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Integrated clinicopathological and molecular profiling of papillary thyroid carcinoma identifies predominant *BRAF* and *TERT* promoter mutations: a cross-sectional study

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Key words: papillary thyroid carcinoma; *BRAF* mutation; *TERT* promoter mutation; body mass index; stress.

Abstract.

Papillary Thyroid Carcinoma (PTC) exhibits diverse genetic alterations that influence tumor behavior and clinical outcomes. This study aimed to characterize the spectrum of somatic mutations in PTC using targeted Next-Generation Sequencing (NGS) and to evaluate their associations with clinicopathological, metabolic, and stress-related factors. A Prospective study was conducted on 45 patients newly diagnosed with PTC who underwent total thyroidectomy between December 2024 and February 2025. Formalin-Fixed, Paraffin-Embedded (FFPE) tumor tissues were analyzed using a targeted NGS panel encompassing 35 PTC-related genes. Bioinformatics analysis was performed using Genome Analysis Toolkit (GATK) and ANNOtate VARiation (ANNOVAR) for variant detection and annotation. Clinicopathological variables age, sex, Body Mass Index (BMI), tumor size, laterality, thyroid ultrasound findings, Thyroid Imaging Reporting and Data System (TI-RADS) level and stress exposure were statistically

correlated with detected mutations using the chi-square test; a $p < 0.05$ value was considered as significant. All patients exhibited at least one genetic alteration, with 171 somatic mutations identified (average 3.8 per patient). The most frequent mutation was *BRAF V600E* (73.3%), followed by *TERT* promoter (28.9%), *MET* (24.4%), *NRAS* (22.2%), and *DICER1* (20%). *BRAF V600E* mutations were significantly more common in tumors ≤ 1.2 cm ($p = 0.037$) and in right-lobe lesions ($p = 0.049$). *RAS* mutations correlated with smaller tumor size ($p = 0.021$), while *TERT* mutations were associated with stress exposure ($p = 0.001$). *BRAF*, *RAS*, and *TERT* mutations were also significantly more frequent among overweight and obese patients ($p = 0.047$, 0.046, and 0.040, respectively). Age-wise, *BRAF* and *RAS* mutations predominated in younger patients (≤ 40 years), whereas *TERT* mutations occurred unexpectedly more often in this group. *BRAF V600E* and *TERT* promoter mutations represent the dominant molecular events in PTC and demonstrate significant associations with tumor size, BMI, stress status, and patient age, suggesting that both metabolic and psychosocial factors may influence thyroid tumorigenesis.

Introduction

Thyroid cancer is the most prevalent endocrine malignancy and one of the fastest-growing cancers worldwide, with Papillary Thyroid Carcinoma (PTC) representing nearly 80–85% of all thyroid malignancies.[1,2] Globally, thyroid cancer ranks as the ninth most common malignancy, with an estimated 821,173 new cases reported in 2022.[3] In the United States, the American Cancer Society estimated approximately 44,020 new thyroid cancer cases and 2,170 deaths in 2024, reflecting a continuing rise in incidence, particularly among women and younger individuals.[4] At the regional level, epidemiological data from 2013 to 2019 indicated 97 newly diagnosed thyroid cancer cases in Erbil and 22 in Duhok, highlighting the regional burden of the disease within the Kurdistan Region.[5]

Although PTC generally follows an indolent clinical course, a subset of cases exhibits aggressive behavior, including extrathyroidal extension, recurrence, and distant metastasis.[6] This variability

in clinical outcome reflects the molecular heterogeneity of PTC, which is largely driven by genetic alterations affecting key signaling pathways, most notably the Mitogen-Activated Protein Kinase (MAPK) and phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathways.[7] The implementation of Next-Generation Sequencing (NGS) has enabled comprehensive detection of somatic mutations in PTC, facilitating the identification of oncogenic drivers and co-occurring genetic events. Prior studies have identified *BRAF V600E* as the most prevalent mutation, together with recurrent alterations in *RAS* genes, the *TERT* promoter, and TP53, many of which are associated with adverse clinicopathological features.[8,9] Notably, co-occurrence of *BRAF V600E* and *TERT* promoter mutations has been linked to particularly aggressive disease phenotypes.[10]

In recent years, more evidence has highlighted metabolic dysregulation and psychosocial stress as factors in thyroid carcinogenesis. Non-genetic modifiers, beyond genetic alterations, may influence the development and progression of PTC. Several epidemiological studies show that increased Body Mass Index (BMI) is linked with higher thyroid cancer risk and more aggressive tumors and obesity and insulin resistance are associated with chronic inflammation, oxidative stress, and alterations in growth factor signaling.[11,12] These factors can promote DNA damage, genomic instability, and abnormal activation of cancer pathways in thyroid follicular cells.[11,12] Previous reports suggest that high BMI may be associated with increased BRAF mutations in papillary thyroid carcinoma, suggesting that adiposity may influence tumor mutations.[12,13] In addition to adiposity, chronic psychological stress has also been implicated in cancer progression through activation of the hypothalamic-pituitary-adrenal axis and sustained inflammatory responses, which may impair DNA repair mechanisms and promote mutation accumulation.[14] However, despite these insights, data examining the relationship between stress exposure and somatic mutation patterns in PTC remain limited. The present study aimed to characterize the somatic mutational landscape of PTC using targeted next-generation sequencing and to investigate its associations with clinicopathological features, body mass index, and stress-related factors. All cited references were thoroughly reviewed and their eligibility verified.[15]

Materials and Methods

Study design and population

This cross-sectional prospective study included 45 patients newly diagnosed with PTC. All patients underwent total thyroidectomy at the Smart Health Tower Center, a single private-sector hospital in the Kurdistan Region of Iraq that collaborates with the University of Sulaimani on clinical and research activities.

Inclusion and exclusion criteria

Patients were eligible for inclusion if they had a histopathologically confirmed diagnosis of PTC, underwent total thyroidectomy during the study period, and had available Formal Fixed, Paraffin-Embedded (FFPE) tumor tissue suitable for molecular analysis. Only cases with complete clinicopathological data, including demographic, biochemical, and tumor-related parameters, were enrolled. Patients were excluded if they had any other histological type of thyroid cancer, presented with distant metastasis at the time of diagnosis, or had insufficient or poor-quality FFPE samples that failed DNA extraction or sequencing quality control. Individuals with recurrent thyroid cancer, previous thyroid surgery, radiotherapy, or systemic therapy were also excluded, as well as cases with incomplete medical records that could not be used for statistical evaluation.

Sample collection

Molecular testing was performed on surgical specimens preserved as FFPE tissue samples. The diagnosis of PTC in each FFPE specimen was independently confirmed by two expert pathologists to ensure diagnostic accuracy. FFPE tissue blocks were collected for analysis from surgical specimens obtained between December 2024 and February 2025.

Clinical and pathological data collection

Comprehensive clinicopathological data were collected for all patients, including age, sex, BMI, stress status, tumor size, tumor laterality, ultrasound findings, Thyroid Imaging Reporting and Data System (TI-RADS) level, and Bethesda system classification. For analytical purposes, patients were categorized into two age groups: ≤41 years and >41 years. Stress exposure was

assessed using a structured questionnaire administered at the time of clinical evaluation. Participants were asked about recent and chronic stress-related experiences, and responses were categorized based on predefined criteria.

Ethical considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee of the College of Medicine, University of Sulaimani (Approval No. 352; Meeting No. 25; dated 23 October 2024). All procedures were conducted in accordance with the ethical standards of the institutional and national research committees.

Patient consent

Written informed consent was obtained from all participants prior to their inclusion in the study. Each participant was informed about the study objectives, procedures, and confidentiality of their data. Consent was obtained before sample collection and molecular testing. All data were anonymized, and the study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Next generation sequencing

DNA extraction was performed using the ReliaPrep™ FFPE gDNA Miniprep System (Promega Co. Ltd., Madison, Wisconsin, USA). DNA concentration was measured with a Quantus™ Fluorometer (Promega Co. Ltd., Madison, Wisconsin, USA). Sequencing libraries were generated using the ThermoFisher PTC multigene panel, following the manufacturer's recommended protocol. The sequencing panel targeted 35 genes (*BRAF*, *TPO*, *TP53*, *RET*, *PTEN*, *MEN1*, *APC*, *FGFR1*, *FGFR2*, *FGFR3*, *KIT*, *KRAS*, *HRAS*, *NRAS*, *CTNNB1*, *PIK3CA*, *GNAS*, *ATM*, *PRKAR1A*, *CHEK2*, *MET*, *AKT1*, *FLT3*, *ALK*, *CREBBP*, *TERT*, *WRN*, *DICER1*, *RARA*, *CD44*, *THADA*, *ROS1*, *SPOP*, *FOXA2* and *FOXE1*) that are closely associated with PTC, including point mutations and insertions/deletions. A portion of the extracted DNA was used for multiplex PCR to amplify the target regions. Each amplified library was then subjected to PCR

to attach a unique index and universal adapter. Library quality was analyzed utilizing the LabChip GX Touch24 system (PerkinElmer, Waltham, Massachusetts, USA) to assess fragment size distribution and integrity. Subsequently, the qualified libraries were subjected to 200 bp paired-end read (Ion 540^{TM} Chip) sequencing using the Ion GeneStudio S5 Sequencer (ThermoFisher Scientific, Waltham, Massachusetts, USA).

Bioinformatics

Raw NGS data were processed using a standard bioinformatics pipeline. Quality control was performed with FastQC (v0.11.9 – Babraham Bioinformatics, Cambridge**,** UK), and reads were trimmed using Trimmomatic (v0.39 – Usadel Lab, RWTH Aachen University, Aachen, Germany). Cleaned reads were aligned to the human reference genome (GRCh38) using Burrows–Wheeler Aligner-Maximal Exact Match (BWA-MEM)(v0.7.17 – Heng Li, Broad Institute, Cambridge, Massachusetts, USA). Variant calling was performed with the Genome Analysis Toolkit (GATK) HaplotypeCaller tool (v4.4.0.0 – Broad Institute, Cambridge, Massachusetts, USA), and variants were functionally annotated using ANNOtate VARiation (ANNOVAR) (Release June 2023 – Wang Genomics Lab, University of Pennsylvania, Philadelphia, USA). Annotation references included ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), COSMIC (https://cancer.sanger.ac.uk/cosmic), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), and gnomAD (https://gnomad.broadinstitute.org/). Only exonic and splice-site variants with potential functional effects were retained. Variants were classified according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines and filtered for known PTC-related genes.

Statistical analysis

Data was obtained from patient medical records and structured questionnaires. All data were entered, coded, and organized using Microsoft Excel Version 2017 (Microsoft Corporation, Redmond, WA, USA). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) Version 26 (IBM Corp., Armonk, NY, USA). Each patient sample represented a single independent case; therefore, technical or biological replicates were not

applicable. Data were presented as frequencies and percentages. The relationships between clinicopathological variables and gene mutations were assessed using the χ^2 (Chi-square) test. When more than 20% of the cells in a contingency table had an expected count of ≤5, Fisher's exact test was applied to ensure statistical validity. Statistical significance was defined as $p < 0.05$.

Results

Clinical and pathological characteristics of the study population

Forty-five non-metastatic patients with PTC were enrolled in the study. The patients were aged between 18 and 78 years, with a mean age of 46 years. Twenty-three patients (51.1%) were aged ≤40 years, while 22 patients (48.9%) were older than 40 years. Most patients were women (35 cases, 77.8%), resulting in a woman-to-man ratio of 3.5:1. Tumor laterality showed right-sided tumors in 19 cases (42.2%) and left-sided tumors in 26 cases (57.8%). Ultrasound imaging revealed that most patients (37 - 82.2%) fell into the high-suspicion category for PTC. Cytological analysis based on the Bethesda System confirmed 26 (57.8%) cases as malignant (Category VI) and 12 (26.7%) cases as suspicious for malignancy (Category V) (Table 1).

Somatic mutation profile identified by targeted NGS

A targeted 35-gene NGS panel was used to identify somatic mutations in PTC tumor samples. At least one genetic alteration was detected in every patient, yielding a total of 171 mutations across 45 cases, with an average of 3.8 mutations per patient. The most frequent alteration was *BRAF V600E*, identified in 33 cases (73.3%), followed by *TERT* promoter mutations in 13 cases (28.9%), *MET* in 11 cases (24.4%), NRAS in 10 cases (22.2%), and *DICER1* in nine cases (20%). Other recurrently mutated genes included *ALK* (17.8%), *ROS1* (15.6%), *CHEK2* (13.3%), *APC* (13.3%), and *PTEN* (11.1%). The prevalence of mutations in other genes is presented in Figure 1.

Association between genetic alterations and clinicopathological variables

BRAF V600E mutations were more frequent among women (25/33, 75.8%) compared to men (8/33, 24.2%); however, there was no significant difference between *BRAF V600E* mutation and sex. RAS mutations were significantly higher in men (8/15, 53.3%) than in women (7/15, 46.7%; $p < 0.001$). Conversely, *TERT* promoter mutations were more common in women (7/13, 53.9%) than in men (6/13, 46.1%; $p = 0.022$).

With regard to age, *BRAF V600E* mutations predominated in patients aged ≤ 40 years (20/33, 60.6%) compared to those > 40 years (13/33, 39.4%; $p = 0.037$). Similarly, RAS mutations were more frequent in younger patients (12/15, 80%) than older ones (3/15, 20%; $p = 0.007$). *TERT* promoter mutations also showed a higher occurrence in younger individuals (10/13, 76.9%) compared with older patients (3/13, 23.1%; $p = 0.029$). Regarding stress exposure, *TERT* mutations were significantly more common in patients reporting stress ($p = 0.001$). In terms of BMI, *BRAF V600E*, *RAS*, and *TERT* mutations demonstrated significant associations ($p = 0.047$, $p = 0.046$, and $p = 0.040$, respectively), being more frequent in obese and overweight individuals. *BRAF V600E* mutations were significantly more prevalent in tumors ≤ 1.2 cm (17/33) than > 1.2 cm (16/33; $p = 0.037$). *RAS* mutations also showed a significant association with smaller tumor size ($p = 0.021$). Laterality analysis revealed that *BRAF V600E* mutations were more frequent in right-lobe tumors (22/33) than left-lobe tumors (11/33; $p = 0.049$). However, according to the Bethesda system, *BRAF V600E* mutations were significantly associated with categories indicative of malignancy ($p = 0.017$) (Table 2).

Discussion

This study provides a comprehensive molecular and clinicopathological analysis of 45 patients with PTC using a targeted NGS panel. The sequencing approach enabled the detection of mutations across 35 PTC-related genes, reflecting the well-recognized molecular heterogeneity of the disease.

The mean age in this cohort (46 years) aligns with previously published data on PTC, where patient age typically ranges from the early to late 40s. Similar studies have reported median ages

of 44 and 45 years in large patient populations, confirming that PTC is most diagnosed in middle-aged adults.[16,17]

In this study, the woman-to-man ratio was approximately 3.5:1 (77.8% women), consistent with the well-established women predominance in PTC. Previous reports describe a ratio of about 3:1 in middle-aged adults, with higher ratios around 4:1 observed in younger patients, small papillary tumors, and during the reproductive age period.[18-20] Therefore, the sex distribution in our cohort aligns with published data.

The 36-gene targeted assay detected at least one mutation in all patients, with 171 somatic alterations identified mean 3.8 per tumor, which is consistent with the upper range of mutation burdens reported for PTC.[21,22]

In our cohort, *BRAF V600E* was the most frequent alteration (73.3%), consistent with its role as the dominant driver mutation in adult PTC, with reported frequencies ranging from ~30% to 88% depending on population and tumor characteristics.[23] *BRAF V600E* activates MAPK signaling and is associated with aggressive features, including extrathyroidal extension, lymph node metastasis, and radioiodine resistance, particularly when co-occurring with *TERT* promoter mutations, which together constitute a high-risk molecular signature in PTC.[24]

TERT promoter mutations were identified in 13/45 cases (28.9%), a rate higher than that reported in most unselected PTC cohorts (<5–10%), where these alterations are typically associated with older age, extrathyroidal extension, and advanced stage.[25] *TERT* mutations promote telomerase reactivation and synergize with *BRAF V600E* via MAPK/ETS-mediated transcription, contributing to aggressive tumor behavior and poorer outcomes.[24] The elevated prevalence observed here likely reflects enrichment for clinically high-risk PTC rather than indolent disease. Mutations in other MAPK/RAS pathway genes were also frequent.

NRAS alterations were detected in 10/45 cases (22.2%). Although *RAS* mutations are typically associated with follicular-patterned and more differentiated thyroid tumors, they also occur in PTC, particularly the follicular variant.[26] The coexistence of BRAF- and RAS-driven tumors in this cohort underscores the molecular heterogeneity of PTC and likely reflects inclusion of different histologic subtypes.

BRAF V600E is among the most common alterations in PTC, occurring in approximately 50–80% of cases across populations. While many studies report no significant sex-based difference, others have observed a slightly higher prevalence in women, consistent with our findings (75.8% in women), likely reflecting the overall predominance of PTC in women. Several reports indicate that *BRAF V600E* is more frequent in younger patients, peaking before 40 years of age, in line with our observation of a 60.6% prevalence in individuals ≤40 years.[27-29]

TERT promoter mutations occur less frequently but are key prognostic markers for aggressiveness and recurrence. These mutations typically increase with age and are more frequent in advanced or poorly differentiated carcinomas. However, some recent data highlight variable sex distribution, with certain cohorts showing a slightly higher frequency in women, paralleling the study's observation (53.9% in women). Interestingly, while *TERT* mutations are generally age-related, the reported predominance in younger patients (76.9%) may indicate a distinct subset of aggressive early-onset tumors co-harboring BRAF or RAS lesions.[30,31]

Stress exposure was found to be significantly associated with a higher frequency of *TERT* promoter mutations in patients with PTC ($p = 0.001$). This observation is consistent with previous studies indicating that *TERT* promoter mutations are linked to aggressive clinicopathological features and poor prognosis in PTC patients. Chronic psychological stress has been shown to drive hormonal and oxidative changes, activating the hypothalamic-pituitary-adrenal axis and increasing reactive oxygen species, which may induce genomic instability and facilitate oncogenic mutations such as those in the *TERT* promoter. The present findings add to growing evidence that stress-related biological pathways can influence tumor biology by promoting genetic alterations that enhance telomerase activity and support cancer cell survival.[32-36] Although this association is preliminary, it highlights a possible biological interaction between stress and genetic alterations in PTC that warrants further investigation.

In this study, *BRAF*, *RAS*, and *TERT* mutations were significantly more frequent among overweight and obese patients with PTC ($p = 0.047$, $p = 0.046$, and $p = 0.040$, respectively). This finding supports prior evidence that excess adiposity contributes to thyroid carcinogenesis through metabolic, hormonal, and inflammatory mechanisms. Several studies have reported that obesity is associated with a greater risk of *BRAF V600E*–mutated PTC, implying that metabolic dysregulation and chronic low-grade inflammation may create a genetic milieu favoring

oncogenic mutations.[12] *RAS* and *TERT* mutations, similarly, have been linked to advanced disease features and worse outcomes, and their coexistence with *BRAF* alterations further amplifies tumor aggressiveness.[37,38] The interplay between adipokines, insulin resistance, and oxidative stress in obese individuals may enhance DNA damage and telomerase activation, providing a potential explanation for the observed molecular associations.[12,37,38] Thus, the clustering of *BRAF*, *RAS*, and *TERT* mutations in individuals with higher BMI underscores the role of obesity not only as a clinical risk factor but also as a modulator of the molecular landscape in PTC.

In the present study, the observation that *BRAF* mutations were significantly more prevalent in smaller tumors (≤1.2 cm; 17/33) compared to larger ones (>1.2 cm; 16/33; $p = 0.036$), and that *RAS* mutations were similarly associated with smaller tumor size ($p = 0.019$), aligns with certain strands of the published literature but contrasts with others. Some reports, including studies by Semsar-Kazerooni *et al*., indicate that *BRAF* positive tumors tend to be smaller than RAS-like positive tumors, suggesting a molecular signature independent of gross tumor size.[39] Similarly, Vianello *et al*. found that smaller thyroid cancers exhibited a higher frequency of *BRAF* mutations, whereas *RAS* mutations were comparatively less common in larger lesions.[40] However, other research, such as the large-scale analysis by Cong *et al*., reported that *BRAF* mutations were instead associated with larger tumors and features of aggressiveness, including extrathyroidal extension and multifocality.[23] Furthermore, contrasting studies have described either null or inverse associations between *BRAF* and tumor size due to methodological and population-based differences.[41] Thus, while the current finding supports literature indicating a higher *BRAF* prevalence among microcarcinomas, the broader evidence remains heterogeneous, reflecting variability across patient cohorts and detection methods.

This study has provided new information on the molecular characteristics of PTC. Gene alterations and their clinicopathological associations were identified using next-generation sequencing, offering a broader understanding than previously reported studies.[42] Data from the Kurdistan Region of Iraq have been limited to histopathological and clinical observations, which reported a predominance of PTC among thyroid malignancies and higher incidence in women.[43,44] Therefore, the present study adds novel regional molecular data and highlights the importance of genetic profiling in improving diagnosis and personalized management of PTC.

This study has several limitations that should be acknowledged. First, the sample size was relatively small, which may limit the statistical power to detect subtle associations between genetic mutations and clinicopathological features. Second, stress exposure and lifestyle factors such as body mass index were based on self-reported data, which may introduce recall or reporting bias. Third, the molecular analysis was limited to a targeted 35-gene panel; therefore, other potentially relevant genetic or epigenetic alterations influencing PTC pathogenesis might not have been captured. In addition, the study did not assess serum or tissue biomarkers of oxidative stress or telomerase activity, which could have provided mechanistic insight into the observed associations, particularly the link between stress exposure and *TERT* promoter mutations. Finally, as this was a single-center study, the findings may not be generalizable to broader or ethnically diverse populations.

Conclusions

In conclusion, this study confirms that *BRAF V600E* is the most prevalent genetic alteration in papillary thyroid carcinoma, followed by *TERT* promoter, *RAS*, and *MET* mutations, underscoring the molecular heterogeneity of PTC. Significant associations between these genetic alterations and clinicopathological characteristics were identified, suggesting that metabolic and psychosocial factors may contribute to shaping tumor biology. Notably, the relatively high frequency of *TERT* promoter mutations in younger and stressed individuals highlights a potentially distinct molecular subset and supports the need for further investigation into the interaction between environmental influences and molecular drivers in thyroid carcinogenesis. Larger, multi-center studies are recommended to validate these findings and clarify their clinical implications.

References

1- Liu J, Li H, Du R, et al. Targeted next-generation sequencing in papillary thyroid carcinoma of Chinese Han population. J Clin Oncol 2019;37:e17574.

2- Bonjoc KJ, Young H, Warner S, et al. Thyroid cancer diagnosis in the era of precision imaging. J Thorac Dis 2020;12:5128.
3- Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74:229-63.
4- Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2024. CA Cancer J Clin 2024;74:7–33.
5- Karwan M, Abdullah OS, Amin AM, et al. Cancer incidence in the Kurdistan region of Iraq: results of a seven-year cancer registration in Erbil and Duhok governorates. Asian Pac J Cancer Prev 2022;23:601.
6- Zhang J, Xu S. High aggressiveness of papillary thyroid cancer: from clinical evidence to regulatory cellular networks. Cell Death Discov 2024;10:378.
7- Du Y, Zhang S, Zhang G, Hu J, et al. Mutational profiling of Chinese patients with thyroid cancer. Front Endocrinol 2023;14:1156999.
8- Shi D, Yao M, Wu D, Jiang M, et al. Detection of genetic mutations in 855 cases of papillary thyroid carcinoma by next generation sequencing and its clinicopathological features. Diagnostic Pathol 2024;19:146.
9- Wang Q, Zhao N, Zhang J. Gene mutation analysis in papillary thyroid carcinoma using a multi-gene panel in China. Int J Gen Med 2021;14:5139-48.
10- Mady LJ, Grimes MC, Khan NI, et al. Molecular profile of locally aggressive well differentiated thyroid cancers. Sci Rep 2020;10:8031.
11- Zhang BT, Guo M, Yang LR, et al. Mechanistic aspects of inflammation and oxidative stress and their association with thyroid cancer risk. Cancer Med 2025;14:e71030.
12- Park H, Heo J, Ryu HJ, et al. The association between BMI and BRAFV600E mutation may differ by primary tumor size. Eur Thyroid J 2024;13:e230255.
13- Jin L, Chen E, Dong S, et al. BRAF and TERT promoter mutations in the aggressiveness of papillary thyroid carcinoma: a study of 653 patients. Oncotarget 2016;7:18346.
14- Akay GG. Telomeres and psychological stress: perspective on psychopathologies. Noro Psikiyatr Ars 2022;59:330.
15- Abdullah HO, Abdalla BA, Kakamad FH, et al. Predatory publishing lists: a review on the ongoing battle against fraudulent actions. BMJ 2024;2:26-30.

16- Adam MA, Thomas S, Hyslop T, et al. Exploring the relationship between patient age and cancer-specific survival in papillary thyroid cancer: rethinking current staging systems. J Clin Oncol 2016;34:4415-20.
17- Huang H, Xu S, Wang X, et al. Patient age is significantly related to distant metastasis of papillary thyroid microcarcinoma. Front Endocrinol 2021;12:748238.
18- Limaiem F, Rehman A, Mazzoni T. Papillary Thyroid Carcinoma. (Updated 2024). In: StatPearls (Internet). Treasure Island (FL): StatPearls Publishing; 2025.
19- LeClair K, Bell KJ, Furuya-Kanamori L, et al. Evaluation of gender inequity in thyroid cancer diagnosis: differences by sex in US thyroid cancer incidence compared with a meta-analysis of subclinical thyroid cancer rates at autopsy. JAMA Intern Med 2021;181:1351-8.
20- Moleti M, Sturniolo G, Di Mauro M, et al. Female reproductive factors and differentiated thyroid cancer. Front Endocrinol 2017;8:111.
21- Lu Z, Zhang Y, Feng D, et al. Targeted next generation sequencing identifies somatic mutations and gene fusions in papillary thyroid carcinoma. Oncotarget 2017;8:45784.
22- Chauhan A, Sen S, Amin K, Burmeister LA. Papillary Thyroid Carcinoma with 5 unique point mutations and typical behavior. JCEM Case Rep 2025;3:luaf062.
23- Cong R, Ouyang H, Zhou D et al. BRAF V600E mutation in thyroid carcinoma: a large-scale study in Han Chinese population. World J Surg Oncol 2024;22:259.
24- Nhung NT, Mussazhanova Z, Kurohama H, et al. BRAF V600E and TERT promoter mutations and their impact on recurrent papillary thyroid carcinoma progression. Endocr Connect 2025;14:e250116.
25- Kim SY, Kim T, Kim K, et al. Highly prevalent BRAF V600E and low-frequency TERT promoter mutations underlie papillary thyroid carcinoma in Koreans. J Pathol Transl Med 2020;54:310-7.
26- Perou C. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014;159:676-90.
27- Huang J, Wang J, Xv J, et al. Genetic alterations and allele frequency of BRAF V600E and TERT mutation in papillary thyroid carcinoma with intermediate-to-high recurrence risk: a retrospective study. Clin Exp Med 2024;24:76.

28- Brumfield A, Azar SA, Nordgren R, et al. Prevalence and clinical impact of BRAF p. V600E mutation in papillary thyroid carcinoma. Endocr Pathol 2025;36:1-4.
29- Bandoh N, Goto T, Kato Y, et al. BRAF V600E mutation co-existing with oncogenic mutations is associated with aggressive clinicopathologic features and poor prognosis in papillary thyroid carcinoma. Asian J Surg 2024;47:413-9.
30- Yang H, Park H, Ryu HJ, et al. Frequency of TERT promoter mutations in real-world analysis of 2,092 thyroid carcinoma patients. Endocrinol Metab 2022;37:652-63.
31- Melo M, da Rocha AG, Vinagre J, et al. TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. J Clin Endocrinol Metab 2014;99:E754-65.
32- Amen AM, Fellmann C, Soczek KM, et al. Cancer-specific loss of TERT activation sensitizes glioblastoma to DNA damage. Proc Natl Acad Sci 2021;118:e2008772118.
33- Muzza M, Pogliaghi G, Colombo C, et al. Extra-nuclear TERT counteracts oxidative stress and promotes progression in papillary thyroid carcinoma. Transl Res 2024;271:1-2.
34- Kyriacou A, Tziaferi V, Toumba M. Stress, thyroid dysregulation, and thyroid cancer in children and adolescents: proposed impending mechanisms. Horm Res Paediatr 2023;96:44-53.
35- Lin J, Epel E. Stress and telomere shortening: Insights from cellular mechanisms. Ageing Res Rev 2022;73:101507.
36- Rusinek D, Pfeifer A, Cieslicka M, et al. TERT promoter mutations and their impact on gene expression profile in papillary thyroid carcinoma. Cancers 2020;12:1597.
37- Rahman ST, Pandeya N, Neale RE, et al. Obesity is associated with BRAFV600E-mutated thyroid cancer. Thyroid 2020;30:1518-27.
38- Censi S, Cavedon E, Bertazza L, et al. Frequency and significance of Ras, Tert promoter, and Braf mutations in cytologically indeterminate thyroid nodules: a monocentric case series at a tertiary-level endocrinology unit. Front Endocrinol 2017;8:273.
39- Semsar-Kazerooni K, Morand GB, Payne AE, et al. Mutational status may supersede tumor size in predicting the presence of aggressive pathologic features in well differentiated thyroid cancer. J Otolaryngol Head Neck Surg 2022;51:9.
40- Vianello F, Censi S, Watutantrige-Fernando S, et al. The role of the size in thyroid cancer risk stratification. Sci Rep 2021;11:7303.

41- Wikanta ER, Prajoko YW, Issakh B, Istiadi H, Puspasari D. Relationship between the BRAF V600E and tumor size, lymph node, and distant metastasis in papillary thyroid carcinoma. Russ Open Med J 2022;11:216.
42- Junainah EM. Genetic alterations in papillary thyroid cancer: clinicopathological correlations and diagnostic implications. Folia Histochem Cytobiol 2025;63:113-20.
43- Mustafa DH, Ahmed BS, Haweizy RM, Dewana AM. Evaluation of anaplastic thyroid carcinoma in the Kurdistan region of Iraq. BMC Surg 2022;22:364.
44- Shakor FN, Abdalqader MH, Saeed AH. Histopathology analysis of thyroid cancer tumor size in Sulaymaniyah-Kurdistan Region-Iraq. Iraq Med J 2020;4.52-7.

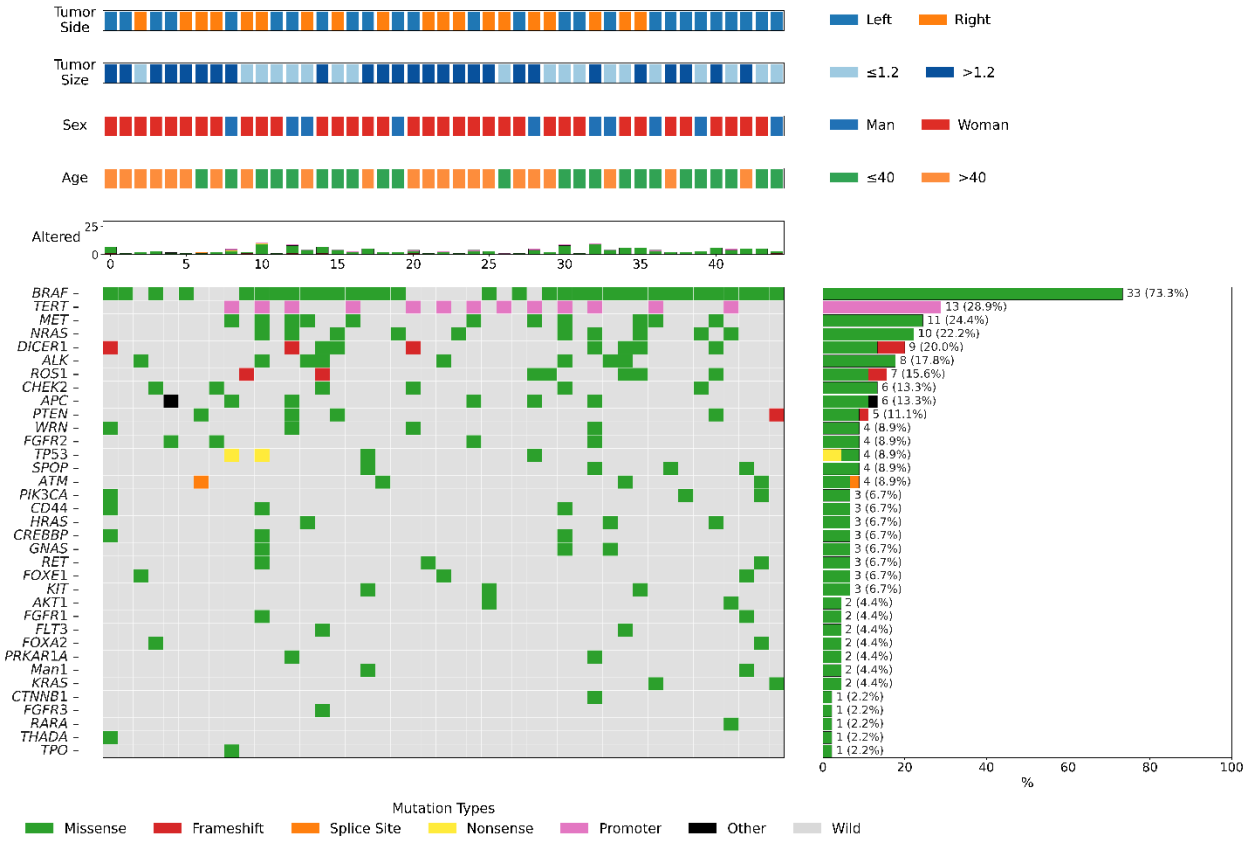

Figure 1. Oncoprint of somatic mutations and clinicopathological features in 45 papillary thyroid carcinoma cases. Columns represent individual patients and rows indicate the 35 analyzed genes. Clinicopathological features included tumor side, tumor size, sex, age. Mutated genes included *BRAF*, *TERT*, *MET*, *NRAS*, *DICER1*, *ALK*, *ROS1*, *CHEK2*, *APC*, *PTEN*, *WRN*, *SPOP*, *TP53*, *FGFR2*, *ATM*, *CD44*, *GNAS*, *RET*, *PIK3CA*, *KIT*, *CREBBP*, *HRAS*, *FOXE1*, *FLT3*, *PRKARIA*, *KRAS*, *MEN1*, *FOXA2*, *AKT1*, *FGFR1*, *TPO*, *THADA*, *RARA*, *CTNNB1*, *FGFR3*.

Table 1. Association between clinicopathological variables and *BRAF V600E*, *RAS*, and *TERT* mutations in 45 PTC patients**.** The table shows mutation and wild-type distribution across variables with corresponding p values.

Variable	N (%)
Sex	
Men	10 (22.2)
Women	35 (77.8)
Age	
≤40	23 (51.1)
>40	22 (48.9)
BMI	
Underweight (Below 18.5)	0 (0)
Healthy weight (18.5-24.9)	16 (35.6)
Overweight (25-29.9)	16 (35.6)
Obese (30 or higher)	13 (28.9)
Stress	
Yes	9 (20)
Occasionally	28 (62.2)
No	8 (17.8)
Tumor size (cm)	
≤1.2	19 (42.2)
>1.2	26 (57.8)
Tumor side	
Right	26 (57.8)
Left	19 (42.2)
Ultrasound Thyroid Gland	
High Suspicion for PTC	37 (82.2)
Intermediate Suspicion for PTC	8 (17.8)
TI-RADS level	
TR3	5 (11.1)

TR4	22 (48.9)
TR5	18 (40)
Bethesda system Classification	
AUS or FLUS	2 (4.4)
SN FN/SFN	5 (11.1)
SM	12 (26.7)
Malignancy	26 (57.8)

BMI, Body Mass Index; PTC, Papillary Thyroid Cancer; TI-RADS, Thyroid Imaging Reporting and Data System; AUS, Atypia of Undetermined Significance; FLUS, Follicular Lesion of Undetermined Significance; FN/SFN, Follicular Neoplasm or Suspicious for Follicular Neoplasm; SM, Suspicious for Malignancy.

Table 2. Clinicopathological characteristics of 45 patients with papillary thyroid carcinoma. Variables include sex, age, body mass index, stress, tumor size, tumor side, ultrasound findings, TI-RADS level, and Bethesda classification. Data are presented as percentage.

	BRAF N			RAS N			TERT N		
Variable	Mutation	Wild-type	p value	Mutation	Wild-type	p value	Mutation	Wild-type	p value
Sex									
Men	8	2	0.589	8	2	<0.001	6	4	0.014
Women	25	10		7	28		7	28	
Age									
≤40	20	3	0.035	12	11	0.006	10	13	0.027
>40	13	9		3	19		3	19	

Stress									
Yes	5	4		4	5	0.654	7	2	
	22	6	0.39	8	20		4	24	0.00
Occasionally	6	2	5	3	5		2	6	1
No									
BMI									
Healthy	15	1		9	7		6	10	
weight (18.5-						0.046			0.04
24.9)	11	5	0.04	4	12		1	15	0
Overweight	7	6	7	2	11		6	7	
(25-29.9)									
Obese (30									
or higher)									
Tumor size									
(cm)	17	2	0.03	10	9	0.019	8	11	0.09
≤1.2	16	10	6	5	21		5	21	4
>1.2									
Tumor side									
Right	22	4	0.04	8	18	0.945	8	18	0.74
Left	11	8	5	6	13		5	14	5
Ultrasound									
Thyroid									
Gland	26	11	0.31	11	26	0.270	12	25	0.25
High	7	1	8	4	4		1	7	9
Suspicion for									
PTC									
Intermediate									
Suspicion for									
PTC									
TI-RADS									
level	4	1		1	4		0	5	

TR3 TR4 TR5	18 11	4 7	0.31 7	10 4	12 14	0.240	5 8	17 10	0.10 2
Bethesda system Classification AUS or FLUS FN/SFN SM Malignancy	0 5 11 17	2 0 1 9	0.01 7	0 2 6 7	2 3 6 19	0.379	1 2 4 6	1 3 8 20	0.73 5

BMI, Body Mass Index; PTC, Papillary Thyroid Cancer; TI-RADS, Thyroid Imaging Reporting and Data System; AUS, Atypia of Undetermined Significance; FLUS, Follicular Lesion of Undetermined Significance; FN/SFN, Follicular Neoplasm or Suspicious for Follicular Neoplasm; SM, Suspicious for Malignancy

Contributions: Rawezh Qadir M. Salih was involved in the conception and design of the study, in the literature review and in the writing of the manuscript. Dlnya A. Mohammed was major contribution to the conception of the study, as well as to the literature search for related studies. Nizar M.T. Hamawandi was involved in the literature review, in the study design, in the critical revision of the manuscript and in the processing of the tables. All authors have read and approved the final manuscript.

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Ethics approval and consent to participate ethical approval for this study was obtained from the Institutional Ethics Committee of the College of Medicine, University of Sulaimani (Approval No. 352; dated 23 October 2024). Written informal consent was obtained from the patients for their participation in the present study. Written informal consent was obtained from the patients for the publication of the study and any accompanying images.

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