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Detection of genetic mutations in 855 cases of papillary thyroid carcinoma by next generation sequencing and its clinicopathological features

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Abstract

Objective To investigate the genetic mutations in patients with papillary thyroid carcinoma (PTC) and their clinicopathological features by next generation sequencing (NGS).

Methods NGS technology was used to detect genetic mutations in PTC patients, and clinicopathological features were collected.

Results ①Among 855 PTC patients, 810 patients had genetic mutations, and 45 patients had no genetic mutation. ②*BRAF* mutation was associated with tumor diameter ($P<0.001$) and histological subtypes ($P=0.002$). The abundance of *V600E* mutation was associated with gender ($P=0.004$), tumor diameter ($P<0.001$), bilateral presentation ($P=0.001$), extrathyroidal extension ($P<0.001$), lymphatic metastasis ($P<0.001$), histological subtypes ($P=0.002$) and TNM staging ($P=0.000$); The different mutation abundance of *V600E* was associated with tumor diameter ($P<0.001$), multifocal presentation ($P=0.047$), bilateral presentation ($P=0.001$), extrathyroidal extension ($P=0.001$), lymphatic metastasis ($P<0.001$), histological subtypes ($P=0.022$) and TNM staging ($P=0.000$). ③*RET* fusion was associated with tumor diameter ($P<0.001$) and lymphatic metastasis ($P=0.005$). ④*TERT* mutation was associated with gender ($P=0.043$), tumor diameter ($P<0.001$), extrathyroidal extension ($P=0.028$) and TNM staging ($P=0.017$). ⑤*RAS* mutation was associated with histological subtypes ($P<0.001$). ⑥*NTRK* and *PIK3CA* mutations were not associated with clinicopathological features.

Conclusion NGS technology can comprehensively analyze the genetic mutations in PTC patients, which provides important prompts for the occurrence, development, diagnosis and treatment of PTC. In addition, *BRAF V600E* mutation, *RET* fusion and *TERT* mutation are associated with a number of high-risk clinicopathological features. Detection of genetic mutations in PTC patients by NGS is of great significance.

Keywords Papillary thyroid carcinoma, Next generation sequencing, *BRAF*, *RET*, *RAS*, *TERT*

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Introduction

Papillary thyroid carcinoma (PTC) is the most common malignant tumor of the endocrine system. In recent decades, the incidence rate of PTC has risen rapidly worldwide, and is expected to become the fourth malignant tumor in the incidence rate within 10 years [1–3]. According to histological subtypes, thyroid cancer can be divided into PTC, medullary thyroid cancer, follicular thyroid cancer, and undifferentiated thyroid cancer, with PTC being the most common, accounting for approximately 80–85% [4, 5]. The occurrence of thyroid cancer is closely related to factors such as activation of oncogenes, inactivation of tumor suppressor genes, excessive iodine intake, estrogen, ionizing radiation, and obesity, but the specific mechanisms have not been elucidated [6, 7].

Next Generation Sequencing (NGS) technology has the characteristics of high throughput, high depth and high sensitivity, and has been widely used in the research of neoplastic diseases [8, 9]. In this study, NGS technology was used to clarify the genetic mutations in PTC patients, and to explore the relationship between key genes and clinicopathological features of PTC.

Materials and methods

Clinical data selection

PTC patients in Fujian Medical University Union Hospital from January 2021 to September 2022 were selected. A total of 855 patients were confirmed to have PTC by pathology, and surgical resection samples and clinicopathological features were collected. Among the 855 patients, there were 189 males and 666 females; Age range from 7 to 83 years old, with 665 cases≤55 years old and 190 cases>55 years old; The diameter of the tumor ranges from 0.1 to 5.0 cm, with 620 cases≤1 cm and 235 cases>1 cm; 557 patients had single lesion, while 298 patients had multiple lesions; 716 patients were on the single side, and 139 patients were on both sides; 204 patients had absent or incomplete capsule, while while 651 patients had complete of the capsule;401 patients had extrathyroidal extension, while 454 patients had no extrathyroidal extension; 478 patients had lymphatic metastasis, and 377 patients had no lymphatic metastasis; 676 patients had classical subtype, 120 patients had follicular subtype, and 59 patients had both classical subtype and follicular subtype; There were 728 patients with TNM stage I, 110 with stage II, and 7 with stage III. The different histological subtypes of PTC patients are shown in Fig. 1.

NGS sequencing

For paraffin-embedded samples, a nucleic acid extraction kit (DKJ28-01, Guangzhou Meiji Biotechnology Co., Ltd.) was utilized for extraction. The concentration was detected by employing the gene sequencing universal library kit (dsDNA quantification) (230228C03Z, Xiamen Aide Biological Company) and the Quantus fluorescence photometer (E6150, Peomega Company, USA). The human tumor multi-gene mutation detection kit (reversible end termination sequencing) (220701C01Z, Xiamen Aide Biological Company) was used to construct a library. DNA fragmentation was accomplished through enzyme digestion, followed by end repair, splicing, and PCR amplification to obtain an amplified library. The amplified library was subjected to liquid-phase hybridization with probes, capture enrichment via the magnetic bead method, and PCR amplification to yield the captured library. Quality control of pre-library and captured library fragments was conducted using the 2100 Bioanalyzer System (Agilent Technologies, Germany). Sequencing was performed using the Illumina MiseqDx sequencer (Illumina, USA). After filtering the raw sequencing data, the Aide Bioinformatics Analysis System was adopted for bioinformatics analysis. Using GRCh37/hg19 as the reference sequence, analyze and detect all hot exon regions and some introns of human *AKT1*, *ALK*, *BRAF*, *CTNNB1*, *EGFR*, *EIF1AX*, *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, *GNAS*, *HRAS*, *KDR*, *KIT*, *KRAS*, *MET*, *NRAS*, *NTRK1*, *NTRK2*, *NTRK3*, *PAX8*, *PDGFRA*, *PIK3CA*, *PTEN*, *RASAL1*, *RET*, *TERT*, *TP53*, *TSC2*, *TSHR*; The types of genetic mutations detected include point mutation, insertion deletion variation, fusion, copy number variation, etc.

Genetic mutations with an abundance of 1% or more were considered positive. For hot spot mutation below 1%, PCR validation was required. The primer sequence for *BRAF V600E* mutation and internal reference are

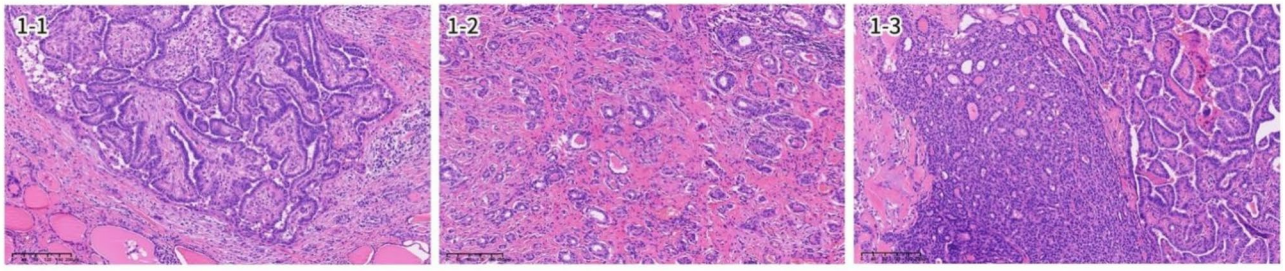

Fig. 1 (**1-1**: classical subtype; **1-2**: follicular subtype; **1-3**: classical and follicular subtype)

shown in Table 1. The methodology of PCR technology is as follows. After nucleic acid extraction, DNA was obtained from tissue samples. A PCR reaction system was established according to the kit instructions. Then PCR amplification is performed on a fluorescence quantitative PCR instrument. Finally, the results were interpreted to complete the validation.

Bioinformatics analysis

The following analysis should be carried out for the detection data: ①Data quality control: Perform quality control on the data obtained through sequencing to eliminate low-quality sequences and possible contaminated data. ②Data alignment: Align the sequencing data with the reference genome to determine the position of each sequencing fragment on the genome. ③Sorting and deduplication: After comparison, sort the sequence and remove duplicate sequences to enhance the accuracy of subsequent analysis. ④Obtaining BAM file: After the above steps, a BAM file was generated, which contains the information of sequencing data and reference genome alignment. ⑤Annotation and filtering: Annotate the test results to determine gene, location and other information, and filter to remove false positive results to obtain the results.

This study only detected somatic variants of tumors and did not conduct germline gene detection using peripheral blood. The variants information obtained after sequencing were compared with public databases such as 1000 Genomes (http://www.internationalgenome.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), COSMIC (http://cancer.sanger.ac.uk) to rule out the possibility of germline mutation. Simultaneously, the Aide Bioinformatics Analysis System we utilize contains a white blood cell database which can assist us in screening for SNP loci, benign SNP loci can be removed.

Variation classification

Referring to the "Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer" by the Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO), College of American Pathologists (CAP), by integrating the relevant research evidence of variants in treatment, diagnosis, and prognosis, the variants were divided into: Tier I (variants with strong clinical significance), Tier II (variants with potential clinical significance), Tier III (variants with unknown clinical significance), and Tier IV (variants deemed benign or likely benign). In this study, variants of Tier I and Tier II were included [10].

Statistical analysis

The experimental data was analyzed by SPSS 26.0 statistical software. The chi-square test was employed to compare the counting data. Specifically, when the total sample size $n \geq 40$ and the theoretical frequencies of all cells $n \geq 5$, the Pearson chi-square test was utilized; In cases where $n \geq 40$ but some theoretical frequencies $n<5$, a continuity correction test was applied. For sample sizes $n<40$ or theoretical frequencies $n<1$, Fisher's exact probability method should be used. Comparison of measurement data was conducted using a T-test with statistical significance defined as $P<0.05$.

Results

Among the 855 PTC patients, 810 patients were found to have genetic mutations, while 45 patients were found to have no genetic mutation. Among the 810 patients with genetic mutations, 775 patients had single genetic mutation, while 35 had two genetic mutations. The highest mutation rate was for the *BRAF* ($n=743$), with a mutation rate of 86.90%. The other genes in order of mutation rate were *RET* ($n=43$), *KRAS* ($n=10$), *TERT* ($n=9$), *NTRK3* ($n=8$), *NRAS* ($n=7$), *TP53* ($n=5$), *PIK3CA* ($n=7$), *NTRK1* ($n=4$) and *ALK* ($n=2$) etc. The detail of genetic mutations are shown in Table 2.

BRAF

Among the 743 *BRAF* mutation, there were 732 cases of *V600E* mutation, 1 case of V600-K601delinsE mutation, 1 case of V601E mutation, and 9 fusion (with *IGF2BP2*, *OSBPL9*, *WARS1*, *MKRN1*, *KATNA1*, *MBP*, *B1CD2*, *MBNL2*, *TRIM24*, respectively). In addition, 33 cases of *V600E* mutation patients had co-mutation with other genes, including *AKT1*, *EGFR*, *E1FIAX*, *KRAS*, *NRAS*, *HRAS*, *NTRK1*, *TP53*, *PIK3CA*, *TERT* and *FGFR3* (Table 2). We divided 855 cases of PTC patients into the *BRAF* mutation group ($n=743$) and the *BRAF* wild-type group ($n=112$), and then compared the clinicopathological features in the two groups. The results showed

Table 1 Primer sequence of PCR

Gene		Primer sequence
BRAF V600E	Forward	5′-TCTGTAGCAGCCCTCAGTAGCGAAGCAGTGATTTTGGTCTAGCTACAGA-3′
	Reverse	5′-AGCCCTCAGTAGCGAAGCAACTCAGCAGCATCTCAGG – 3′
	Probe	5′-TTCAAACCATCAGTTTGAACAGTTGTCTGGATCAACTG-3′
Internal Reference	Forward	5′-GTACCTGCAAGGTGTGGAGTTAC-3′
	Reverse	5′-GCCATCCTGAATTCTGTAAACAGC-3′
	Probe	5′-AGACCTCTCATCATCAGTGC-3′

Table 2 Genetic mutations in PTC

Genetic mutations	Total(*n*)	Mutation/Fusion (*n*)
BRAF	710	*V600E (699), V601E (1), V600_K601delinsE (1), IGF2BP2-BRAF (1)*$^{\Delta}$*, OSBPL9-BRAF (1)*$^{\Delta}$*, WARS1-BRAF (1)*$^{\Delta}$*, MKRN1-BRAF (1)*$^{\Delta}$*, KATNA1-BRAF (1)*$^{\Delta}$*, MBP-BRAF (1)*$^{\Delta}$*, B1CD2-BRAF (1)*$^{\Delta}$*, MBNL2-BRAF (1)*$^{\Delta}$*, TRIM24-BRAF(1)*$^{\Delta}$
RET	41	*CCDC6-RET (22), NCOA4-RET (11), ERC1-RET (4), GOLGA5-RET (2)*$^{\Delta}$*, ANKRD26-RET (1)*$^{\Delta}$*, AFAP1L2-RET (1)*$^{\Delta}$
KRAS	9	*Q61K (8), G12V (1)*
NTRK3	8	*ETV6-NTRK3 (8)*
NTRK1	3	*TPM3-NTRK1 (1), BPNT1-NTRK1 (1)*$^{\Delta}$*, TFG-NTRK1 (1)*$^{\Delta}$
ALK	2	*EML4-ALK (1), STRN-ALK (1)*$^{\Delta}$
TP53	1	*R248W (1)*
NRAS	1	*Q61R (1)*
*BRAF + TERT**	9	*V600E + C228T (7), V600E + C250T (2)*
BRAF + NRAS	6	*V600E + Q61R (6)*
BRAF + PIK3CA	6	*V600E + E545A(1), V600E + H1047R(2), V600E + E545K(1), V600E + N1044K(1)*$^{\Delta}$*, V600E + M1043I(1)*$^{\Delta}$
BRAF + TP53	4	*V600E + R273H (1), V600E + W146R (1)*$^{\Delta}$*,V600E + M246V (1)*$^{\Delta}$*, V600E + C176F (1)*$^{\Delta}$
BRAF + AKT1	1	*V600E + E17K (1)*
BRAF + EGFR	1	*V600E + M137R (1)*$^{\Delta}$
BRAF + EIF1AX	1	*V600E + K10E (1)*$^{\Delta}$
BRAF + FGFR3	1	*V600E + CNV (1)*
BRAF + HRAS	1	*V600E + Q61R (1)*
BRAF + KRAS	1	*V600E + G12V (1)*
BRAF + NTRK1	1	*V600E + G517E (1)*$^{\Delta}$
BRAF + PTEN	1	*V600E + R335 (1)*
RET + EGFR	1	*CCDC6-RET + CNV (1)*
RET + PIK3CA	1	*NCOA4-RET + V344M (1)*$^{\Delta}$

*This means that 9 patients have co-mutations of the *BRAF* and *TERT*. Specifically, there are 7 patients with *BRAF (V600E)* and *TERT (C228T)*, and 2 patients with *BRAF (V600E)* and *TERT (C250T)*

$^{\Delta}$This means that genetic mutations are relatively infrequent in PTC

that the *BRAF* mutation was only associated with tumor diameter ($P<0.001$) and histological subtypes ($P=0.002$) (Table 3). Furthermore, we divided PTC patients into the *V600E* mutation group and the *V600E* wild-type group, the result was consistent with above.

To further investigate the relationship between the abundance of *V600E* mutation and clinicopathological features, *V600E* mutation patients were divided into different groups according to their clinicopathological features. The results showed that the abundance of *V600E* mutation was related to gender($P=0.004$), tumor diameter($P<0.001$), bilateral presentation($P=0.001$), extrathyroidal extension ($P<0.001$), lymphatic metastasis($P<0.001$), histological subtypes($P=0.002$), and TNM staging ($P=0.000$) (Fig. 2).

In 732 patients with *V600E* mutation, the mutation abundance ranged from 0.09 to 38.93%, and 26 cases of PTC patients with mutation abundance below 1% were verified by PCR. According to the 20% mutation abundance as the limit, 732 patients were divided into low mutation abundance group (abundance≤20%) and high mutation abundance group (abundance>20%), and the differences of clinicopathological features in each group were compared. The results showed that the different abundance of *V600E* mutation was correlated with the tumor diameter($P<0.001$), multifocal presentation($P=0.047$), bilateral presentation($P=0.001$), extrathyroidal extension ($P=0.001$), lymphatic metastasis($P<0.001$), histological subtypes($P=0.022$), TNM staging ($P=0.000$).

RET

Among the 855 cases, 43 cases of *RET* fusion were detected, with a mutation rate of 5.03%. Among them, there were 23 cases of *CCDC6-RET* fusion, 12 cases of *NCOA4-RET* fusion, 4 cases of *ERC1-RET* fusion, 2 cases of *GOLGA5-RET* fusion, 1 case of *AFAP1L2-RET* fusion, and 1 case of *ANKRD26-RET* fusion. The clinicopathological features of 43 patients with *RET* fusion are shown in Table 3.

The PTC patients were divided into *RET* fusion group ($n=43$) and *RET* wild-type group ($n=812$). The differences of clinicopathological features between the two groups were compared. The results showed that *RET* fusion was associated with tumor diameter ($P<0.001$) and lymphatic metastasis ($P=0.005$) (Table 3). PTC patients were also divided into *CCDC6-RET* fusion group ($n=23$) and non *CCDC6-RET* fusion group ($n=832$). The

Table 3 Relationship between BRAF/RET/TERT mutations and clinicopathology features of PTC, while the clinicopathology features of RAS/NTRK/PIK3CA mutations in PTC

Parameter	Total	*BRAF*				*RET*				*TERT*				*RAS*	*NTRK*	*PIK3CA*
		Mutation($n=743$)	Wild type ($n=112$)	χ^2-value	*P-value*	Fusion($n=43$)	Wild type ($n=812$)	χ^2-value	*P-value*	Mutation($n=9$)	Wild type ($n=846$)	χ^2-value	*P-value*	Mutation ($n=18$)	Mutation ($n=12$)	Mutation ($n=7$)
Gender																
Male	189	170	19	1.978	0.16	8	181	0.322	0.57	5	184	4.11	**0.043***	4	2	1
Female	666	573	93			35	631			4	662			14	10	6
Age																
≤55	665	573	92	1.421	0.233	36	629	0.925	0.336	6	659	0.162	0.687	13	12	6
>55	190	170	20			7	183			3	187			5	0	1
Tumor diameter(cm)																
≤1	620	556	64	15.28	**<0.001***	11	609	50.04	**<0.001***	1	619	18.05	**<0.001***	12	7	3
>1	235	187	48			32	203			8	227			6	5	4
Multifocal presentation																
No	557	483	74	0.049	0.826	24	533	1.737	0.188	5	552	0.065	0.798	11	7	4
Yes	298	260	38			19	279			4	294			7	5	3
Bilateral presentation																
No	716	622	94	0.003	0.954	32	684	2.891	0.089	6	710	0.887	0.346	15	9	7
Yes	139	121	18			11	128			3	136			3	3	0
Tumor capsule																
Absent/Incomplete	204	175	29	0.293	0.588	12	192	0.408	0.523	3	201	0.082	0.775	12	2	3
Complete	651	568	83			31	620			6	645			6	10	4
Extrathyroidal extension																
Yes	401	351	50	0.264	0.608	21	380	0.068	0.794	8	393	4.848	**0.028***	7	7	3
No	454	392	62			22	432			1	453			11	5	4
Lymphatic metastasis																
Yes	478	407	71	2.93	0.087	33	445	7.975	**0.005***	6	472	0.1	0.752	9	9	5
No	377	336	41			10	367			3	374			9	3	2
Subtypes																
Classical	676	598	78	12.93	**0.002***	34	642	0.001	1	8	668	1.621	0.445	8	9	7
Follicular	120	92	28			6	114			0	120			9	3	0
Classical/Follicular	59	53	6			3	56			1	58			1	0	0
TNM Staging																
I	728	629	99	2.021	0.364	39	689	4.485	0.108	6	722	8.802	**0.017***	15	11	6
II	110	100	10			2	108			1	109			3	1	1
III	17	14	3			2	15			2	15			0	0	0

The data of mutation/fusion group and wild-type group in the table are n (%). Comparison between groups, *: $P<0.05$

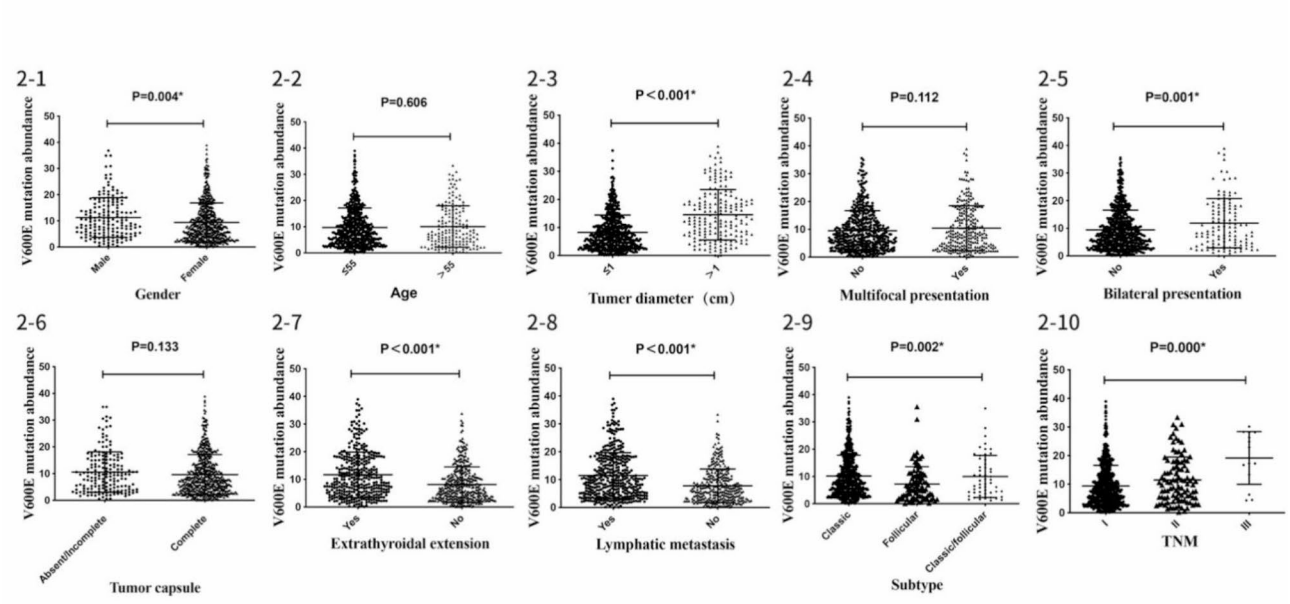

Fig. 2 Relationship between the abundance of *V600E* mutation and the clinicopathological features of PTC
2-1 Gender; **2-2** Age; **2-3** Tumor diameter(cm); **2-4** Multifocal presentation; **2-5** Bilateral presentation; **2-6** Tumor capsule; **2-7** Extrathyroidal extension; **2-8** Lymphatic metastasis; **2-9** Histological subtypes; **2-10** TNM staging
Comparison between groups, *: $P<0.05$

results showed that *CCDC6-RET* fusion was only associated with the tumor diameter ($P<0.001$) and lymphatic metastasis ($P=0.029$). PTC patients were also divided into the *NCOA4-RET* fusion group ($n=12$) and the non *NCOA4-RET* fusion group ($n=843$). The results show that *RET-NCOA4* fusion was only associated with the tumor diameter ($P=0.001$).

According to the 20% mutation abundance threshold, the *RET* fusion patients were divided into low mutation abundance group ($n=31$) and high mutation abundance group ($n=12$). Comparing the clinicopathological features of different mutation abundance of *RET* afterward, it was found that different mutation abundance of *RET* was related to tumor capsule($P=0.044$).

TERT

Among the 855 PTC patients, 9 cases of *TERT* mutation were detected, with a mutation rate of 1.05%, and all these 9 patients had *BRAF V600E* co-mutation. In which,7 had the *C228T* mutation, and 2 had the *C250T* mutation. The clinicopathological features of *TERT* patients are shown in Table 3.

Comparing the clinicopathological features of the *TERT* mutation group ($n=9$) with *TERT* wild-type group ($n=846$), the results showed that *TERT* mutation was associated with gender ($P=0.043$), tumor diameter ($P<0.001$), extrathyroidal extension ($P=0.028$), and TNM staging ($P=0.017$) (Table 3).

RAS/NTRK/PIK3CA

Among the 855 PTC patients, 18 cases of *RAS* mutation were detected, with a mutation rate of 2.11%. Among the *RAS* mutation patients, 10 had *KRAS* mutation, 7 had *NRAS* mutation, and 1 had *HRAS* mutation. 12 cases of *NTRK* fusion were detected, with a mutation rate of 1.40%. Among the *NTRK* fusion patients, 4 had *NTRK1* fusion, and 8 had *NTRK3* fusion. 7 cases of *PIK3CA* mutation was detected, with a mutation rate of 0.82%. The clinicopathological features of *RAS/NTRK/PIK3CA* are shown in Table 3.

After comparing the clinicopathological features of the *RAS* mutation group ($n=18$) with *RAS* wild-type group ($n=837$), we found that *RAS* mutation was only related to histological subtypes ($P<0.001$). The same methodology was applied to the comparison of *NTRK/PIK3CA* and clinicopathological features, and the results showed that *NTRK*/*PIK3CA* mutations were not associated with clinicopathological features of PTC patients.

Discussion

NGS has high throughput, high depth, and high sensitivity, and it has been widely used in basic and clinical research. Compared with PCR technology and first-generation sequencing technology, NGS can not only detect common mutations, but also detect rare mutations, which is of great significance [8, 9, 11]. Apart from the commonly observed mutations in PTC, in this study, we have also detected some rare mutations, including fusion

and point mutation. The detailed information regarding these rare mutations can be found in Table 2. Applying NGS to the detection of tumor diseases provides important hints and guidance for the occurrence and development of tumor diagnosis and treatment [8, 12]. In this study the most common mutations were found in the *BRAF*, *RET*, *TERT* and *RAS*. The *BRAF*, *RET* and *RAS* are involved in the mitogen-activated protein kinase (MAPK) signaling pathway, which is the most common carcinogenic mechanism in PTC. The *BRAF*, *RET*, and *RAS* genes usually do not co-mutation, but in our experimental results, we found 6 cases of *BRAF* with *NRAS* co-mutation, 1 case of *BRAF* with *KRAS* co-mutation, and 1 case of *BRAF* with *HRAS* co-mutation. In addition, we detected *EGFR*, *TP53*, *PIK3CA*, *TERT*, *E1F1AX1*, *FGFR3*, *AKT1*, *E1F1AX*, and *NTRK1* co-mutation with *BRAF*, these mutations are difficult to be detected by PCR methodology or first-generation sequencing technology.

BRAF was discovered by Ikawa S et al. [13] in neuroblastoma in 1988, and it is located on human chromosome 7 (7q34), encoding a RAF family serine/threonine protein kinase. *BRAF* plays a role in regulating the MAPK signaling pathway, promoting continuous cell division, proliferation, and tumor formation [14, 15]. The relationship between *BRAF* and the clinicopathological features of PTC patients has not been clearly established, and different research results vary [16–21]. In this study, *BRAF* mutation was only related to tumor diameter and histological subtypes. Further studies have revealed that the abundance of *V600E* mutation was associated with gender, tumor diameter, bilateral presentation, extrathyroidal extension, lymphatic metastasis, histological subtypes and TNM staging. Numerous studies had confirmed that *BRAF* mutation was linked to high-risk clinicopathological features such as lymphatic metastasis, age and extrathyroidal extension (Table 4) [18, 21]. It can be seen that *BRAF* mutation is an important independent prognostic factor for PTC.

RET is located on chromosome 10q11.2 of human and was identified as an oncogene through the transfection of human lymphoma DNA into mouse NIH3T3 cells [22]. *RET* gene encodes the RET protein, which can activate signaling pathways such as RAS, STAT, and PI3K, thereby participating in the proliferation, invasion, and migration of tumor cells [23]. *RET* fusion account for only 6% of *RET* variation, but is related to the occurrence, development, and biological behaviors of tumor such as invasion and migration [24]. In this study, *RET* fusion was detected in 43 patients. The common fusion types among patients with PTC are *CCDC6-RET* and *NCOA4-RET*, which is in line with the current consensus as described in references [25, 26]. Presently, there is inconsistency in research findings regarding the correlation between *RET* and clinicopathological features of PTC patients [27–29]. Our investigation revealed an association between *RET* fusion and tumor diameter as well as lymphatic metastasis, while no significant link existed with other clinicopathological features. Current studies hold that *RET* fusion is related to factors such as gender, TSH level, lymphatic metastasis, immune microenvironment, progressive histopathological features, later T stages, and patients with *RET* fusion are more likely to experience recurrence [27–29] (Table 4).

TERT is the catalytic protein subunit of telomerase. The abnormal activation of *TERT* in telomeres is of great significance to the biological behavior of tumor cells,

Table 4 Relationship between *BRAF/RET/TERT* genes and clinicopathological characteristics of PTC patients in different studies

Gene	Study	Sample Size(*n*)	Related clinicopathological features
BRAF	This study	855	tumor diameter, histological subtypes
	Li C et al. [16]	6372	lymphatic metastasis, TNM staging, extrathyroidal extension, tumor diameter, male, multifocal presentation, tumor capsule, classic of PTC, and tall-cell variant of PTC
	Chung JH et al. [17]	8315	extrathyroidal extension, lymphatic metastasis, TNM staging
	Wei X et al. [18]	9908	age, male, multifocal presentation, lymphatic metastasis, extrathyroidal extension, TNM staging
	Xing M et al. [19]	219	extrathyroidal extension, lymphatic metastasis, TNM staging
	Scheffel RS et al. [20]	134	This paper is based on single center data, and they did not identify any meaningful pathological parameters
	Xing M et al. [21]	507	male, tumor diameter, extrathyroidal extension, lymphatic metastasis, TNM staging
RET	This study	855	tumor diameter, lymphatic metastasis
	Khan et al. [27]	48	gender, TSH level, lymphatic metastasis
	Huang et al. [28]	108	lymphatic metastasis, the immune microenvironment
	Scholfield et al. [29]	41	aggressive histopathological features, later T stages
TERT	This study	855	gender, tumor diameter, extrathyroidal extension, TNM staging
	Liu's et al. [33]	408	age, tumor diameter, extrathyroidal extension, PTC advanced stages III/IV
	Zhao L et al. [34]	8338	lymphatic metastasis, extrathyroidal extension, PTC advanced stages III/IV
	Sang Y et al. [35]	382	age, extrathyroidal extension, lymphatic metastasis, PTC advanced stages III/IV
	Li M et al. [36]	143	age, PTC advanced stages III/IV

such as proliferation, invasion and migration [30]. The most common mutations are *C228T* and *C250T*. The mutation of these two sites will produce a new set site E-26 transcription factor, thus promoting the transcriptional activity of telomerase [31]. The results of this study showed that *TERT* mutation was related to gender, tumor diameter, extrathyroidal extension and TNM stating. Current research indicates that *TERT* mutation is related to the occurrence of PTC, along with features like age, tumor diameter, extrathyroidal extension, and advanced stages III/IV in PTC patients [32, 33]. Co-mutation of *TERT* and *BRAF* are more common and have a stronger connection with clinicopathological aggressiveness. The present study also agrees that *TERT* mutation is an independent factor for a poor prognosis of PTC [17, 21, 34–36] (Table 4). This suggests that *TERT* detection should be included in the pathological assessment of PTC.

RAS was first discovered in thyroid tumors by Lemoine et al. [37] in 1988, and it is an oncogene that participates in the formation and development of various tumors. Point mutation or insertion mutation in the coding region of the *RAS* can activate the gene, and the activated *RAS* can increase the expression of its product, P21 protein. P21 protein participates in regulating cell growth and differentiation, it also can promote the abnormal proliferation of normal cells and ultimately transform them into tumor cells [38, 39]. *RAS* mutation was detected in 18 patients with PTC, with a mutation rate of 2.11%. The results showed that *RAS* mutation was related to the histological subtypes and was unrelated to other clinicopathological features. *KRAS*, *NRAS*, *HRAS* are common in PTC, especially in the follicular subtype, but lack significant and independent prognostic effects [40]. Additionally, we statistically analyzed the *NTRK* and *PIK3CA*, and found no correlation between the genetic mutations and the clinicopathological features of PTC patients.

The NGS test report includes the genetic "mutation abundance" indicator, but previous studies have not clarified the meaning of this indicator [41, 42]. In this study, *BRAF V600E* and *RET* patients were divided into low mutation abundance and high mutation abundance groups based on a threshold of 20%. The results showed that different *V600E* mutation abundance was associated with tumor diameter, multifocal presentation, bilateral presentation, extrathyroidal extension, lymphatic metastasis, histological subtypes and TNM staging. Different *RET* mutation abundance was associated with tumor capsule, but not associated with other clinicopathological features. The relationship between mutation abundance and clinicopathological features has not been reported in previous studies, and the experimental results obtained by NGS testing PTC patients' genetic mutations have higher clinical reference value. In future studies, we can combine mutation abundance with patient prognosis information to establish a prognostic model and obtain the mutation abundance threshold. This could potentially be an independent prognostic factor.

However, this study has certain limitations. We only included 30 genes associated with PTC in our research, rather than performing whole exome sequencing. This may lead to the failure to detect some rare genes. In the future, whole exome sequencing should be conducted on PTC patients, and the sequencing results need to be combined with clinicopathological features and prognosis information to gain a more comprehensive understanding of the genetic mutations status of PTC. Furthermore, the histological subtypes included in our study are mainly classical subtype, follicular subtype, and a mixture of classical subtype and follicular subtype. Among the 855 patients, one patient was of tall-cell subtype (combined with classical subtype, and *BRAF V600E* mutation was detected), and one patient was of cribriform morular subtype (combined with classical subtype, and *BRAF V600E* mutation was detected). Due to the small number of cases and the presence of classical subtype in both, we classified them as classical subtype for analysis. In the future, large-scale research is needed to incorporate subtype such as tall-cell subtype, diffuse sclerosing subtype, and columnar-cell subtype into the study in order to further analyze and grasp the relationship between genetic mutations and histological subtypes in patients with PTC.

Conclusion

NGS technology can comprehensively analyze the genetic mutations of PTC patients, which provides important hints for the occurrence, development, diagnosis and treatment of PTC. In addition, *BRAF V600E* mutation, *RET* fusion, *TERT* mutation are associated with a number of high-risk clinicopathological features. Detection of genetic mutations in PTC patients by NGS is of great significance.

Abbreviations

BRAF	B-Raf proto-oncogene, serine/threonine kinase
HRAS	Harvey Rat Sarcoma Viral Oncogene Homolog
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
NGS	Next Generation Sequencing
NRAS	Neuroblastoma RAS Viral Oncogene Homolog
NTRK	Neurotrophic Receptor Tyrosine Kinase
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PTC	Papillary Thyroid Carcinoma
RAS	Rat Sarcoma
RET	Rearranged during Transfection
TERT	Telomerase Reverse Transcriptase

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Author contributions
YHY and YHZ designed the study; DLS wrote the manuscript; DLS and MHY collected samples and clinical information; JKL and DW performed the experiments and acquired data; DLS and MCJ analyzed the data and drew the picture; all authors revised and approved the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The research ethics was approved by the ethics committee of Fujian Medical University Union Hospital(2024KJCX105).

Consent for publication
Written informed consent for publication was obtained from each participant.

Competing interests
The authors declare no competing interests.

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