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Relationship between Somatic Mutations and Clinic Pathological of Follicular Variant Papillary Thyroid Carcinoma: A Meta-Analysis

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Abstract

Background and aims: The incidence of somatic mutations and clinicopathological features in Follicular Variant Papillary Thyroid Carcinoma (FVPTC) demonstrate inconsistent findings.

Materials and methods: A total of 104 publications from PubMed/MEDLINE, Embase and The Cancer Genome Atlas (TCGA) were included in this meta-analysis. One meta-analysis assessed the histology-specific prevalence, while another examined the clinical features of mutant carriers. Furthermore, the mutational landscape of FVPTC was analyzed using data from TCGA database.

Results: Our meta-analysis included data from 7971 individuals, comprising a total of 2097 clinical samples. The study summarised the overall and subgroup somatic mutation rates, and identified four subtypes as well as clinical features associated with FVPTC. Among the mutant types, *RAS* (34.8%) was the most prevalent, followed by *BRAF* (19.9%), *PAX8-PPARG* (8.1%), *RET-PTC* (5.6%), *TSHR* (4.1%), *TERT* (2.3%), and *EIF1AX* (1.1%). In our subgroup meta-analysis, the highest incidence of *BRAF* (31.0%) and *RAS* (37.5%) mutations were observed in invasive FVPTC and non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), respectively. Furthermore, we found that *BRAF* mutations were associated with an increased incidence of multifocality, extrathyroid extension, lymph node metastases, high TNM stage (AJCC), and tumor recurrence in FVPTC, but not *RAS* mutations.

Conclusion: Based on the somatic mutational landscape of FVPTC, patients with *BRAF* mutations have been found to be at a higher risk of experiencing poor clinical outcomes, whereas *RAS* mutations do not show such an association.

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Keywords: Follicular variant papillary thyroid carcinoma; Somatic mutation; Frequency; Histology; Clinical feature; Meta-Analysis.

Introduction

Thyroid Cancer (TC) has experienced a rise in incidence since the early 1980s and now ranks as the fifth most common cancer among women in the United States. It is projected to become the most commonly diagnosed cancer in people aged 15 to 29 and the fastest-growing cancer in many countries, largely due to an increase in Papillary Thyroid Cancer (PTC) [1-3]. The Follicular Variant of Papillary Thyroid Carcinoma (FVPTC) is the second most common histological subtype within PTC, accounting for 9-27% of all PTC patients [4-6].

FVPTCs are classified into two subgroups: encapsulated and infiltrative forms [7]. Encapsulated EFVPTC is further divided into non-invasive EFVPTC and invasive EFVPTC, based on the invasion of the capsule by tumor cells [8]. Overall, non-invasive EFVPTC has been found to exhibit a less aggressive recurrence and metastasis rate than other PTC variants [9,10]. In 2016, Nikiforov et al. [11] suggested renaming non-invasive EFVPTC to exclude the word "carcinoma" from its nomenclature and introduced the term "Non-Invasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features" (NIFTP). The 2017 World Health Organization classification of neoplasms removed NIFTP from the list of cancers, emphasizing its favorable treatment outcomes and limited malignant potential, which result in a mild course of the disease during follow-up [12].

Somatic mutational profiling has identified driver mutations that are believed to contribute to early carcinogenesis, diagnosis, and therapy [13]. In recent decades, research on human cancer genetics has greatly benefitted from new technologies such as Sanger sequencing, Polymerase Chain Reaction (PCR), and Next-Generation Sequencing (NGS) approaches. These include Whole-Genome Sequencing (WGS), whole-exome sequencing (WES), and targeted panels, which have identified mutations with prognostic significance [14-17].

Recent large-scale whole-genome and whole-exome sequencing studies have aimed to identify the genetic causes of FVPTC, with varying degrees of success. In FVPTC, one of the most common genetic alterations is Rat Sarcoma viral oncogene (*RAS*) mutations, which is a key protein in many signaling pathways that regulates normal cell growth and malignant transformation and occurs at a frequency of 15-40% [18-20]. *V-raf* murine sarcoma viral oncogene homolog B(*BRAF*) is a serine/threonine protein kinase activated by the Ras-GTP protein [21]. The most frequent *BRAF* mutation in FVPTC is the T1799A transversion mutation in exon 15 of the gene, which causes a V600E amino acid substitution in the protein [22]. Additionally, Telomerase Reverse Transcriptase (*TERT*) mutations occur in two hotspot positions located 124 and 146 bp upstream from the ATG start site (124 G4A and 146 G4A, C4T on opposite strand), enhancing *TERT* promoter activity [23]. The Cancer Genome Atlas identified additional driver alterations present at a lower frequency, including *EIF1AX*, *PPM1D*, and *CHEK2* [24]. The presence or absence of each of these genetic markers may have therapeutic and/or prognostic implications for patients with FVPTC.

The frequency of somatic mutations in FVPTC varies considerably, and many literature studies have reported molecular abnormalities in FVPTC. While *BRAF* mutations have been shown to have a strong positive correlation with poor clinical characteristics of FVPTC [25-28], others found no such connection [29-32]. Similarly, *RAS* mutations in FVPTC have yielded contradictory outcomes [16,33-37]. Additionally, a wide range of mutation landscapes has been observed for other mutations due to objective factors such as sample size, ethnicity, and mutation analysis methodologies [23,38-41]. However, a comprehensive or pooled meta-analysis of the entire somatic mutational landscape of FVPTC is currently lacking.

Here, we present a meta-analysis of the somatic mutation landscape of FVPTC, assessing the prevalence and clinical characteristics of these mutations. The analysis includes ethnicity, tumor preservation conditions, gene sequencing methods, and reference quality to demonstrate the potential clinical significance of these mutations.

Materials and methods

This systematic review was conducted following the methods outlined in the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines [42].

Search strategy

To conduct this research, we selected articles from the Pubmed/MEDLINE, Embase, and The Cancer Genome Atlas databases between January 2013 and December 2023. Our search included both indexing terms (MeSH terms in PubMed and ENTREE terms in Embase) and keyword terms such as "Genomic [Mesh]" or "Mutation" and ("Follicular Variant of Papillary Thyroid Carcinoma" or "Encapsulated Follicular Variant of Papillary Thyroid Carcinoma" or "Invasive Encapsulated Follicular Variant of Papillary Thyroid Carcinoma" or "Non-Invasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features" or Infiltrative Follicular Variant of Papillary Thyroid Carcinoma" or "FVPTC" or "EFVPTC" or "IEFVPTC" or "NIFTP" or "IFVPTC"). We manually searched the reference lists of all included articles to identify any potentially related studies, and used EndNote software to manage references and remove duplicates. Furthermore, we reviewed the references cited in the searched articles and relevant studies to ensure that no eligible articles were missed.

Eligibility criteria

Our objective was to conduct a meta-analysis on genomic data obtained from FVPTCs to assess the prevalence of gene mutations and clinical features. We used a modified PICOS (participants, interventions, comparators, outcomes, and studies) approach to guide our screening of studies for eligibility in our analysis.

Details of the inclusion and exclusion criteria can be found in the Supplementary methods [43].

Study selection

Two authors (Fan and Zhang), screened the retrieved papers independently. They first screened them by title, then by abstract, and finally by full text. Any disagreements during screening were

resolved through discussion and consensus. In cases where disagreements persisted, a third researcher (Huang) was consulted. The following information was extracted from each study using a predefined worksheet: title, journal, publication year, study design, country, institution, time of enrollment, sequencing method used, type of FVPTC, and mutational genes involved. If required, the authors of each trial were contacted for additional information.

Quality assessment

Qgenie-tool was used to perform a literature quality assessment for all included articles [44].

Statistical analysis

The prevalence of somatic mutations, including point mutations, was presented using forest plots with 95% confidence intervals in R Studio version 1.3 and the "Meta" package. Heterogeneity was assessed using x^2-based Q statistics and I^2, where *P* values <0.05 for the Cochran Q test and I^2 exceeding 50% were considered significant. Publication bias was evaluated using funnel plot of standard error against the effect estimate, and statistical significance was determined by a *P* value <0.05 using the Egger linear regression test method. Subgroup analyses were conducted for FVPTC subtypes, tumor preservation conditions, ethnicity, gene test method, Q-genie quality score, and research center. The Z-test was used to evaluate differences between pooled proportions of prevalence, and statistical significance was set at $P<0.05$. The "maftools" R package [45] was used to visualize oncoplot, somatic interaction, and position-based cancer driver analytics, and to calculate the number of somatic non-synonymous point mutations within each sample.

Results

Eligible studies: After conducting our initial literature search and removed 86 duplicates, we found a total of 197 relevant abstracts on PubMed, 319 on Embase, and an additional record from the TCGA database (Figure 1). Following the elimination of duplicates and screening of titles and abstracts, we were left with 130 publications. After a careful review of full-text articles, 281 irrelevant records were removed, leaving us with 104 articles for frequency meta-analysis. Unfortunately, due to a lack of available clinical data, 89 papers were excluded. Ultimately, our clinical feature-related meta-analysis was based on 15 independent studies, in addition to mutation data from the TCGA database.

Study characteristics: In summary, publication details for the studies are provided in the supplementary reference list in the Supplementary Table S1 [43]. Our meta-analysis included a total of 7971 FVPTC patients, with the majority being women. The genotype data were primarily obtained from Formalin-Fixed Paraffin-Embedding samples (FFPE) from patients, and direct sequencing was the most common method used. Our analysis consisted of 92 single-center studies and 12 multi-center studies, which are described in Table S1 [43] along with their basic features and enrollment details. And 31 high-quality studies were identified, as shown in Table S2 [43].

Somatic mutation frequencies of FVPTC: Our frequency meta-analysis included a total of 104 studies. We selected 35 mutated genes using a priori mutation prevalence threshold of 1%.

In our pooled meta-analysis of FVPTCs, we grouped mutations and compared them with total mutations and high mutational points. As shown in Figure 2, the prevalence of somatic mutations ranged from 1% to 35% in FVPTCs. The most commonly mutated gene was *RAS* (34.8%; 95%CI, 30.4%-39.3%), which had three main mutational sites: *NRAS* (23.5%; 95% CI, 20.3%-26.7%), *HRAS* (10.1%; 95% CI, 7.9%-12.6%), and *KRAS* (3.6%; 95% CI, 2.1%-5.4%). The other four most frequently studied genes were *BRAF*, *RET-PTC*, *TERT*, and *PAX8-PPARG*, with mutation prevalences of 19.9%, 5.6%, 2.3%, and 8.1%, respectively. Notably, parts of the mutation gene (THDA1, TET2, SMARCB1, etc) only having one to three articles described, were summarized in Table S3 [43].

Generally, our pooled meta-analysis involved a diverse range of research studies, leading to significant heterogeneity. Therefore, we focused our subgroup analysis on *RAS*, *BRAF*, *TERT*, *RET-PTC*, and *PAX8-PPARG*. We aimed to identify the reasons for this heterogeneity by examining various parameters including tumor preservation conditions (formalin-fixed vs Fresh-FNA), ethnicity (Western vs Asian), gene test method (Direct Sequencing vs Immunohistochemistry vs Sanger sequencing vs Next-Generation Sequencing), Q-genie quality score (High vs other), and research center (single vs multiple centers). Furthermore, we observed a diverse mutation landscape in other mutations, which could be attributed to several objective factors such as sample size, ethnicity, and mutation analysis methods.

Table S4 [43] shows that FFPE is the primary preservation method for mutation testing samples. However, there is no significant difference in mutation frequency between FFPE and FNA samples. Furthermore, ethnicity subgroup analyses revealed similar population-level differences for *BRAF* and *RAS* mutations. Direct sequencing was the most commonly used test for these two mutations, with a *BRAF* mutation frequency of 17.2% in Westerners compared to 24.4% in Easterners and 21.7% by direct sequencing. In contrast, the multi-center study reported a *TERT* mutation frequency of 10.6%, which was significantly higher than the 1.8% reported in the uni-center study. Lastly, there was no significant difference in mutation frequency between the two levels scores.

Prevalence of individual mutations by histology: Interestingly, the *RAS* gene (rate 37.5%; 95% CI, 28.0%-47.5%) had the highest occurrence of mutations among all genes listed in Figure 3. The frequency was significantly greater in the NIFTP subgroup compared to other histology groups. Similarly, *BRAF* mutations (rate 31.0%; 95% CI, 23.8%-38.6%) were relatively common in the IFVPTC subgroup. However, IFVPTC showed only about a 10% prevalence in *RAS* mutations. Additionally, the prevalence of *BRAF* mutations was relatively low in NIFTP (rate 3.4%; 95% CI, 0.7%-7.3%) and NIEFVPTC (rate 5.0%; 95% CI, 2.3%-8.3%).

Association between mutations and FVPTCs' clinical feature: The basic characteristics of the clinical feature-related eligible studies are summarized in Table S5 [43]. Fourteen and four articles respectively dealt with clinical features associated with *BRAF* and *RAS* mutations.

The *BRAF* and *RAS* mutations were characterized in eight and four trials, respectively, with a total of 1079 and 324 patients. The *BRAF* mutation was discovered in 262 patients with a positive mutational status, while the *RAS* mutation was found in 118 patients (Figure 4). In relation to multifocality in FVPTC, the *BRAF*

mutation was linked with an Odds Ratio (OR) of 1.630 (95% Confidence Interval [CI], 1.115-2.385; Z=2.52; P=0.012), whereas there were no significant differences between *RAS* mutation groups (OR, 1.174; 95% CI, 0.719-1.917; Z=0.64; P=0.522). Regarding extrathyroid extension, eight studies involving 559 individuals for *BRAF* mutations, and four studies involving 324 individuals for *RAS* mutations were discovered, with 160 and 118 patients having a positive mutational status, respectively. The *BRAF* mutant was linked with extra thyroid extension (OR, 1.986; 95% CI, 1.088-3.626; Z=2.23; P=0.025), but not the *RAS* mutation (OR, 0.931; 95% CI, 0.365-2.376; Z=-0.15; P=0.882). With respect to lymph node metastases, ten studies encompassing 942 patients for *BRAF* mutations and four studies including 400 patients for *RAS* mutations were discovered, with 248 and 167 individuals having a positive mutational status, respectively. The *BRAF* mutation was related to lymph node metastases (OR, 1.958; 95% CI, 1.153-3.323; Z=2.49; P=0.013), but there were no significant differences between *RAS* mutation groups (OR, 0.964; 95% CI, 0.582-1.598; Z=-0.14; P=0.888). Eight investigations encompassing 751 patients for *BRAF* mutations and four studies including 399 patients for *RAS* mutations in relation to advanced TNM stage were discovered, with 152 and 170 patients having a positive mutational status, respectively. The *BRAF* mutation was related to advanced TNM stage (OR, 2.724; 95% CI, 1.753-4.232; Z=4.46; P<0.001), but there were no significant differences between *RAS* mutation groups (OR, 0.8960; 95% CI, 0.562-1.684; Z=-0.1; P=0.922). Both *BRAF* and *RAS* mutations were found in two investigations encompassing 298 patients each, in terms of vascular invasion, with 66 and 134 individuals having a positive mutational status, respectively. However, neither *BRAF* nor *RAS* mutations were significantly related to vascular invasion (OR, 0.775; 95% CI, 0.133-4.531; Z=-0.28; P=0.777 | OR, 0.6571; 95% CI, 0.1789-2.4138; Z=2.49; P=0.527). Three studies encompassing 669 patients for *BRAF* mutations and one study including 101 patients for *RAS* mutations in relation to tumor recurrence were found, with 141 and 34 patients having a positive mutational status, respectively. The *BRAF* mutation was linked to tumor recurrence (OR, 3.2460; 95% CI, 1.7481-6.0272; Z=2.49; P=0.0002), but not the *RAS* mutation (OR, 5.8182; 95% CI, 0.5823-58.1373; Z=2.49; P=0.1338). Regarding distant metastases, three investigations covering 324 patients for *BRAF* mutations and one study including 172 patients for *RAS* mutations were discovered, with 41 and 89 patients having a positive mutational status, respectively. However, both *BRAF* and *RAS* mutations were unrelated to vascular invasion (OR, 2.193; 95% CI, 0.246-19.508; Z=0.7; P=0.481 | OR, 2.775; 95% CI, 0.39-19.729; Z=1.02; P=0.308).

The landscape of somatic mutation in FVPTCs: In the TCGA-THCA cohort, a total of 102 FVPTC patients were detected, and their basic characteristics are summarized in Table S6 [43]. As shown in the waterfall map, 70 out of 102 FVPTC patients had somatic mutations, accounting for 68.63%. The *NRAS*, *BRAF*, and *HRAS* mutations were the three most highly mutated genes in FVPTC samples, with frequencies of 25%, 17%, and 9%, respectively (Figure 5A). Missense mutations had an absolute position among the total mutation classification (Figure 5Ba), and Single Nucleotide Polymorphisms (SNPs) accounted for a higher proportion than deletions or insertions (Figure 5b and e). Additionally, C > A had the highest frequency, 1066 times, among the variant types of SNVs (Figure 5b,c). Figure 5d showed that the number of variants per sample and the median value of mutation variants was 11. Furthermore, the top 10 genetically varied genes in the TCGA-FVPTC cohort were *NRAS*, *BRAF*, *HRAS*, *TTN*, *EIF1AX*, *TG*, *MUC16*, *RYR1*, *NAV3*, and *CEP350* (Figure 5bf). The distribution of SNVs in FVPTC was classified into six transition and transversion events, as displayed in the transition and transversion plot (Figure 5c). The stacked bar plot at the bottom shows the distribution of mutation spectra for every sample in the MAF file. To further elucidate the intrinsic connection between these genetically altered genes, the exclusive and co-occurrence correlations were presented in Figure 5D. *HRAS* and *HERC1* had the highest co-occurrence frequency.

Heterogeneity and publication bias: We performed several subgroup analyses on the top 5 mutational genes investigated to understand the variation in mutation prevalence among primary

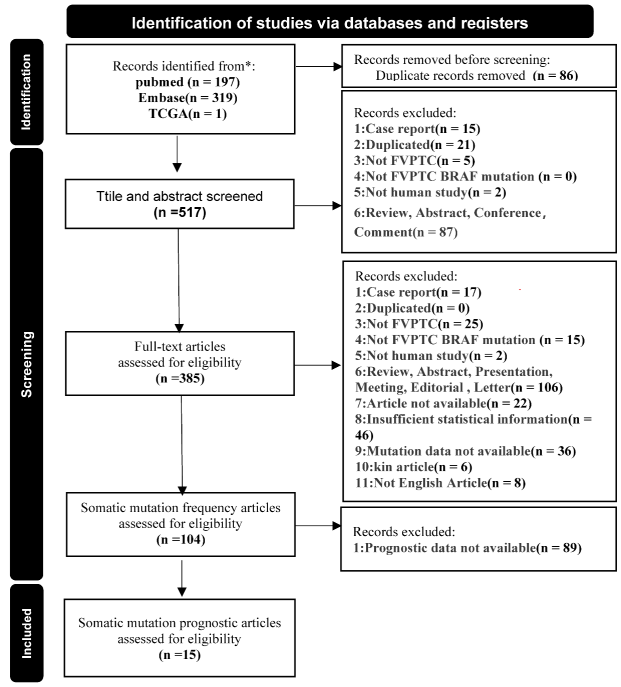

Figure 1: Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flow diagram for the study selection process.

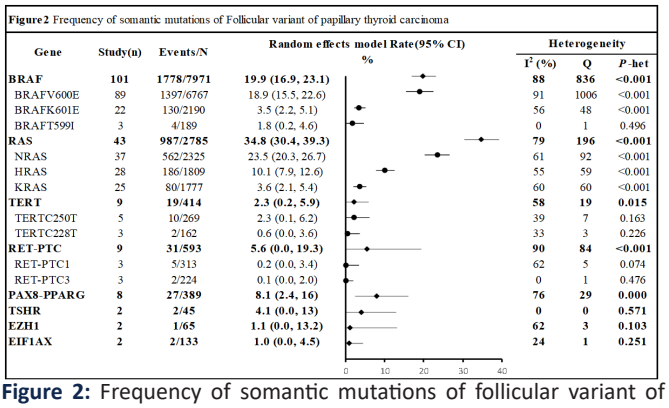

Figure 2 Frequency of somantic mutations of Follicular variant of papillary thyroid carcinoma

Gene	Study(n)	Events/N	Random effects model Rate(95% CI) %	Heterogeneity I^2 (%)	Q	*P*-het
BRAF	**101**	**1778/7971**	**19.9 (16.9, 23.1)**	**88**	**836**	**<0.001**
BRAFV600E	89	1397/6767	18.9 (15.5, 22.6)	91	1006	<0.001
BRAFK601E	22	130/2190	3.5 (2.2, 5.1)	56	48	<0.001
BRAFT599I	3	4/189	1.8 (0.2, 4.6)	0	1	0.496
RAS	**43**	**987/2785**	**34.8 (30.4, 39.3)**	**79**	**196**	**<0.001**
NRAS	37	562/2325	23.5 (20.3, 26.7)	61	92	<0.001
HRAS	28	186/1809	10.1 (7.9, 12.6)	55	59	<0.001
KRAS	25	80/1777	3.6 (2.1, 5.4)	60	60	<0.001
TERT	**9**	**19/414**	**2.3 (0.2, 5.9)**	**58**	**19**	**0.015**
TERTC250T	5	10/269	2.3 (0.1, 6.2)	39	7	0.163
TERTC228T	3	2/162	0.6 (0.0, 3.6)	33	3	0.226
RET-PTC	**9**	**31/593**	**5.6 (0.0, 19.3)**	**90**	**84**	**<0.001**
RET-PTC1	3	5/313	0.2 (0.0, 3.4)	62	5	0.074
RET-PTC3	3	2/224	0.1 (0.0, 2.0)	0	1	0.476
PAX8-PPARG	**8**	**27/389**	**8.1 (2.4, 16)**	**76**	**29**	**0.000**
TSHR	**2**	**2/45**	**4.1 (0.0, 13)**	**0**	**0**	**0.571**
EZH1	**2**	**1/65**	**1.1 (0.0, 13.2)**	**62**	**3**	**0.103**
EIF1AX	**2**	**2/133**	**1.0 (0.0, 4.5)**	**24**	**1**	**0.251**

Figure 2: Frequency of somantic mutations of follicular variant of papillary thyroid carcinoma.

Abbreviations: CI: Confidence Interval; het: heterogeneity; Q: Standardized weighted sum of the squares of variations across different studies; 12: Proportion of observed between-study variation.

Figure3 Frequency of somantic mutations in FVPTC in sub-group analyses

Gene	Study(n)	Cases/N	Random effects model Rate(95% CI) %	Heterogeneity I^2 (%)	Q	*P*-het
BRAF						
IFVPTC	21	244/761	31.0 (23.8, 38.6)	69.1	65	<0.001
EFVPTC	12	70/422	11.2 (4.3, 19.9)	72	39	<0.001
IEFVPTC	16	118/525	16.2 (9.4, 24.3)	79.3	72	<0.001
NIEFVPTC	6	25/326	5.0 (2.3, 8.3)	0	2	0.809
NIFTP	25	66/854	3.4 (0.7, 7.3)	78.3	111	<0.001
RAS						
IFVPTC	12	60/352	11.4 (6.4, 17.3)	8.8	12	0.359
EFVPTC	6	61/187	30.8 (18.8, 44)	62.8	13	0.020
IEFVPTC	11	116/316	35.2 (25.4, 45.6)	65.4	29	0.001
NIEFVPTC	3	16/72	26.6 (8.5, 49.2)	68.4	6	0.042
NIFTP	17	253/633	37.5 (28.0, 47.5)	83.9	99	<0.001
RET-PTC						
IFVPTC	1	6/52	11.5 (4, 21.9)	0	-	0.000
EFVPTC	2	1/54	1.3 (0, 7.3)	0	1	0.464
IEFVPTC	1	0/51	0 (0, 3.3)	0	-	0.000
NIEFVPTC	-	-	-	-	-	-
NIFTP	1	0/74	0 (0, 2.3)	0	-	0.000
PAX8-PPARG						
IFVPTC	1	0/29	0 (0, 5.9)	0	-	0.000
EFVPTC	3	3/56	1.2 (0, 8.9)	0	1	0.620
IEFVPTC	1	2/57	3.5 (0.1, 10.3)	0	-	0.000
NIEFVPTC	-	-	-	-	-	-
NIFTP	-	-	-	-	-	-

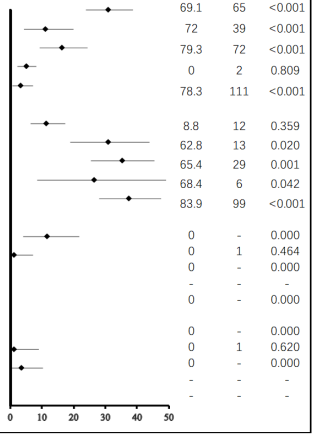

Figure 3: Frequency of somantic mutations in FVPTC in sub-group analyses.
Abbreviations: CI: Confidence Interval; het: heterogeneity; Q: Standardized weighted sum of the squares of variations across different studies; 12: Proportion of observed between-study variation.

Figure 4 Random model of the odds ratio (ORs) with 95% of BRAF and RAS mutation associated with prognostic clinical factor

Gene	Study(n)	Case(N)	Mutation type(n/N) Mutated	Mutation type(n/N) WT	Random effects model OR (95% CI)	*p*-value	Heterogeneity I^2 (%)	Q	*P*-het
BRAF									
Multifocality	8	1079	233/817	96/262	1.630 (1.115, 2.385)	0.012	29	10	0.199
Extrathyroid extension	7	559	82/399	48/160	1.986 (1.088, 3.626)	0.025	24	8	0.243
Lymph node metastases	10	942	145/694	88/248	1.958 (1.153, 3.323)	0.013	49	18	0.040
TNM stage(AJCC)	8	751	103/599	52/152	2.724 (1.753, 4.232)	<0.001	0	5	0.665
Vascular invasion	2	298	9/232	1/66	0.775 (0.133, 4.531)	0.777	0	0	0.487
Tumor recurrance	3	669	28/528	20/141	3.246 (1.748, 6.027)	<0.001	0	1	0.621
Distant metastasis	3	324	5/283	1/41	2.193 (0.246, 19.508)	0.481	35	3	0.216
RAS									
Multifocality	4	324	65/206	41/118	1.174 (0.719, 1.917)	0.522	0	1	0.857
Extrathyroid extension	4	324	38/206	21/118	0.931 (0.365, 2.376)	0.882	32	4	0.221
Lymph node metastases	4	400	60/233	39/167	0.964 (0.582, 1.598)	0.888	0	2	0.667
TNM stage(AJCC)	4	399	48/229	33/170	0.973 (0.562, 1.684)	0.922	0	3	0.397
Vascular invasion	2	298	6/164	4/134	0.657 (0.179, 2.414)	0.527	0	0	0.766
Tumor recurrance	1	101	1/65	3/36	5.818 (0.582, 58.137)	0.134	-	-	-
Distant metastasis	2	172	1/83	3/89	2.775 (0.39, 19.729)	0.308	0	0	0.919

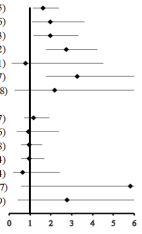

Figure 4: Random model of the Odds Ratio (ORs) with 95% of BRAF and RAS mutation associated with prognostic clinical factor.
Abbreviations: WT: Wild Life; CL: Confidence Interval; het: heterogeneity; Q: Standardized weighted sum of squares of variations across different studies; 12: Proportion of the observed between-study variation.

tumors. Unfortunately, our findings were inconsistent (Table S4 [43]), but the complete funnel plots can be viewed in Figure S1 [43]. As per the sensitivity analysis, none of the studies significantly affected the pooled Odds Ratios (ORs) and Confidence Intervals (CIs). Additionally, **Figure S2 [43] shows the sensitivity assessment results for the response assessment outcomes.**

Discussion

We present a meta-analysis of somatic mutations in FVPTC, which offers more robust findings for gene mutation prevalence compared to data from individual studies. Understanding somatic mutations in FVPTC may aid in categorizing individuals based on their clinicopathological risk factors. Genes with a higher frequency of mutations ought to be included in future genomic and functional investigations to gain a better understanding of their role in FVPTC, as well as in sequencing panels.

We found a high incidence of *RAS* mutations (34.8% in patients at baseline), as shown in Figure 2. These mutations are central to the development of FVPTC cancer, but they do not appear to be related to the clinical characteristics of malignancy. *RAS* oncogenes encode a family of guanine nucleotide-binding proteins and play a critical role in carcinogenesis and progression [18,19]. As such, they are considered an important target for therapeutic intervention. The *RAS* family is composed of three small GTP proteins, specifically *HRAS*, *NRAS*, and *KRAS*. Studies show that among the three, *NRAS* mutations occur more frequently compared to *HRAS* mutations. Meanwhile, *KRAS* mutations are considered rare, accounting for less than 1% of cases [28]. Interestingly, in FVPTC subtype brackets, we found that *RAS* mutations were most commonly mutated in NIFTP (Rate, 37.5%; 95% CI, 28.0%-47.5%), but not in IFVPTC (Rate, 11.4%; 95% CI, 6.4%-17.3%). As Nikiforov et al. [11] in 2016 suggested, NIFTPs were detected in more than 45,000 patients each year and have a very low risk of adverse outcomes. On the other hand, IFVPTC is more aggressive than both EFVPTC types for most clinicopathological features [9,25,46]. Although *RAS* mutations were most commonly found in NIFTP, our analysis showed no significant association between *RAS* mutation and malignancy-related clinical features in FVPTC (Figure 4).

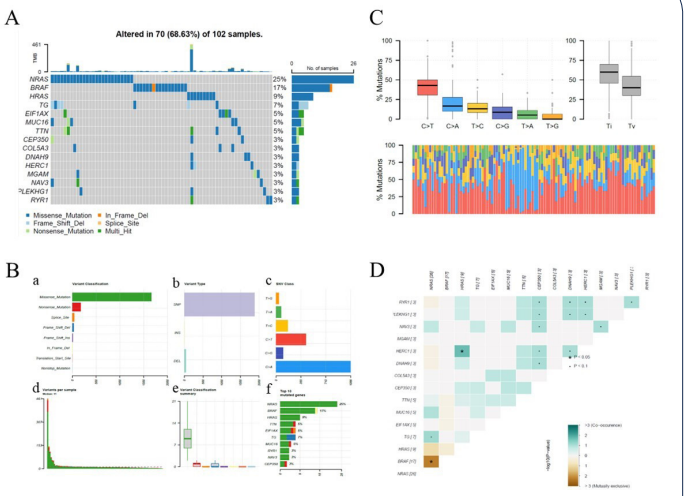

Figure 5: Landscape of somatic mutation profiles in TCGA-THCA of FVPTC samples. **A** The mutation information of each gene in each sample was shown in the waterfall plot. **B** Cohort summary plot displaying the distribution of variants according to variant classification, type, and SNV class. **C** Transition and transversion plot displaying the distribution of SNVs in FVPTC classified into six transition and transversion events. **D** The coincident and exclusive associations across mutated genes.

Figure 2 shows that the frequency of *BRAF* mutations followed that of *RAS* mutations in FVPTC. *BRAF* is one of the three isoforms of *RAF*, which has activating missense point mutations clustered in the kinase domain (exons 11 and 15) [47,48]. The c.T1799A is the most commonly detected mutation in PTC, resulting in a valine-to-glutamic acid amino acid substitution (*BRAFV600E*). This constitutive activation of *BRAF* kinase may play a role in initiating tumorigenesis of FVPTC (Figure 5) [49]. Our *BRAF* mutation results in different subgroups (Figure 3) indicate that the frequency of *BRAF* mutations was lowest in NIFTP (rate: 3.4%; 95% CI: 0.7%-7.3%), and highest in IFVPTC (rate: 31.0%; 95% CI: 23.8%-38.6%). The higher the frequency of *BRAF* mutation, the more aggressive the histological subtype of FVPTC.

The occurrence and development of FVPTC are also associated with three other mutated genes: *TERT*, *RET-PTC*, and *PAX8-*

PPARG. Telomerase, a ribonucleoprotein complex that maintains the length of telomeres at the end of chromosomes, plays a vital role in cellular immortality and tumorigenesis [50,51]. The C228T and C250T *TERT* promoter mutations were detected in follicular-derived thyroid cancers, but they were not present in benign or medullary thyroid cancers [23,52]. Translocation t(2;3) (q13;p25) that causes the fusion of the DNA-binding domain of the thyroid transcription factor *PAX8* to domains A to F of the peroxisome Proliferator-Activated Receptor (*PPAR*) [53]. The *RET* gene encodes a transmembrane Receptor Tyrosine Kinase (RTK) that is involved in numerous cellular mechanisms. Its extracellular domain features four repeats of approximately 110 amino acids, which bear similarities to cadherins. The loss of these genes promotes genetic instability and is an early event in the carcinogenesis of FVPTC.

There are multiple clinicopathological risk factors associated with the recurrence of thyroid cancer. Among them are particular histologic variations, such as the tall cell variant, substantial tumor size, the presence of lymph node metastasis, extrathyroidal extension, and distant metastasis; all of which are tumor-related factors [54,55]. Traditional staging methods are not adequate for assessing recurrence, and recurrent thyroid cancer requires additional therapy and more effective clinical management strategies. This will have a significant impact on the quality of life of patients [56]. Understanding somatic mutations in patients with FVPTC may aid in prognostic risk stratification. Some hospitals recommend the use of targeted next-generation sequencing techniques to identify thyroid cancer in postoperative tissues as part of determining a patient's prognosis. Our systematic review indicates that for FVPTC patients who exclusively harbor *RAS* mutations without *BRAF* or other mutations which are related to malignant prognosis, ultrasound monitoring and regular follow-up can be adopted to avoid irreparable damage from overtreatment, such as direct surgical removal of thyroid tissue. This approach is beneficial in terms of preserving medical resources and reducing the medical burden on patients. Although we employed various subgroup analysis methods, including tumor preservation conditions, ethnicity, gene test methods, centers, and quality score, to address the heterogeneity of the meta-analysis concerning frequency, challenges still remained. In contrast, clinical feature-related meta-analysis showed no heterogeneity across studies, except for lymph node metastases in *BRAF* mutation. This finding is a significant result of our study since it underscores the need for a more detailed understanding of the specific roles of different mutations in the disease for effective medical treatment. It also highlights the importance of prioritizing *BRAF* mutation testing over *RAS* mutation testing in FVPTC patients.

Our meta-analysis has some limitations. The research lacked comprehensive clinical information on treatment techniques and outcomes for patients. We used eligibility criteria to identify baseline patient features, but we could not determine whether they underwent surgical or medicinal treatment. Additionally, despite various subgroup analyses to address this limitation, the heterogeneity of studies regarding mutation frequency is a noteworthy aspect of our meta-analysis. In contrast, we observed no heterogeneity across studies in relation to clinical features, except for LNM in BRAF mutation. This finding is significant since patients with BRAF mutations are found to be at a higher risk of experiencing poor clinical outcomes, unlike RAS mutations which do not show such an association. Another disadvantage of the study is that some of the research used older techniques for monitoring gene alterations, such as Sanger sequencing and pyrosequencing, which only identify 5% to 25% of mutant alleles.

In summary, this study established a somatic mutational landscape for FVPTC. The evidence suggests that FVPTC patients with *BRAF* mutations but not *RAS* mutations have an elevated likelihood of poor clinical characteristics. With its huge sample size, this study can be used as a reference and guidance for the development of therapeutically targeted treatment medications, as well as for inclusion in corresponding sequencing panels that physicians and healthcare regulatory bodies may use.

Declarations

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Data availability: All data used in this study are publicly available summary-level data, with the relevant studies cited. Data that support the findings of our study are available on request from the corresponding author.

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