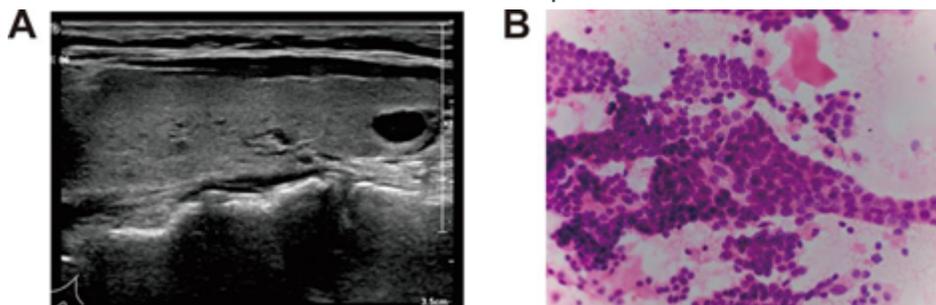


Figure 2

Preoperative conventional color ultrasound diagnosis (A) and US-FNA diagnosis (B) (A: The echo in the right lobe was uneven, and a 0.7 cm×0.5 cm mass was visible. B: A lesional biopsy specimen of the thyroid nodules was obtained (hematoxylin-eosin, 400×.)

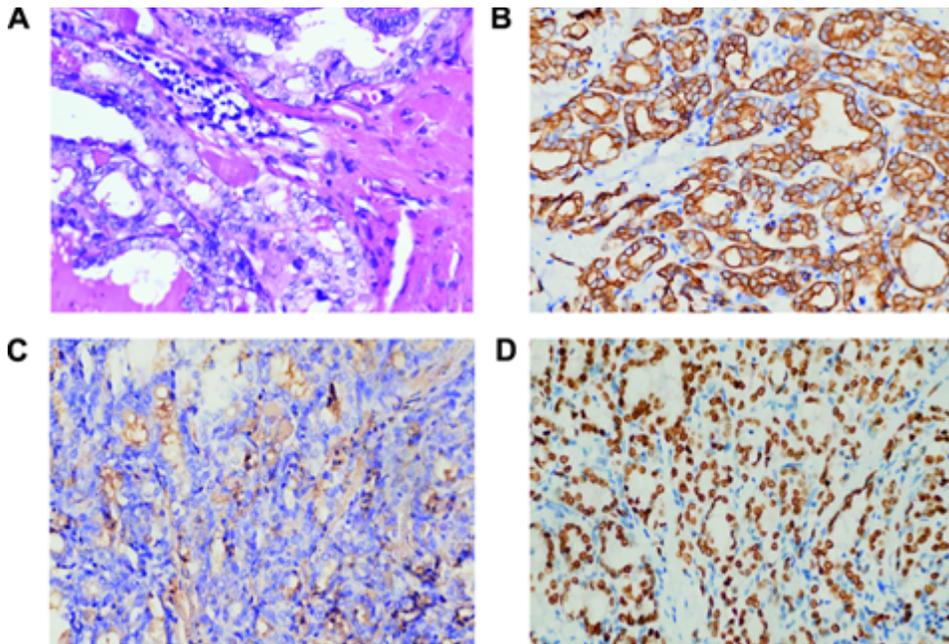


Figure 3

The HE and IHC staining of the intracranial metastases (400×) (A: Tumor cells are relatively uniform in size, with round nuclei, small nucleoli visible in part, cytoplasmic staining and mitotic divisions that are not easily seen. B: CK19 staining showed brownish yellow particles on the cell membrane (+++). C: TG staining showed brownish yellow particles on the cell membrane (+). D: TTF1 staining showed nucleus showing brownish yellow particles (+++).)

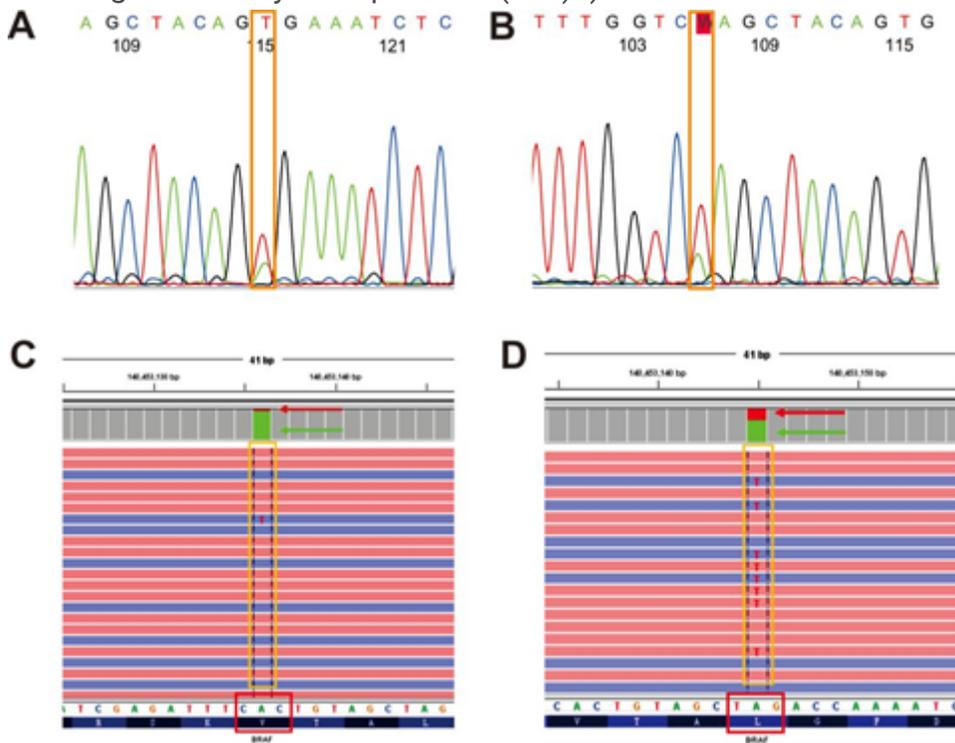
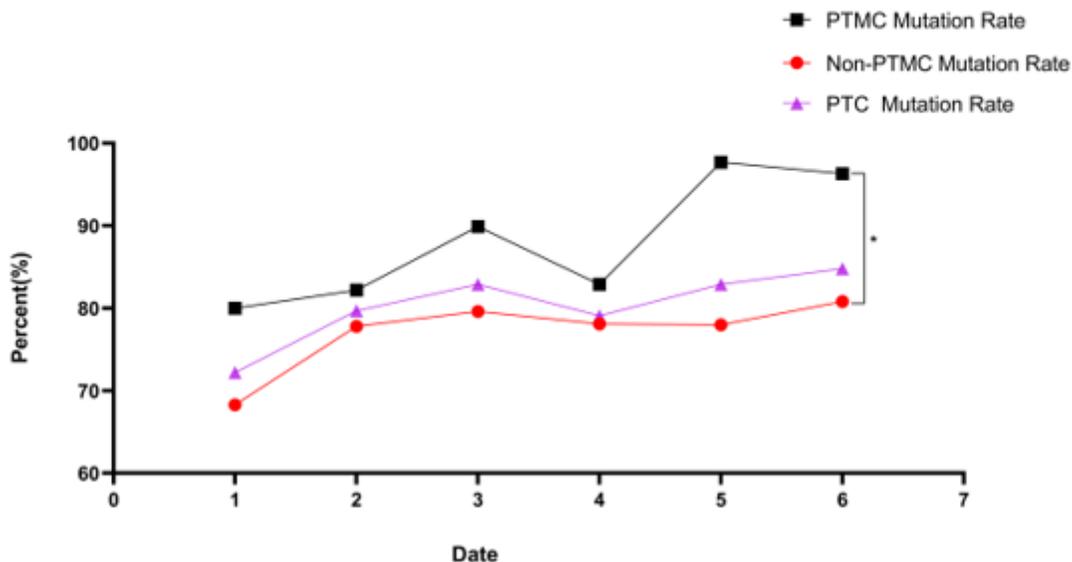


Figure 4

Genetic diagnosis results (A: BRAF V600E (c.1799T>A) mutation tested by Sanger sequencing in situ. B: BRAF L597Q (c.1790T>A) mutation tested by Sanger sequencing in metastases. C: BRAF V600E (c.1799T>A) mutation tested by NGS in situ, and D: BRAF L597Q (c.1790T>A) mutation tested by NGS in metastases.)



Date	2016.8-2017.1(1)	2017.2-2017.7(2)	2017.8-2018.1(3)	2018.2-2018.7(4)	2018.8-2019.1(5)	2019.2-2019.8(6)
non-PTMC	69 (68.32%)	77 (77.78%)	133 (79.64%)	125 (78.13%)	99 (77.95%)	63 (80.77%)
PTMC	40 (80.00%)	60 (82.19%)	71 (89.87%)	34 (82.93%)	42 (97.67%)	26 (96.30%)
PTCs	109 (72.19%)	137 (79.65%)	204 (82.93%)	159 (79.10%)	141 (82.94%)	89 (84.76%)

Figure 5

Comparison of mutation rates between PTMC and non-PTMC (Comparison of mutation rates between PTMC and non-PTMC over 3 years; Data were showed as N (%); The difference in mutation rate between PTMC groups and non-PTMC groups were highly significant.)