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Molecular Pathological Markers and *TERT Promoter* Mutations: Correlations with Clinicopathological Features and Distant Metastasis in Turkish Patients with Papillary Thyroid Carcinoma

Ezgi Yılmaz[1]
Gaye Güler[2]
Olcay Kurtulan[2]
Jale Karakaya[3]
Tuğçe Telli[4]
Murat Tuncel[4]
Alper Gürlek[5]

[1]Department of Internal Medicine, Hacettepe University Faculty of Medicine, Ankara, Turkey
[2]Department of Pathology, Hacettepe University Faculty of Medicine, Ankara, Turkey
[3]Department of Biostatistics, Hacettepe University Faculty of Medicine, Ankara, Turkey
[4]Department of Nuclear Medicine, Hacettepe University Faculty of Medicine, Ankara, Turkey
[5]Division of Internal Medicine, Department of Endocrinology and Metabolism, Hacettepe University Faculty of Medicine, Ankara, Turkey

Corresponding author:
Alper Gürlek
✉ agurlek2001@yahoo.com

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ABSTRACT

Objective: Studies concerning the frequency of *BRAF*^{V600E}, *TERT promoter*, and *NRAS* mutations, *RET/PTC* and *PAX8/PPARγ* rearrangements, and their correlations with distant metastasis and several clinicopathological parameters are lacking in Turkish papillary thyroid carcinoma (PTC) patients.

Methods: Mutations were detected by real-time polymerase chain reaction (PCR) from paraffin-embedded tumor tissues obtained from 42 PTC patients (16 with and 26 without distant metastasis, median age 52 years, range 21-80).

Results: The follow-up period was a median of 41.38 (range 1-168) months. A relationship was found between distant metastasis and aggressive histological variant ($P = .008$), capsular ($P = .001$), lymphovascular ($P = .001$) and extrathyroidal invasion ($P = .002$), advanced stage ($P = .002$), and recurrence ($P = .008$). Mortality was greater in the distant-metastatic group ($P = .002$). The frequency of *BRAF*^{V600E} mutation was 67.5% (27/40). Of the *BRAF*^{V600E} mutation-positive group, 22.2% (6/27) had distant metastasis and 77.8% (21/27) had no metastasis ($P = .006$). No significant difference existed between *BRAF*^{V600E} mutation-positive and -negative groups concerning the clinicopathological features. The mortality rate was higher in the *BRAF* mutation-negative group compared with the positive group ($P = .03$). The frequency of *TERT* mutation (all at position C228T) was 9.5% (4/42), showing no correlation with any clinicopathological feature. *NRAS* mutation and *PAX8/PPARγ* rearrangement were not observed. *RET/PTC* gene rearrangement was detected in only 2 patients.

Conclusion: Our findings suggest that molecular changes, contrary to previous observations in different populations, are not related with aggressive behavior and distant metastasis in Turkish PTC patients. Low number of patients and short follow-up, however, might have hindered the ability to draw accurate conclusions regarding molecular markers and poor prognosis in such a slow-growing carcinoma type.

Keywords: Molecular markers, papillary thyroid carcinoma, TERT promoter

Introduction

Papillary thyroid cancer (PTC) is the commonest subtype representing 80%-85% of all thyroid cancers.[1] PTC tends to have a slow biological course and has an excellent prognosis (10 years life expectancy >95%).[1] Distant metastasis is not common in PTC (2%-5%) and is associated with increased mortality. The American Thyroid Association (ATA) guidelines express distant metastasis as a high-risk criterion. Several clinicopathological features (age, large tumor size, vascular invasion, and extrathyroidal spread) have been implicated as risk factors for distant metastasis.[2] The mortality rate in cases with distant metastasis is 70%. Certain genetic traits have been linked with poor prognosis and aggressive behavior for PTC.[2] Elucidation of gene changes or protein expression in thyroid cancer will provide early and aggressive treatment by identifying risky groups for disseminated disease and positively affect life expectancy.[3]

The *BRAF*^{V600E} mutation is the commonest mutation in PTC, and its frequency was reported as 36%-83%.[4] Various studies have been conducted to elucidate the relationship between *BRAF*^{V600E} mutation and distant metastasis, yielding conflicting results. The *RAS* mutation is a mutation that acts through the mitogen-activated protein kinase (MAPK) and phosphatydil inositol 3-kinase-Akt (PI3K-AKT) pathways from the guanosine tri-phosphate (GTP)-binding protein family. It has a role in cellular growth, differentiation, and survival. The *RAS* mutation is a dual activator of the MAPK- and PI3K-AKT-signaling pathways in the pathogenesis

of thyroid cancer.[5] *RAS* mutations are among the other frequently reported ones in PTC. It has been noted that the frequency of RAS mutations ranges between 0% and 20% in PTC and between 17% and 25% in follicular variant of papillary thyroid carcinoma (FVPTC). The *NRAS* mutation is usually associated with follicular and classical variants.[6] There is no consensus in studies examining the relationship between *RAS* mutation and distant metastasis. As far as we know, no study exists examining the prognostic and predictive value of *NRAS* mutation in determining distant metastasis in the Turkish population. The *RAS* mutation is associated with an aggressive phenotype, risk for recurrence, distant metastasis, and death.[7] However, in another study, no relationship has been found between *RAS* mutation and gender, size of the tumor, histological subtype, being multifocal, age, lymph node spread, and tumor, node, metastasis (TNM) stage.[8]

The *TERT promoter* region is a crucial regulator of telomerase activation,[9] and the prognostic value of *TERT promoter* mutation in PTC has been a popular research area in recent years. The *TERT promoter* mutation in thyroid cancer has been shown to be associated with poor prognostic signs (advanced age, tumor size, advanced stage, distant metastasis, no response to treatment, and reduced life expectancy).[10] However, some observations offer the opposite opinion.[11] *RET*/*PTC* gene fusion is seen in 10%-20% of PTC patients. Various frequencies of PAX8/PPARγ rearrangements ranging from 1%-5%[12] to as high as 37%[13] have been reported.

To develop new treatment methods and strategies, reliable predictive molecular markers that detect distant metastasis in PTC cases are needed, and data on this subject are insufficient. Detection of aggressive tumors and distant metastasis with molecular markers is important in determining the initial treatment and the necessity of aggressive treatment that may affect the patient's life expectancy.

Therefore, we sought to examine the frequencies of *BRAF*^{V600E}, *TERT promoter*, and *NRAS* mutations in PTC and as well as the relationships between these mutations and distant metastasis and clinicopathological characteristics. Such a discrete evaluation involving multiple molecular markers has not been performed previously in a Turkish PTC group.

MAIN POINTS

- A thorough evaluation of a cluster of molecular markers and mutations in papillary thyroid carcinoma (PTC) with respect to aggressive clinicopathological features and distant metastasis is lacking in the Turkish population.
- Our results indicate that, contrary to some observations in other populations from different countries, *BRAF* and *TERT promoter* mutations and *RET*/*PTC* gene rearrangement are not related with the aggressive features and distant metastasis, at least as it pertains to the particular patient group we studied.
- The aggressive behavior and potential for causing distant metastasis seem to be differentially modulated by these markers/mutations among patient groups from different ethnic backgrounds and populations. Our results need to be confirmed in larger patient groups from our population.

Materials and Methods

Patients and Clinicopathological Characteristics

Forty-two PTC cases (16 with distant metastasis and 26 without) were retrospectively analyzed. PTC cases diagnosed after age 18 years and followed between 2004 and 2021 were considered for selection. The group with distant metastasis included all patients who had available paraffin blocks, and the group without distant metastasis was randomly selected to match with demographic characteristics of the distant-metastatic group. All of the PTC patients have undergone a total thyroidectomy. According to the World Health Organization (WHO) criteria, the histological diagnosis was confirmed by experienced pathologists. The staging was done as recommended by American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) TNM staging. Demographic (e.g., age, gender, and additional malignancy), histopathological (tumoral size and subvariant, being multifocal, extending beyond the thyroid, lymphovascular invasion and capsular invasion, lymph node metastasis, and metastatic spread to distant organs), postoperative radioactive iodine (RAI) doses, surgical methods applied, recurrence, survival status, and follow-up data were obtained through the University Hospital *Nucleus*® Patient Data Recording System. DNA/RNA extraction of patients was made from the original-site thyroid tissue in 33 cases, metastatic nodes in 6, and tissue from the distant-metastatic site (1 lung and 2 bones) in 3 patients. Aggressive/nonaggressive variant differentiation of PTC cases included in the study was made as to whether they contain tall cell, columnar, or hobnail variants accepted as aggressive variants.

Tumor Tissues and DNA and RNA Isolation

DNA purification was done by nucleic acid isolation kit for paraffin blocks (GeneAll® Exgene™ DNA FFPE Tissue Kit). RNA purification was performed using Qiagen® RNeasy FFPE Kit.

Mutation Analysis

Following polymerase chain reaction (PCR) procedures, mutation was investigated by EntroGen Thyroid Cancer Mutation Detection Kit for *BRAF*^{V600E} and *NRAS* mutations (absent or present). *TERT promoter* mutation analysis was performed by using GenMark *TERT promoter* Mutation Detection Kit (absent or present). *RET*/*PTC* and *PAX8*/*PPARγ* gene fusion analysis was performed using EntroGen Thyroid Cancer Fusion Gene Detection Kit.

Statistical Analysis

IBM Statistical Package for the Social Sciences software version 25.0 (IBM Corp.; Armonk, NY, USA) was used. The relationship between categorical variables was evaluated with the "Pearson's chi-square" or the "Fisher's exact probability test." "Significance test of the difference between the two means" was used when the numerical variables were normally distributed for the 2 groups, and the "Mann–Whitney *U*-test" was used if they are not normally distributed. A *P* below .05 indicated significance.

Ethics Committee Approval

Our research protocol has been approved by the ethical committee of Hacettepe University Faculty of Medicine (registration number: GO 21/569; approval number: 2021/09-08) and adhered to Declaration of Helsinki. Informed consent was obtained from each participant.

Results

The median follow-up period of the cases was 41.38 (range 1-168) months. The median age was 52 (range 21-80) years, and the mean tumor size was 2.25 (SD = 1.25) cm. Distant-organ metastasis was present in 38.1% (n = 16) of the cases, while there was no distant metastasis in 61.9% (n = 26). Clinicopathological characteristics of all patients with PTC are presented in Table 1.

Eight (50%) of 16 patients with distant metastasis had lung metastasis, 5 (31.2%) had bone metastasis, and 3 (18.8%) had lung and bone metastasis. No relationship was found between distant metastases and sex, age at the diagnosis, tumoral size, being multifocal, and lymph node metastasis. An aggressive histological variant, capsular, lymphovascular, and extrathyroidal invasion, advanced stage, and relapsed metastases were detected more frequently in the distant-metastatic group. Comparisons of clinicopathological features of patients with and without distant metastasis are given in Table 2.

In 2 samples, *BRAF* mutation analyses could not be performed due to technical reasons; therefore, the frequency of $BRAF^{V600E}$ mutation was 67.5% (27/40) for the entire group. *TERT promoter* mutation was positive in 9.5% (4/42) of the cases. Mutations *TERT promoter* region were all at position C228T. We did not observe *NRAS* mutation in any case (0/42). $BRAF^{V600E}$ *and TERT promoter* mutation positivity in patients with and without distant metastasis is presented in Table 3. Of the $BRAF^{V600E}$-positive group, 22.2% (6/27) had distant metastasis and 77.8% (21/27) had no distant metastasis ($P = .006$).

As shown in Table 4, no significant differences existed between *BRAF* mutation-positive and -negative groups with respect to gender, age distribution, tumoral size, histologically aggressive subtype, and capsule and lymphovascular invasion, being multifocal, extrathyroidal spread, lymph node metastasis, tumor stage, or recurrence. A significant difference existed between the $BRAF^{V600E}$ mutation-positive and -negative ones in terms of mortality (the mortality was increased in the latter) ($P = .03$).

As shown in Table 5, when the *TERT promoter* mutation-positive group was compared with the negative group, no difference existed with respect to gender, age distribution, capsule/lymphovascular involvement, lymph node spread, tumor stage, as well as recurrence.

As shown in Table 6, the $BRAF^{V600E}$- and *TERT promoter*-positive group was comparable with the one having only one type of mutation in terms of sex, age distribution, tumor size, histologically aggressive subtype distribution, and presence of capsule and lymphovascular invasion, being multifocal, extrathyroidal spread, lymph node metastasis, tumor stage, and recurrence.

RET/PTC gene rearrangement was investigated from paraffin blocks of 42 patients and was found only in 2 patients. *PAX8/PPARγ* rearrangement was not detected in any patient.

Discussion

PTCs are usually slowly progressive tumors with low growth rates and metastases. Nonetheless, a small fraction of PTCs (5%-10%) may exert aggressive behavior, as exemplified by developing distant metastases and possibly causing death.[14] Identifying prognostic markers that can discriminate aggressive PTCs from ones with good prognosis would be important to better manage the patient with appropriate follow-up to avoid overtreatment. As far as we know, we report for

Table 1. Clinicopathological Features of the Entire PTC Group (n = 42)

Feature	n (%)
Gender	
Female	21 (50)
Male	21 (50)
Age (years)	
≤45	14 (33.3)
>45	28 (66.7)
Other malignancy	
Yes	5 (11.9)
No	38 (88.1)
Tumor size (cm)	
>1-≤2	18 (42.9)
>2-≤4	18 (42.9)
>4	6 (14.2)
Histological variants	
Aggressive variants*	7 (16.7)
Other variants	35 (83.3)
Capsule invasion	
Yes	16 (38.1)
No	26 (61.9)
Lymphovascular invasion	
Yes	18 (42.9)
No	24 (57.1)
Multifocality	
Yes	27 (61.9)
No	16 (38.1)
Extrathyroidal invasion	
Yes	24 (57.1)
No	18 (42.9)
Lymph node metastases	
Yes	38 (90.5)
No	4 (9.5)
Distant metastases	
Yes	16 (38.1)
No	26 61.9)
Stage (TNM, AJCC7/AJCC8)	
Stages 1-2	12 (28.5)
Stages 3-4	30 (72.5)
Recurrence	
Yes	7 (16.7)
No	35 (83.3)
Total thyroidectomy	42 (100)
Radioiodine (mCi) (median, range)	150 (150-450)
Follow-up time (months) (median, range)	34 (1-168)
Postoperative Tg (ng/mL) (median, range)	8.3 (0.2-8947)
Mortality	6 (14.3)

Demographic and clinicopathological features of 42 PTC cases are shown.
AJCC7, American Joint Committee on Cancer, seventh edition; AJCC8, American Joint Committee on Cancer, eighth edition; PTC, papillary thyroid carcinoma; Tg, thyroglobulin; TNM, tumor, node, metastasis.
*Tall cell, columnar cell, and hobnail variants were grouped as aggressive variants according to the ATA guidelines.[7]

Table 2. Clinicopathological Features of Patients With and Without Distant Metastasis

Feature	Distant Metastasis, n (%) Present (n = 16)	Absent (n = 26)	*P*
Gender			
Female	5 (33.7)	10 (59.4)	.525
Male	11 (67.3)	6 (40.6)	
Age			
<45	4 (25)	9 (34.6)	.822
≥45	10 (75)	17 (65.4)	
Tumor size (cm)			.259
Mean (SD)	3.26 (SD = 1.76)	2.44 (SD = 1.34)	.096
>1-≤2	4 (25)	8 (30.8)	
>2-≤4	6 (37.5)	15 (57.7)	
>4	6 (37.5)	3 (11.5)	
Histopathological variants			
Aggressive variant*	6 (37.5)	1 (3.8)	**.008**
Nonaggressive variant	10 (62.5)	35 (96.2)	
Capsule invasion			
Yes	11 (68.8)	5 (19.2)	**.001**
No	5 (31.3)	21 (80.8)	
Lymphovascular invasion			
Yes	13 (81.2)	5 (19.2)	**.001**
No	3 (18.8)	21 (61.8)	
Multifocality			
Yes	11 (73.3)	15 (57.7)	.317
No	15 (26.7)	11 (42.3)	
Extrathyroidal invasion			
Yes	14 (87.5)	10 (38.5)	**.002**
No	2 (12.5)	16 (61.5)	
Lymph node metastasis			
Yes	13 (81.3)	25 (96.2)	.146
No	3 (18.7)	1 (3.8)	
TNM stage (AJCC7/AJCC8)			
Stages 1-2	5 (43.7)	23 (89.1)	**.002**
Stages 3-4	11 (57.3)	3 (11.9)	
Recurrence			
Yes	6 (37.5)	1 (3.8)	**.008**
No	10 (62.5)	25 (96.2)	
Mortality	6 (37.5)	0 (0)	**.002**

Demographic, clinical, and pathological features of 16 PTC cases with distant metastases and 26 without distant metastases are shown.
AJCC7, American Joint Committee on Cancer, seventh edition; AJCC8, American Joint Committee on Cancer, eighth edition; ATA, American Thyroid Association; TNM, tumor, node, metastasis.
*Tall cell, columnar cell, and hobnail variants were grouped as aggressive variants according to ATA guidelines.[7]

Table 3. *BRAF*^{V600E}, *TERT Promoter,* and *NRAS* Mutation Positivity in Patients With and Without Distant Metastasis

Mutation	Patients with Distant Metastasis (n)	Patients Without Distant Metastasis (n)	*P*
BRAF^{V600E} (total n = 27)	6	21	**.006**
TERT promoter (C228T) (total n = 4)	2	2	.628
Both *BRAF*^{V600E}*and TERT Promoter (C228T)* (total n = 3)	1	2	1.000
NRAS	0	0	

The distribution of *BRAF*^{V600E}, *TERT promoter*, and *NRAS* mutation frequencies in patients with and without distant metastases is shown.

the first time the status of *BRAF*^{V600E} combined with *TERT promoter* and *RAS* mutations in PTC from Turkish cases.

We found that aggressive histological variants, capsule invasion, lymphovascular invasion, extrathyroidal spread, advanced stage, and recurrence rate were higher in patients with distant metastasis than in those without. There was no relationship between distant metastases and gender, age at diagnosis, tumoral size, multifocality, and lymph node metastasis. There are studies investigating distant metastases and clinicopathological features in PTC, and their results partially overlap with our findings.[15,16] Liu et al[15] from Taiwan showed that vessel invasion and extrathyroidal spread were similarly correlated with distant-organ metastases in PTC. However, unlike our study, advanced age and tumor size were determined as causative determinants for distant metastases in PTC.[15] Jing et al[16] found a significant relationship between gender (male), multifocality, bilateral disease, extrathyroidal spread, tumoral size, lymph node metastases, and distant-organ metastases in a work conducted in 107 Chinese PTC cases. Distant-organ metastases are rarely observed in PTC, but they considerably reduce the life expectancy if found. Concordantly, our distant-metastatic group had considerably higher mortality.

Although the frequency of *BRAF* mutation differs between studies, it was found to be between 29% and 83%; and *BRAF*^{V600E} (Val600Glu,V600E) constitutes 90% of all these mutations.[17] This difference may depend on histological subtypes, epidemiological factors, and age variability. In our study, the frequency of *BRAF*^{V600E} mutation was 67.5%. Pessôa-Pereira et al[18] in Brazil have reported the frequency of *BRAF* mutation as 65.1%, very close to ours. It can be stated that the frequency of *BRAF*^{V600E} is higher in Korean and Chinese patients compared with others. For example, Yang et al[19] from China has reported the frequency of *BRAF*^{V600E} as 82.5%, which is more common than ours. Only 1 study from Turkey has previously reported the frequency of *BRAF*^{V600E} mutation as 25.4%, which is less than what we found.[20] It can be thought that there exists a correlation with respect to the status of *BRAF*^{V600E} mutation and ethnicity. In addition, the different ratios of histological subtypes in studies seemed to cause these discrepant results. The correlation between clinicopathological characteristics and *BRAF*^{V600E} status is contradictory in the literature. To this end, we did not find any relationship between age, gender, tumor size, histological subtype, capsule invasion, lymphovascular invasion, multifocality, extrathyroidal spread, lymph node involvement, and *BRAF*^{V600E} status, similar to other reports.[21,22] It has been shown that there is a relationship between aggressive clinicopathological features and *BRAF*^{V600E} mutation.[23] Contrary to this expectation, the frequency of *BRAF*^{V600E} mutation was lower in our patients with distant-organ involvement compared with the patients without. The correlation between *BRAF*^{V600E} mutation and distant metastases is also contradictory in the literature. Our results with respect to *BRAF* mutation status might be affected from a selection bias because we randomly selected the patients without distant-organ metastases to match with the distant-metastatic group in terms of demographic characteristics. Probably the *BRAF*^{V600E} mutation rate could be lower if more patients without distant metastasis were included in the study.

Table 4. Relationship Between *BRAF*^{V600E} Mutation Status (Total n = 40) and Clinicopathological Features

Feature	*BRAF*^{V600E} Positive (n, %)	*BRAF*^{V600E} Negative (n, %)	*P*
Gender			
Female	12 (44.4)	8 (61.5)	.500
Male	15 (55.6)	5 (38.5)	
Age			
<45	7 (25.9)	3(40.1)	.822
≥45	20 (74.1)	10 (59.9)	
Tumor size (cm)			
>1-≤2	12 (44.4)	5 (38.5)	.733
>2-≤4	12 (44.4)	5 (38.5)	
>4	3 (11.2)	3 (23.0)	
Histopathological variants			
Aggressive variants*	5 (18.5)	2 (15.4)	1.000
Nonaggressive variants	22 (81.5)	11 (84.6)	
Capsule invasion			
Yes	7 (25.9)	7 (53.8)	.155
No	20 (74.1)	6 (46.2)	
Lymphovascular invasion			
Yes	9 (33.3)	8 (61.5)	.171
No	18 (66.7)	5 (38.5)	
Multifocality			
Yes	19 (70.4)	6 (48.7)	.287
No	8 (29.6)	13 (51.3)	
Extrathyroidal pread			
Yes	15 (55.6)	8 (61.5)	.720
No	12 (44.4)	5 (38.5)	
Lymph node metastasis			
Yes	7 (25.3)	7 (53.6)	.156
No	20 (74.7)	6 (46.4)	
Stage (AJCC)			
Stages 1-2	7 (25.9)	5 (38.5)	.096
Stages 3-4	20 (74.1)	8 (61.5)	
Recurrence			
Yes	4 (14.8)	3 (23.1)	.662
No	23 (85.2)	10 (76.9)	
Mortality			
	1 (3.7)	4 (30.8)	**.03**

The relationship between *BRAF*^{V600E} mutation and clinicopathological features is shown. *P* <.05 is statistically significant.*Aggressive variants, Tall cell, columnar cell and hobnail.
AJCC, American Joint Committee on Cancer.

Table 5. Relationship Between *TERT Promoter* Mutation and Clinicopathological Features

Feature	*TERT Promoter (C228T)* Positive (n, %) (n = 4)	*TERT Promoter (C228T)* Negative (n, %) (n = 38)	*P*
Gender			
Female	3 (75)	20 (52.6)	.125
Male	1 (25)	18 (47.4)	
Age			
<45	0 (0)	14 (36.8)	.283
≥45	4 (100)	24 (63.2)	
Tumor size (cm)			
>1-≤2	0 (0)	18 (47.4)	.230
>2-≤4	3 (75)	15 (39.5)	
>4	1(25)	5 (13.1)	
Histopathological variants			
Aggressive variants*	2 (50)	5 (13.2)	.123
Nonaggressive variants	2 (50)	33 (86.8)	
Capsule invasion			
Yes	3 (75)	13 (34.2)	.146
No	1 (25)	25 (65.8)	
Lymphovascular invasion			
Yes	3 (75)	15 (39.5)	.297
No	1 (25)	23 (60.5)	
Multifocality			
Yes	2 (50)	24 (64.9)	.615
No	2 (50)	14 (35.1)	
Extrathyroidal spread			
Yes	3 (75)	21 (55.3)	.623
No	1 (25)	17 (44.7)	
Lymph node metastasis			
Yes	4 (100)	34 (89.4)	.170
No	0 (0)	4 (10.6)	
Stage (AJCC/IUU)			
Stages 1-2	0 (0)	12 (31.6)	.728
Stages 3-4	4 (100)	26 (68.4)	
Recurrence			
Yes	1 (25)	6 (15.8)	.532
No	3 (75)	32 (84.2)	
Mortality	0 (0)	6 (15.8)	1.000

The relationship between *TERT promoter* mutation and clinicopathological features is shown. *P* <.05 is statistically significant.
AJCC, American Joint Committee on Cancer; ATA, American Thyroid Association.
*Tall cell, columnar cell, and hobnail variants were grouped as aggressive variants according to ATA guidelines.[7]

We suggest further large-scale studies involving more cases with distant metastases to verify this.

In various studies, the frequency of mutations in the *TERT promoter* region was found to be 10% in thyroid cancers and 11% in PTC.[24] We found it to be 9.5%, according to previous reports. All of these mutations were in the form of C228T. To our knowledge, there is no previous report from Turkey investigating the status of *TERT promoter* mutation in PTCs with respect to clinicopathological characteristics. Our findings did not indicate a relationship between *TERT* mutation and aggressive clinicopathological features as well as survival. Our results differed from studies in the literature reporting existence of such a relationship. For example, Melo et al[25] studied PTC patients in 5 different university hospitals in Portugal and Spain and found the frequency of *TERT promoter* mutation to be 7.5%. They found a relationship between age, gender, tumor size, distant metastasis, and *TERT promoter* mutation at diagnosis. They also showed that *TERT promoter* mutation-positive cases need more cumulative dose RAI

Table 6. Comparison of *BRAF*^{V600E} and *TERT Promoter* Mutation Coexisting Cases with *BRAF*^{V600E} or *TERT Promoter* Mutation-Only Cases

Feature	*BRAF*^{V600E} and *TERT Promoter* Mutation Coexisting Cases (n, %) (n = 3)	*BRAF*^{V600E} or *TERT Promoter*-Only Cases (n, %) (n = 39)	*P*
Gender			
Female	2 (66.7)	20 (51.3)	.126
Male	1 (33.3)	19 (48.7)	
Age			
<45	0 (0)	9 (23.1)	.210
≥45	3 (100)	30 (76.9)	
Tumor size (cm)			
>1-≤2	0 (0)	18 (46.2)	.191
>2-≤4	3 (100)	15 (38.5)	
>4	0 (0.0)	6 (15.3)	
Histopathological variants			
Aggressive variants*	1 (33.3)	6 (15.3)	.430
Nonaggressive variants	2 (66.7)	33 (84.7)	
Capsule invasion			
Yes	2 (66.7)	14 (35.9)	.547
No	1 (33.3)	25 (66.6)	
Lymphovascular invasion			
Yes	2 (66.7)	16 (41)	.567
No	1 (33.3)	23 (59)	
Multifocality			
Yes	1 (33.3)	22 (56.4)	.543
No	2 (66.6)	17 (43.6)	
Extrathyroidal spread			
Yes	2 (66.7)	22 (56.4)	1.000
No	1 (33.3)	17 (43.6)	
Lymph node metastasis			
Yes	3 (100)	35 (89.7)	.156
No	0 (0)	4 (10.3)	
Stage (AJCC/IUU)			
Stages 1-2	0 (0)	12 (30.8)	.819
Stages 3-4	3 (100)	27 (69.2)	
Recurrence			
Yes	1 (33.3)	6 (15.3)	.430
No	2 (66.7)	33 (84.7)	
Mortality	6 (15.3)	0 (0)	1.000

BRAF^{V600E} *and TERT promoter* mutation coexistence or having only one type of mutation in relation to clinicopathological features are shown. *P*-values < .05 are statistically significant.
AJCC, American Joint Committee on Cancer; ATA, American Thyroid Association.
*Tall cell, columnar cell, and hobnail variants were grouped as aggressive variants according to ATA guidelines.[7]

treatment and alternative treatments.[25] In a study by Gandolfi et al[26] from Italy, the *TERT* mutation correlated with distant metastases. The relatively small number of mutations can explain our different results in a relatively small population. We found the frequency of *TERT promoter* mutation more frequent in cases with distant metastasis (12.5%) than in patients without (7.7%), but we could not obtain a statistically significant difference, possibly due to the insufficient number of subjects.

We also examined whether the co-occurrence of *BRAF*^{V600E} mutation and *TERT* mutation increases tumor aggressiveness in PTC but could not find a relationship, including distant metastasis. Our results contradict the studies suggesting that the coexistence of the 2 mutations worsens tumor aggressiveness and overall prognosis. For example, Liu et al from China have shown that co-occurrence of *BRAF*^{V600E} and *TERT* mutations is related to advanced age, large tumor size, and extrathyroidal invasion.[27] Furthermore, Xing et al[28] from China have shown that the coexistence of these mutations indicates to poor prognostic outcomes in PTC.

There is no previous study examining the relationship between *NRAS* mutation and clinicopathological characteristics and distant-organ metastasis in Turkish PTC cases and whether it is predictive for distant metastases. No *NRAS* mutations in any PTC case with or without distant metastasis were observed in the present study. We think this could be due to the histological subtypes we included. Trials investigating the prognostic and clinical importance of *RAS* mutations in PTC are not sufficient in the literature. The frequency of *RAS* mutations has been reported to be 0%-20% in PTC. The *NRAS* mutation is usually associated with the follicular and classical variant.[6] Although the prognostic role of *RAS* mutations has not been fully explained, studies are showing their relationship with distant metastases. Hara et al[7] have found *NRAS* gene codon 61 mutation as a distinct prognostic sign in PTC.

We found *RET/PTC* gene rearrangement in 4.76% of PTC cases. Leeman-Neill et al[29] reported an increased incidence of *RET/PTC* mutation (35%) in 62 cases affected by Chernobyl nuclear accident. In our opinion, it can be stated that *RET/PTC* gene rearrangement may be associated with ionizing radiation.

The frequency of *PAX8/PPARγ* rearrangement in PTC is conflicting in the series as previously stated, showing a wide range. Klemke et al[30] stated that the genetic change was absent in their samples. The geographical difference in the existence of the rearrangement requires more investigation.

Among the study's limitations, the number of patients presenting with distant-organ involvement is limited because of rare distant metastases in PTC cases due to its natural indolent course. Furthermore, given the fact that *TERT* mutation is detected in only 4 cases, it is not easy to examine its relationship with clinicopathological features. Another limitation is that most of the samples used have not been obtained from the distant-metastatic sites per se due to technical difficulties.

In conclusion, our results reveal that *BRAF*^{V600E} mutation is not necessarily indicative of aggressive clinicopathological characteristics and distant metastases in this particular group of Turkish cases. Frequency of *TERT* mutation is 9.5%, concordant with the literature's reported frequencies. We could not find a relationship between *TERT promoter* mutation, aggressive clinicopathological features, and distant metastases, either. The co-occurrence of *BRAF*^{V600E} and *TERT* mutations was also not related with distant-organ metastasis. We did not observe any *NRAS* mutation irrespective of the presence or absence of distant-organ metastasis. Our study is a preliminary

one due to a relatively small number of subjects and should be validated in further larger-scale studies on the Turkish population. Low number of patients and short follow-up, however, might have hindered the ability to draw accurate conclusions regarding molecular markers and poor prognosis in such a slow-growing carcinoma type.

It may be emphasized that previous observations attributing a role to these molecular markers for poor prognosis and aggressive disease course are not universally applicable in different populations and ethnic backgrounds.

Data Availibility: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Hacettepe University Faculty of Medicine (project number: GO 21/569; approval number: 2021/09-08) and was conducted according to the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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