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ORIGINAL RESEARCH

Genetic and Clinicopathologic Characteristics of Papillary Thyroid Carcinoma in the Chinese Population: High *BRAF* Mutation Allele Frequency, Multiple Driver Gene Mutations, and *RET* Fusion May Indicate More Advanced TN Stage

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Purpose: To describe the genetic landscape and clinical characteristics of Chinese patients diagnosed with papillary thyroid cancer (PTC) and to determine which high-risk genetic characteristics suggest a likelihood of lymph node metastasis (LNM) and lateral lymph node metastasis (LLNM).

Patients and Methods: Data from previously untreated patients with PTC collected between May 2018 and December 2020 from 14 hospitals in China were analyzed retrospectively. High-risk pathologic characteristics were defined as T3/T4, N(+), and N1b(+) stages. All patients were tested for 57 genes by second-generation sequencing. The *t*-test, chi-square test, and Fisher's exact test were performed for statistical analysis.

Results: Overall, 395 patients were enrolled in this study. The prevalence of *BRAF* mutation was 78.53%. *BRAF* mutant allele frequency (MAF) >16.93% was associated with a significantly higher risk of LNM, LLNM, and T3 + T4 stage compared with a low-risk group, defined by a MAF <2.54% (odd ratios [ORs] for each risk=3.38, 3.46, and 8.54, respectively), and an intermediate-risk group, defined by a MAF of 2.54% to 16.93% (ORs=2.04, 2.07, and 4.07, respectively). The population with *RET* fusion had higher T, N, and N1b stages (ORs for each stage=10.40, 7.60, and 8.77, respectively) compared with a *RET*-negative population. Similar conclusions about T, N, and N1b stages were observed in relation to multiple driver gene mutations (ORs for each stage=7.48, 2.80, and 7.04, respectively) compared with population without multiple driver mutations. These genetic characteristics may be suggestive of high clinical risk. However, regardless of genetic profiles, patients younger than age 45 years had greater rates of LNM and LLNM.

Conclusion: The main driver gene in this study, *BRAF*, differs significantly between the United States (79% vs 51%) and other countries. The Chinese population in this study that experienced more aggressive tumor biology had a *BRAF* MAF greater than 16.93%, exhibited *RET* fusion events, and had multiple driver gene mutations; thus, these traits may be considered high-risk genetic characteristics in PTC that could warrant aggressive treatment in such population.

Keywords: papillary thyroid cancer, lymph node metastasis, high risk clinicopathological characteristics, genotypes

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Introduction

Thyroid cancer is the most common malignant endocrine cancer both worldwide and in China. Its incidence has been gradually increasing in East Asia in recent years.[1] The age-specific incidence of thyroid cancer in the standard population of China was 8.82/100,000 in 2013,[2] and the mortality rate was 0.52/100,000.[3] In the past few decades, the incidence of papillary thyroid cancer (PTC)—the most common thyroid cancer—has been increasing steadily; now, PTC may account for >99% of thyroid cancers in China.[4] Patients diagnosed with PTC often undergo surgical treatment and postoperative thyroid-stimulating hormone suppressive therapy. The 5-year survival rate after surgery of PTC is >95%.[5] However, contralateral central lymph node metastasis (LNM) is still confirmed in 3.88% to 30.63% of the patients who have no LNM detected by preoperative ultrasound or physical examination (cN0 patients) after undergoing total thyroidectomy.[6,7] Therefore, it is controversial whether prophylactic lymph node prophylaxis in the contralateral central lymph node is required for patients with a preoperative ultrasound diagnosis of cN0.

Genetic mutations could serve as potential predictive factors for LNM or for therapeutic efficacy in PTC.[8,9] Previous study have revealed that the major mutations in PTC are located in the *MAPK* pathway (eg, *BRAF* and *RAS* mutations, gene fusion events such as *RET, NTRK1*, and *BRAF* gene fusions).[9] Recent research indicates that patients with PTC who have *BRAF*-positive/*TERT*-positive co-mutations have a significantly high probability and prognosis of distant LNM than do those without detectable gene mutations.[10,11] Therefore, preoperative genetic testing may help clinicians predict the prognosis of patients and guide clinical decisions.

A large-scale genetic landscape study of PTC in a Chinese population (involving 355 patients) was performed in 2018.[12] According to this study, the genetic characteristics of the reported population differed significantly from those of patients from the United States and Saudi Arabia, suggesting that the genetic or epigenetic characteristics of PTC may differ across ethnic backgrounds. However, additional studies are needed to confirm this hypothesis. Moreover, this single-center study did not focus on the relationships between genotype and clinical characteristics (eg, LNM). In this retrospective, multicenter study, we reported the clinical and pathologic characteristics of PTC and the genetic profile of patients with primary PTC in a Chinese population. We also explored the association between genetic mutations and clinical or pathologic features to identify high-risk genetic characteristics that may propose a likelihood of LNM and provide data to support individualized diagnosis and treatment strategies.

Patients and Methods

Patient Enrollment and Screening

Data from patients diagnosed with thyroid cancer who underwent a 57-gene-panel test from May 2018 to December 2020 were retrospectively collected in this study; 451 patients from a total of 14 tertiary hospitals in seven provinces and cities were included. The following clinical characteristics and pathologic features were collected for each patient: age, sex, personal history, family history, specific surgical procedure, detailed pathologic diagnosis, tumor size, and LNM. Fresh tissues or formalin-fixed, paraffin-embedded (FFPE) samples were collected from each patient during radical thyroidectomy. All samples underwent DNA testing for 57 genes with high mutation frequencies in PTC (Figure S1). After all data were collected, they were classified by T, N, and M stages according to the 2015 American Thyroid Association Management (ATA) guidelines.[13] The location of the primary site was classified according to the location of the cancer site, as documented by the pathologic diagnosis after the initial thyroidectomy. In this study, locations were classified as unilateral or bilateral, and unilateral locations were classified as left or right. High-risk genetic characteristics were defined as a T stage ≥T3, an N stage = N1, or an M stage = M1.

The target population was previously untreated patients with thyroid cancer and a clear postoperative pathologic diagnosis of PTC according to the criteria defined by the World Health Organization.[14] During the screening process, 54 patients were excluded: 13 patients underwent non-primary surgery, 17 patients did not have PTC (5 had benign nodules, 8 had medullary carcinomas, 1 had follicular carcinoma, and 4 had undifferentiated carcinomas), and 24 patients had missing pathologic diagnosis data. During next-generation sequencing, 2 patients were excluded from the study because their gene extraction results were ineligible. Overall, 395 patients were eventually included in the data analysis.

The study protocol was approved by the ethical board of the Chinese Academy of Medical Sciences and Peking Union Medical College Hospital, and all participants gave written informed consent at their local hospitals. This study was conducted in accordance with the Declaration of Helsinki.

Second-Generation Sequencing

Genomic DNA was isolated from fresh tumor tissues or FFPE using the QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's instructions. The concentration of the DNA was determined using the Qubit Fluorometer 3.0 (Life Technologies, USA). A custom-designed, 103-kb panel covering exons and partial introns of cancer driver genes, hereditary cancer-related genes, and therapy-related genes was used in this study. First, 50–100 ng of sheared genomic DNAs was subjected to library construction with an MGIEasy Universal DNA Library Kit (MGI, China), and then the DNA underwent hybrid capture using an xGen Hybridization and Wash Kit (IDT, USA). Libraries' quality and concentration were determined using a LabChip® GX Touch™ nucleic acid analyzer (PerkinElmer, USA) and a Qubit Fluorometer 3.0, respectively. Tumor-matched normal samples were sequenced as controls. The qualified libraries were sequenced with 2×100 bp paired-end reads on an MGISEQ-2000 (MGI, China) platform.

Bioinformatic Analysis

The sequencing data were mapped to the GRCh37/hg19 human genome using a Burrows-Wheeler Aligner (BWA, v.0.7.12) MEM algorithm. PCR duplicates were marked using Picard tools (v.1.119). Functional annotations of somatic SNVs and InDels were performed using ANNOVAR. Candidate mutations were filtered if (1) the depth was <200× for tissues and reads supporting alternative alleles were <4; (2) the reads had strand bias; (3) the mutant allele frequency (MAF) was <0.5%; and (4) the variations were synonymous, with unknown significance and introns.

Gene fusions were detected with an in-house algorithm. Briefly, the relative positions of the aligned paired sequences were compared to find the possible structural variation. The abnormally aligned sequences were segmented and aligned by a more relaxed alignment method to map the sequences to possible positions and determine the final alignment position and direction. To determine the positions of the breakpoint, the candidate final alignment positions were marked, and then the sequences were realigned. Only candidate breakpoints with high alignment quality and with supporting reads that covered the points were retained. Breakpoint correction was performed on the basis of alignment scores, sequencing quality, and number of supported sequences. False positives were filtered by tag reads covering the breakpoints and by paired reads supporting the breakpoints. Requirements to consider a gene fusion valid were as follows: (1) depth >500× at the breakpoint; (2) at least eight high-quality unique reads covering the breakpoint; and (3) MAF ≥0.5%.

CNVkit was used to identify copy number variants. The minimum threshold of copy number (CN) gain or loss was CN >2.75 or CN <1.75 for hotspot genes, and CN >3 or CN <1.5 for others. Gene rearrangements were assessed using the Integrative Genomics Viewer. Some important/novel variants were verified by Sanger sequencing or ddPCR, if needed.

Statistics Analysis

Data analysis was conducted with R version 4.0.3. The *t*-test was applied for comparison of means, and certain proportions in specific circumstances. As for categorical variables, we properly take advantage of chi-square test and Fisher's exact test in comparison between two variables. Cochran–Mantel–Haenszel test and exact conditional test, their counterparts in three variables' situation, were used in aforesaid condition. A p-value of <0.05 was considered as significant.

Results

Overview of Clinicopathologic Characteristics Genetic Profile in Chinese PTC Patients: *BRAF*^{V600E} Mutation is the Majority Mutation in Chinese Population

Overall, 395 patients from different provinces in China were included in this study. The mean ± standard deviation age was 44.43±12.25 years, and the gender ratio was nearly 3:1 (296 women and 99 men). The tumors were located on the left, right, and both sides in 133, 166, and 96 patients, respectively. Interestingly, PTC presented less often on

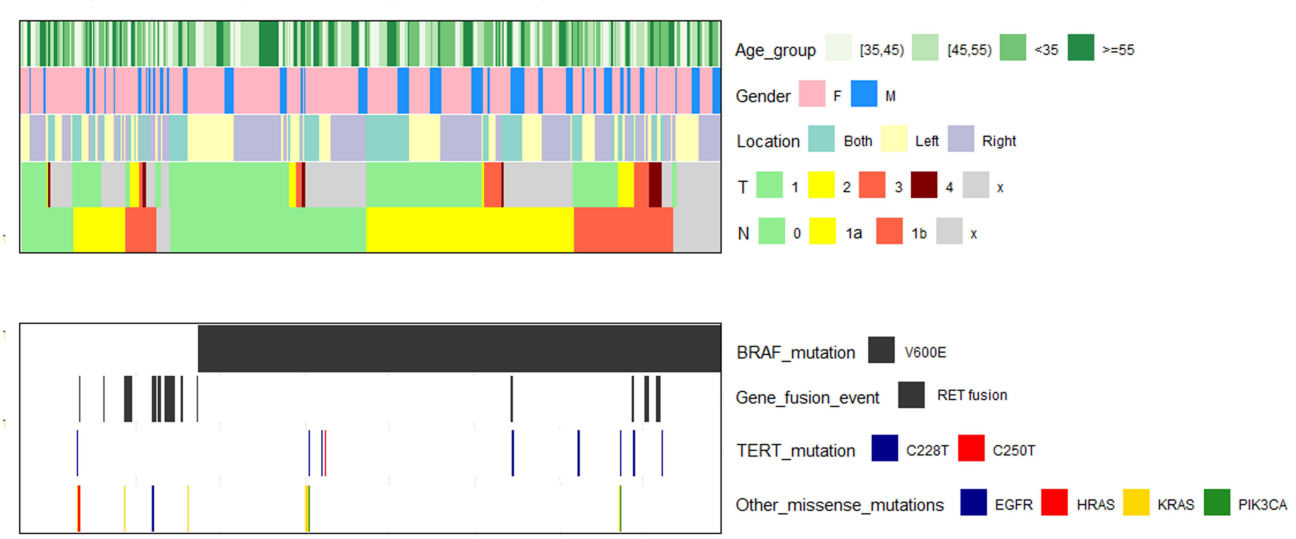

Figure 1 Clinical and genetic profile of 395 Chinese patients with papillary thyroid carcinoma (PTC). Up: clinical profile, including age, gender, T stage, N stage, and location of tumor. Down: genetic profile.

the left side than on the right side (p=0.028, one-sided hypothesis test). All patients in our study had M0 disease (Document S1). The clinical characteristics of all patients are shown in Figure 1 and listed in Table 1.

At least 336 patients (85.07%) had at least one driver gene; 310 patients (78.48% of the enrolled population) had *BRAF* mutations, which represented the majority genotype (310/336, or 92%). All 310 *BRAF* mutations were $BRAF^{V600E}$ mutations. Other driver genes included *RAS* (n=8), *TERT* (n=9), *PIKC3A* (n=2), and *EGFR* (n=1). *RET* fusions (n=28, or 7.09%) were also identified; these included *CCDC6* (n=12), *NCOA4* (n=8), *ACBD5* (n=3), intergenic_*JMJD1C_REEP3:RET_12* (n=2), *CCDC186* (n=1), *AFAP1L2_6* (n=1), *CELF2* (n=1), and *ERC1* (n=1). Among them, *CELF2*, intergenic_*JMJD1C_REEP3:RET_12*, and *CCDC186* fusion events were first reported in patients with PTC. Two or more driver gene mutations were detected in 20 individuals, of which 19 had *BRAF* mutations (95.00%), 9 had *TERT* mutations (45.00%), and 7 had *RET* mutations (35.00%). One patient had three mutations: $BRAF^{V600E}$, *TERT*, and a *RET:ACBD5* gene fusion. Table 1 shows the clinicopathologic characteristics in different genotypes.

Younger Age Was Associated with LNM and LLNM

We performed a cross-sectional analysis to consider the relationship between clinical characteristics (age, gender, and location) and T and N stages (Table S1). According to ATA and American Joint Committee on Cancer (AJCC) guidelines,[13,15] 55 years is crucial for determination of the AJCC stage.[13] Thus, we divided our study population into two groups: ≥55 years and <55 years; we also grouped patients as <45 years and ≥45 years according to the median age of our study (45 years). Fisher's exact test found no significant difference between clinical characteristics and T stage. However, patient age younger than 55 years (vs ≥55 years) was associated with a higher N stage (50.8% vs 43.3%, p=0.009). This finding held true when the cut-point changed to 45 years (65.1% vs 46.0%, p<0.001). When age was considered as a continuous variable, no significant inflection points were identified. To eliminate the influence of gender and location, multi-dimensional contingency analyses were performed, with CMH test and exact conditional test adapted. The association still held true at a cutoff of 55 years (p=0.015) or 45 years (p<0.001), indicating that LNM tended to occur in younger patients in China. We also rule out whether different genotypes (especially *RET* fusions) could influence this phenomenon, but the age association remained in different genotype populations (p=0.005).

Higher BRAF Allele Frequency Reflected Advanced T and N Stages

The *BRAF* mutation was the most frequent mutation in the study (n=310/395). *T*-Test confirmed that compared with the *BRAF*-negative/*RET*-negative population, *BRAF*-positive patients tended to have an advanced T stage. Because the *BRAF*

Table 1 Clinical Features in Different Genotypes

	Overall		*BRAF* Positive		*RET* Positive		*BRAF* & *RET* Negative	
	395		310		28		65	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Mean age, years	44.43*		44.74 (p=0.737)*		36.25 (p=0.001)*		45.89 (p=0.408)*	
<45	188	48.7	142	47.0	22	78.6	29	45.3
≥45	198	51.3	160	53.0	6	21.4	35	54.7
<55	296	76.7	230	76.2	27	96.4	46	71.9
≥55	90	23.3	72	23.8	1	3.6	18	28.1
Gender			p=0.608*		p=0.660*		p=0.119*	
Male	99	25.1	83	26.8	6	21.4	11	16.9
Female	296	74.9	227	73.2	22	78.6	54	83.1
Location								
Bilateral	96	24.3	74	23.9	8	28.6	17	26.1
Right side	166	42.0	130	42.0	14	50	25	38.5
Left side	133	33.7	106	34.1	6	21.4	23	35.4
T stage			p=0.776*		p=0.001*		p<0.001*	
T1	198	78.0	160	78.1	6	27.3	32	91.4
T2	20	7.9	14	6.8	4	18.2	3	8.6
T3	23	9.0	21	10.2	5	22.7	0	0
T4	13	5.1	10	4.9	7	31.8	0	0
N stage			p=0.953*		p<0.001*		p=0.127*	
N0	178	45.1	139	44.8	3	10.7	36	55.4
N1a	145	36.7	116	37.4	8	28.6	23	35.4
N1b	72	18.2	55	17.8	17	60.7	6	9.2

Note: *The *t*-test was performed to examine the mean age, proportion of women, proportion of T3 and T4 stage, and proportion of N1 stage between Overall patients and each subgroups classified by genotypes.

mutation is highly relevant with PTC, we collected the MAF of *BRAF* in 297 patients and explored the association between the MAF and the T and N stages (Figure 2). The MAF varied from 0.20% to 41.78%, with a mean MAF of 11.21% (mean per side: left, 12.47%; right, 10.06%) (Document S1 and Figure S2). Post hoc tests revealed that advanced T and N stages were associated with higher mean *BRAF* frequency in the overall population (p<0.001 and p=0.004 for T and N stage, respectively), especially in left-sided PTC (p=0.001 and 0.007 for T and N stage, respectively). Plotting the cumulative positive rate of high-risk clinicopathologic features that varied with *BRAF* allele frequency (Figure S3) showed that the risk of advanced T and N stage increases linearly as the *BRAF* allele frequency increases. No significant platform or inflection point was found in our analysis.

As a result, patients were grouped to three groups according to MAF by lower quartile (2.54%) and upper quartile (16.93%). MAFs <2.54% were considered the low-risk group, and MAFs >16.93% were considered the high-risk group. Patients with a MAF between 2.54% and 16.93% were considered at intermediate risk. We calculated the odds of T and N stages in each group and determined the odds ratio (OR) between different groups. Results are shown in Table 2. The risks of LNM, LLNM, and T3 + T4 stage were significantly higher in the high-risk group compared with low-risk group (OR=3.38, 3.46, and 8.54, respectively) and the intermediate-risk group (OR=2.04, 2.07, and 4.07, respectively). Therefore, higher *BRAF* allele frequency may be associated with a high risk of clinicopathologic features in Chinese patients with PTC.

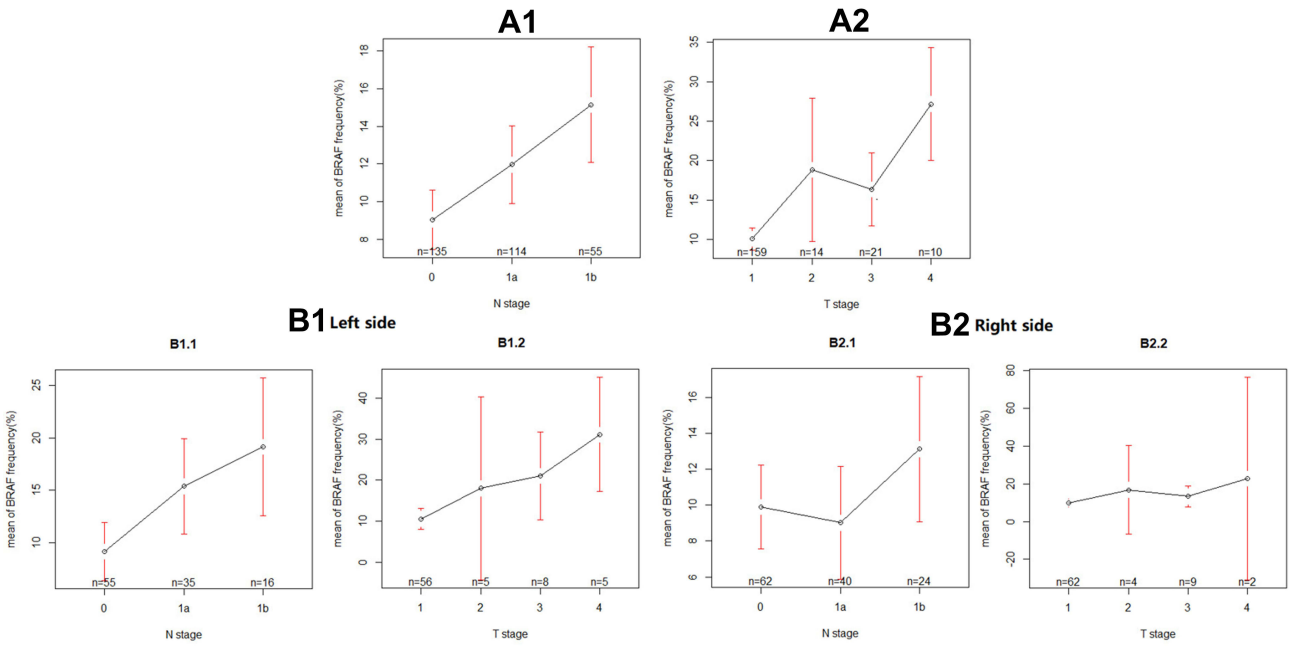

Figure 2 The relationship between mutation frequency at different primary foci locations and T and N staging in the *BRAF*-positive mutation population after correction for age and gender. (**A**) The relationship between gene frequency and T and N stages without considering location. (**A1**) N stage, (**A2**) T stage. (**B**) The relationship between gene frequency and T and N stage considering location. (**B1**) N stage, (**B1.1**) left side, (**B1.2**) right side, (**B2**) T stage, (**B2.1**) left side, (**B2.2**) right side.

RET Fusions Significantly Indicated Advanced T and N Stages

In our population, the *RET*-positive patients (n=28) had quite distinctive clinical characteristics compared with the *RET*-negative population (n=367). They were younger, had larger tumor sizes, and had a significant propensity for LLNM (Table 1). OR calculations clarified the association between *RET* and the clinicopathologic features (ORs=7.60 for LNM, 8.77 for LLNM, and 10.40 for T3 + T4 compared with the *RET*-negative population) (Table 3). No significant effect of the location of the primary site on the clinical characteristics of the *RET*-positive population was found. Because many different genes are fused with *RET*, Fisher's exact test was performed to determine whether different fusions could lead to

Table 2 Odds Ratio Between Different Risk Groups in *BRAF*-Positive Patients

Odds Ratio (N1 vs N0)	**Low Risk (Odds=0.727)**	**Intermediate Risk (Odds=1.202)**	**High Risk (Odds=2.454)**
High risk (n=76) Middle risk (n=152) Low risk (n=76)	3.375 1.654	2.041	
Odds Ratio (N1b)	**Low Risk (Odds=0.118)**	**Intermediate Risk (Odds=0.197)**	**High Risk (Odds=0.407)**
High risk (n=76) Middle risk (n=152)	3.463 1.673	2.070	
Low risk (n=76)			
Odds Ratio (T3 + T4)	**Low Risk (Odds=0.054)**	**Intermediate Risk (Odds=0.113)**	**High Risk (Odds=0.462)**
High risk (n=76) Intermediate risk (n=152) Low risk (n=76)	8.538 2.098	4.070	

Table 3 Odds Ratio in Different Genotypes (A and B)

Clinicopathological Stage	*p* value*	Odds Ratio	Confidence Interval
(**A**) ***RET*-positive vs *RET*-negative disease**			
N1	0.001	7.595	[2.607, 32.296]
N1b	<0.001	8.767	[3.943, 20.266]
T3 + T4	<0.001	10.40	[4.081, 27.217]
(**B**) **Multiple driver genes mutation vs single or no driver gene mutation**			
N1	0.007	2.80	[1.38, 6.18]
N1b	<0.001	7.04	[3.56, 14.07]
T3 + T4	<0.001	7.48	[3.31, 16.98]

Note: *Examined by Fisher's exact test.

different clinicopathologic characteristics (Table S2). *CCDC6*, the major fusion partner with *RET* (n=12/28), may be associated with a younger age of PTC onset and more LLNM (p=0.12).

Multiple Driver Gene Mutations and *TERT* Population

In this study, 5.06% of the population (n=20/395) had at least two driver genes identified simultaneously, and nine patients had *TERT* mutations (Document S1). In a patient (case 444) with no *BRAF* mutation, *HRAS* and *TERT* mutations were identified. No *BRAF*-positive/*RAS*-positive patients were identified. Patients with at least two driver mutations were defined as the multiple driver gene mutated population. They tended to develop T3 or T4 stage disease more often, (p<0.001, OR=7.48, CI=[3.31, 16.98]) and to have more LNM (p=0.007, OR=2.80, CI=[1.38, 6.18]) and LLNM (p<0.001, OR=7.04, CI=[3.56, 14.07]) compared with patients who had single or no driver gene mutations (Table 4).

Interestingly, the *TERT* gene, a widely reported strong driver gene, was only found in the multi-mutant population and was frequently accompanied by almost simultaneous mutations in the *BRAF* gene (n=8/9). The *TERT*-positive population had an older mean age than the *BRAF*-positive population (55.56 years vs 44.74 years) but did not have larger tumor diameters or more LNM events (Table S3). These findings are consistent with previous reports of *BRAF*-positive/*TERT*-positive patients with PTC from Northeast China.[14]

Novel *RET* Gene Fusions

Three rare or novel *RET* fusion partners were identified in our study: *CCDC186, CELF2*, and intergenic region between *JMJD1C* and *REEP3*. The 5′ region of *CCDC186* (exons 1–15) was connected to the 3′ region of *RET* (exons 12–20), and the fusion was in frame. Using the emerging application of NGS, *CCDC186:RET* fusion has been newly noted in Chinese patients with lung cancer and thyroid cancer. In a cohort of Chinese patients with lung cancer, *CCDC186* reportedly attached to *RET* at its intron 10.[16] However, in another two cohorts of Chinese patients with PTC, *CCDC186*

Table 4 Clinical Characteristics of Multiple Driver Gene–Mutated Population

Clinicopathologic Characteristics	Multiple Mutations	Overall	*p* value	No Mutation	*p* value*
Mean age	40.63	44.43	0.080	45.83	0.056
Male	0.268	0.251	0.811	0.136	0.115
T3 + T4	0.441	0.142	0.002	0.000	<0.001
N1	0.756	0.549	0.006	0.458	0.002
N1b	0.688	0.288	<0.001	0.158	<0.001
Bilateral	0.268	0.243	0.732	0.254	0.877
Right side (in unilateral)	0.567	0.555	0.906	0.500	0.579

Note: *The *t*-test was performed to examine the mean value and proportion of male, T3+T4, N1, N1b and locations.

reportedly fused to *RET* at its intron 7.[12,17] *CCDC186*, as shown in its name, harbors coiled-coil domains, which mediate dimerization and the consequent activation of the kinase domain of *RET*.

CELF2, as a member of the *CELF* family, plays an important role in RNA co-transcription and post-transcriptional modifications. In a previous study, *CELF2* inhibited the progression of non-small cell lung cancer by suppressing the binding of *PTEN* to *PREX2*.[18] In addition, inactivation of *CELF2* has been associated with gastric, ovarian, breast, and colorectal cancers.[19–22] The 5′ region of *CELF2* (exons 1–9) has attacked to exons 10–20 of *RET*, and the fusion was in frame.

A novel intergenic rearrangement was also identified in this cohort. *RET* was fused to a point 14.7 kb upstream of the coding region of the *JMJD1C* gene. The protein encoded by this gene interacts with thyroid hormone receptors and is thought to be a coactivator for key transcription factors. Because RNA was not available, the fusion was not confirmed at the RNA level. Several intergenic rearrangements, mostly to exon 12 of *RET*, have also been identified.[23] Intergenic rearrangements accounted for approximately 7.7% of identified *RET* fusions.[23] However, none of these intergenic *RET* rearrangements have responded to *RET*-related tyrosine kinase inhibitors. The significance of these intergenic rearrangements, including whether functional fusion RNAs can be transcribed from these intergenic rearrangements, remains to be determined.

Discussion

To our knowledge, this is the first multicenter study to examine the genetic characteristics of PTC and explore the relationships between the genotype and T and N disease stages in China. In this study, we defined high-risk clinicopathologic features as the presence of lymph node metastases (LNM, N1), the presence of lateral lymph node metastases (LLNM, N1b), and a large tumor diameter or invasion of surrounding tissue (T3 or T4). Here, we confirmed that high MAF of *BRAF, RET* fusions, and multiple driver gene mutations were related to those high-risk clinicopathologic features. Genetic monitoring of preoperative puncture biopsy tissue has been shown to assist in the diagnosis of benign or malignant tumors, therefore this study may help doctors assess the risk of LNM and thus guide the choice of surgical approach.

In the study population, 78.5% of the patients (n=310/395) had a *BRAF* mutation, and all were the $BRAF^{V600E}$ mutation. Compared against data from The Cancer Genome Atlas[24] (Table 5), the prevalence of *BRAF* mutation in this study was significantly higher than in a US PTC population (59.7%, $p<0.001$), and *RAS* (n=8), *TERT* (n=9), and *PIKC3A* (n=2) were significantly less common. In contrast, the distribution of genotypes in this study was very similar to that of a previous single-centered study in China[12] (in which *BRAF* represented 72.4% of the patients), suggesting that the genetic profile of PTC may vary in different regions of the globe. Table S4 summarizes the genotype distribution of mutations in several regions and shows that the *BRAF* mutation represented in all patients is predominant in Korea (72%)[25] but not in Japan (38%)[26,27] or Italy (38.1%).[28] Only 59.5% of the patients in Saudi Arabia carry a *BRAF* mutation.[29] We conclude that *BRAF* is the main driver in this Chinese PTC population.

Table 5 Difference of Clinical and Genetic Profiles Compare Against TCGA Data

	Chinese	TCGA	*p* value*
Mean age	44.43	46.79	0.013
Male (%)	0.251	0.268	0.570
T3 + T4 (%)	0.142	0.347	<0.001
N1 (%)	0.549	0.443	0.002
N1b (%)	0.182	0.141	0.106
Gene fusion events (%)	0.071	0.149	<0.001
RAS mutation (%)	0.020	0.105	<0.001
BRAF mutation (%)	0.785	0.508	<0.001
TERT mutation (%)	0.023	0.073	<0.001

Note: *The *t*-test was performed to examine the mean value between data from this study and TCGA data.
Abbreviation: TCGA, The Cancer Genome Atlas.

Few studies have reported the influence of the MAF of *BRAF* mutations in clinical behavior of PTC. Two previous studies (Guerra et al[30] and Min-Hee Kim[31]) have reported that higher MAF of *BRAF* mutations is related to bigger tumor size and LNM in Italian and Korean people, which conflicted with Gandofi's report.[32] In view of the fact that the genotype distribution (especially BRAF and RAS) in PTC is different in different races, further studies are needed in different races. We certified the influence of MAF of *BRAF* mutations in Chinese PTC population. As a result, the mean MAF of *BRAF* mutation was higher in patients with advanced T and N stages (Figure 2), and the risk of advanced T and N stages increased linearly as the MAF increased (Figure S2). Besides, we firstly reported that increased MAF of *BRAF* is related to LLNM. Those conclusions strongly supported Guerra's finding, which may guide treatment and prognosis.

It has been proved that intratumoral heterogeneity (ITH) plays an important role in genesis and development of cancer, but it is debated in early PTC. Some studies discovered that ITH existed in different area of PTC samples,[33] Masoodi et al[34] studied 79 PTC samples and described the evolutionary patterns to prove existence of ITH in PTC. Fugazzola et al[35] summarized recent studies about ITH in PTC and found that ITH may be not marginal event in early PTC. As a result, the varied MAF of *BRAF* (0.20–41.78%) in our study may could be explained as ITH. But as a clinical study, we could not give a certain answer about this problem without further experiment. However, considering that most patients will not perform any test for ITH or purity of non-tumoral cells during gene detection, we believe that the conclusion that higher MAF of *BRAF* is related with advanced stage may still hold true in clinical practice.

RET fusions have been widely reported as an indicator of poor prognosis in PTC.[36,37] In this study, *RET* fusions were associated with younger age at onset (mean, 37 years), LNM (p=0.001, OR=7.60, CI=[2.61, 32.30]), LLNM (p<0.001, OR= 8.77, CI=[3.94, 20.27]), and T3 + T4 (p<0.001, OR=10.40, CI=[4.08, 27.22]), which coincide with Liang's study.[12] Additional analysis revealed that *CCDC6* fusion may be associated with LLNM (p=0.12). However, previous research showed that, in US and European populations, PTC with a *RET:CCDC6* gene fusion was usually inert and tended to hyper-differentiate.[38] In a recent study, Liu et al[17] noted that no significant difference was found in the Chinese population with *RET:CCDC6* compared with a *RET:non-CCDC6* population. *RET:CCDC6* fusion events are very common in *RET* fusions, but more studies are needed to confirm the role of *CCDC6* in PTC.

We also found that younger patients (<45 years or <55 years) were more likely to have LNM. This conclusion still held true (p=0.005) after a multi-dimensional contingency analysis was performed to control for the influence of genotype, notably *RET* fusions. According to AJCC guidelines,[15] the AJCC stage of young patients (<55 years) with N1 or N1b stage is less advanced than the stage in the older (>55 years) population. However, this Chinese study revealed that the N stage in young patients was usually higher than that in older patients. Zhang et al[39] also found that the Chinese patients with PTC who were <40 years old had greater tendencies to develop LNM (10.7% vs 3.4%, p=0.006). This finding suggests that more aggressive surgical approaches, such as prophylactic bilateral central lymph node dissection and prophylactic lateral lymph node dissection, should be applied for young patients with PTC in China.

There are several limitations to our study. First, this study was retrospective, so the integrity of data could not guaranteed. Second, selection bias may exist, because not every patient diagnosed with PTC was willing to participate in our study. Third, we used a DNA detection technique rather than RNA detection, and the detection mainly included 57 major high-frequency mutations; therefore, some mutations may not have been detected. For example, all gene fusion events in our dataset were associated with *RET*. Previously reported gene fusion events, such as *NTRK, THADA*, and *BRAF*, were not observed. Last, the cross-sectional design precluded exploration of factors associated with prognosis, and the lateral metastases or tumor diameter may not actually be associated with patient prognosis. Prospective study data may be needed to overcome these limitations.

In conclusion, our study reported the clinicopathologic characteristics and somatic gene mutation profile of PTC in a Chinese population, confirming that the main driver mutation is the *BRAF* mutation. This finding was significantly different from results in other countries or regions, therefore the biologic behavior of PTC may also differ significantly. We also confirmed some other conclusions. A high MAF of *BRAF* suggested a high risk of clinicopathologic characteristics. Tumors with *RET* fusion events or multiple driver gene mutations tended to display more malignant biologic behavior than tumors without these changes. Moreover, more LNM developed in young versus older patients in this Chinese population. Our study may help clinicians develop individualized treatment strategies according to genetic

profiles. We will continue to observe these patients and investigate the relationship between genetic characteristics and risk of recurrence in the future.

Ethics Approval Statement

The study protocol was approved by the ethical board of the Chinese Academy of Medical Sciences and Peking Union Medical College Hospital, and all participants gave written informed consent at their local hospitals.

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Author Contributions

All authors contributed significantly to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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