Original Article

# BRAF V600E mutation correlates with aggressive clinico-pathological features but does not influence tumor recurrence in papillary thyroid carcinoma—10-year single-center results

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**Background:** BRAF V600E mutation is common in papillary thyroid carcinoma (PTC) but its prognostic value and influence on tumor recurrence is controversial. We investigated if BRAF V600E mutation influences tumor behavior and recurrence, and if it can be used as surrogate parameter in PTC.

**Methods:** In a single center retrospective study with a median follow-up of 5 years, incidence of BRAF V600E mutation in 186 PTC specimens from 2007–2016 was investigated. Tumor outcome parameters including TNM status, multifocal and invasive growth and tumor recurrence rate were examined.

**Results:** In 98 specimens (52.7%) a BRAF V600E mutation (BRAF+), and in 88 specimens (47.3%) no mutation (BRAF–) was detected. There was no gender specific difference. BRAF+ patients were significantly older (mean 5.6 years; P=0.011). BRAF+ tumors were significantly smaller (14.4 *vs.* 18.3 mm; P=0.018), and more often showed a multifocal (30.6% *vs.* 17%; P=0.031) and extracapsular tumor growth pattern (pT3b and pT4a; BRAF+ 22.4% *vs.* BRAF– 10.2%; P=0.026). Although lymph node-status did not differ in both groups, BRAF+ showed a higher infiltration rate of the lateral lymph node compartment (12.2% *vs.* 5.7%; P=n.s.). Distant metastases occurred only in BRAF+ (3.1% *vs.* 0%). There was no significant difference in terms of tumor recurrence rate.

**Conclusions:** Results regarding the incidence of malignant lymph nodes, tumor growth pattern and tumor multifocality suggest a more aggressive tumor behavior in BRAF+ PTC but this fact does not affect tumor recurrence rate in a five year follow up period. Therefore, the postoperative role of BRAF V600E mutation remains unclear, and a general change of operative procedure and radicality cannot be recommended based on BRAF status alone.

**Keywords:** Papillary thyroid carcinoma (PTC); multifocal tumor growth; extrathyroidal tumor growth; recurrence rate; tumor aggressiveness; BRAF V600E

Submitted Feb 19, 2020. Accepted for publication Jul 09, 2020.

doi: 10.21037/gs-20-244

**View this article at:** http://dx.doi.org/10.21037/gs-20-244

## Introduction

The incidence of thyroid carcinoma has increased worldwide in recent years, with papillary thyroid carcinoma (PTC) representing the highest proportion (1,2).

In adults, women are affected about four times more frequently than men. Although PTC has an excellent prognosis, local lymph node metastases, especially in the central compartment (level 6), are identified in about 60%

of cases (3,4). These lymph node metastases correlate with development of locoregional tumor recurrence resulting in repeated treatment (5).

The surgical therapy of PTC depends on tumor and lymph node status and even differs between continents. The American and European guidelines recommend a total or near total thyroidectomy for PTCs, with a more limited resection only for low-risk tumors (microcarcinoma). In Japan, in case of papillary microcarcinoma an active surveillance with close monitoring without a resection can be recommended in certain cases (6-8). The implementation of a central neck dissection in nodal-positive PTC is generally accepted but a prophylactic lymphadenectomy in clinical nodal-negative cases remains controversial due to increased risk of complications (9,10).

However, the diagnostic options for preoperative assessment of lymph node metastasis, especially in the central compartment, are very limited. Therefore, the stratification for a possibly more aggressive tumor course is based on postoperative histopathological markers (i.e., extrathyroidal extension, larger tumors) (11,12). In recent years, molecular markers have been examined to establish a risk stratification in order to detect potentially more aggressive tumors and to offer a more individualized surgical approach. Among these markers, BRAF V600E (in the following abbreviated as BRAF) has received the widest interest. In PTCs, BRAF mutation is the most common genetic change with 30% to 90% (45–80% in the conventional variant, 5–25% in the follicular variant, 60–95% in the tall-cell variant) (13-15).

BRAF V600E mutation promotes tumorigenesis by activating the MAP kinase pathway leading to an increase in cell growth, proliferation and differentiation (16). Many studies have demonstrated that BRAF mutation status is associated with aggressive tumor features such as capsule invasion, extrathyroidal extension and lymph node metastasis, and that it can increase the risk of persistent and recurrent disease (17). Furthermore, some studies recommend a more radical surgical approach in cases of preoperatively confirmed BRAF mutation in fine needle aspiration (18,19). However, several studies have failed to confirm these findings, leaving the overall significance of the BRAF mutation unclear (20-23). Therefore, the role of BRAF regarding tumor relapse still remains controversial, and standardized BRAF analysis has therefore not yet been included in the relevant guidelines.

This single-centre retrospective study was conducted to investigate the influence of BRAF mutation on tumor course, tumor recurrence and patients' outcome in PTC from 2007 to 2016. We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/gs-20-244).

## Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional/regional/national ethics/committee/ethics board of the Carl von University of Oldenburg (No. 005/2017) and informed consent was taken from all the patients.

### *Patients*

Between 2007 and 2016 n=382 patients with thyroid cancer were treated in the University Clinic for Visceral Surgery, Pius-Hospital, Medical Campus University of Oldenburg; 285 cases of PTC were detected and 270 patients were contacted for permission to determine the mutation status for BRAF mutation (BRAF positive or short BRAF+ *vs.* BRAF negative or short BRAF–). The written consent of 189 patients could be obtained (*Figure 1*). Written consent was needed, because BRAF analysis exceeded the typical diagnostics, and because of handling specimen containing genetic material.

The following data was gathered for all patients for whom consent was obtained: Preoperative TSH (serum thyrotropin), preoperative sonographic features, the amount of postoperative radioiodine therapy (RIA), tumor recurrence and characteristics, and time of re-operation for tumor recurrence were noted.

Postoperative complications, i.e., recurrent nerve paralysis, hypocalcemia, and wound infection were recorded prospectively as part of the quality assurance measures in our thyroid center. As the TNM-classification has changed during the last 10 years, the TNM status was adopted to the actual 8$^{th}$ edition (24).

### *DNA extraction/amplification and BRAF-detection*

Part of the paraffin-embedded tissue was prepared for microscopy, and the areas with carcinoma were marked on the microscope slides. Thus, a small part of the tumor could be removed from the paraffin block. The gathered DNA was cleaned and fluorescence measurements for determining of the DNA concentration were carried out with a

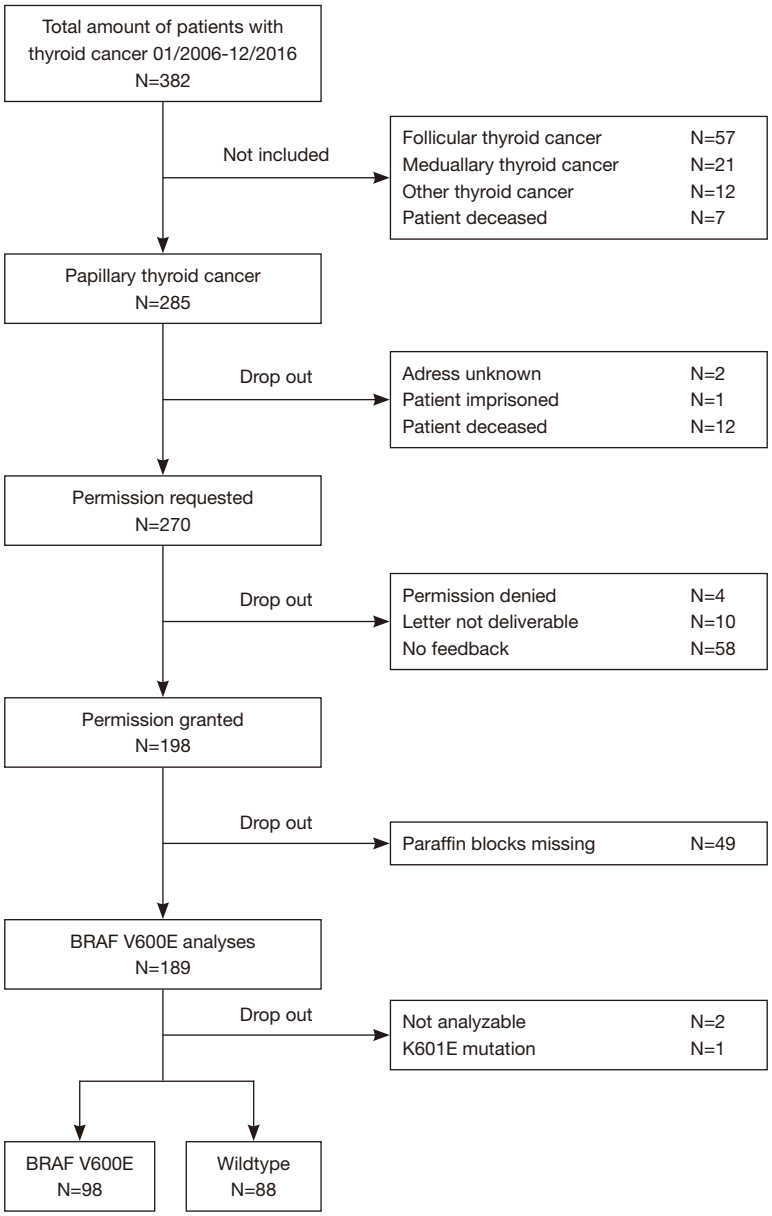


**Figure 1** Flow chart depicting inclusion and exclusion criteria.

spectrophotometer. DNA amplification was done by PCR (polymerase chain reaction) followed by pyrosequencing for determination of the mutation status as already published (25,26).

In summary, the genomic DNA was amplified by standard PCR using the forward primer "5-TCCTTTACTTACTAC ACCTCAGAT-3" and the reverse primer "3-CCCACTCC ATCGAGATT-5". The forward pyrosequencing primer was 5'-TGATTTTGGTCTAGCTACA-3', and pyrosequencing was performed using PyroMark® Q24 System (Qiagen).

### *Statistical analysis*

Data collection was performed using Microsoft Excel, Version 2007 Professional. For statistical analysis the program IBM SPSS Statistic 25 was used. Continuous data was described by calculating mean, standard deviation,

median, minimum, and maximum. The differences in patient characteristics between the BRAF+ and the BRAF– group where analyzed as follows: categorically scaled features with ≤4 characteristic values were tested for independence using non-parametric testing. By default, the Pearson chi-square independence test was used. At an expected frequency <5, Fisher's Exact Test was used instead. For categorically scaled characteristics with >4 feature values, risk ratio with 95% confidence interval was calculated. No tests were performed for variables describing patient characteristics irrelevant to our research question in order to keep the number of tests low. We corrected for multiple testing using the Benjamini-Hochberg method. Metric variables included age, preoperative TSH, sonographic nodule diameter, and thyroid volume, pathologic tumor size, TNM staging. Categorically scaled variables included gender, (extrathyroid) tumor expansion, (multifocal) tumor growth, pathologic variant, lymph node metastasis, distant metastasis and BRAF mutation status. Factors favoring development of recurrence were examined by means of multiple logistic regressions with forward and backward selection. For this analysis the program JASP was used.

## Results

### *Tumor characteristics*

A total of 189 tumor specimens were subjected to BRAF mutation analysis (*Figure 1*). Out of these samples, two cases could not be analyzed even with repeated pyrosequencing and one patient showed a K601E mutation, therefore these three cases were excluded and eventually a number of 186 specimens were analyzed. N=98 (52.7%) specimens were BRAF+ and n=88 (47.3%) were BRAF–.

Patient and tumor characteristics are illustrated in *Table 1*. The mean follow-up time was 5 years for both groups. There was no significant difference in gender distribution between the groups but a tendency for male sex in the BRAF+ group. BRAF+ patients were significantly older (5.6 years) at time of diagnosis (P<0.011). Preoperative thyrotropin (TSH) levels did not differ between the groups.

Despite the significantly smaller tumor size in the BRAF+ group (14.4 *vs.* 18.3 mm; P=0.018), BRAF+ tumors showed a multifocal growth pattern (31.6% *vs.* 17.9%; P=0.031) and a higher rate of extrathyroidal growth, pT3b/pT4a (22.4% *vs.* 10.2%; P=0.026). Although the total number of lymph node metastases was comparable in both groups, the BRAF+ group showed a higher infiltration rate of the lateral lymph node compartment (12.2% *vs.* 5.7%; n.s.). In case of microcarcinoma after hemithyroidectomy, a total resection including the contralateral thyroid tissue plus prophylactic central node dissection was only performed if thyroid nodules were existing in the remnant tissue. Patients with pT1b and more advanced carcinoma were treated by total thyroidectomy and prophylactic central node dissection. An additional ipsilateral or contralateral lymph node dissection was only performed in case of macroscopic proof of potential metastasis. As expected, the rate of remote metastasis was very low in both groups. However, distant metastases occurred only in the BRAF+ group (3.1% *vs.* 0%; n.s.).

BRAF mutation was significantly prevalent in the classic tumor subtype (79% *vs.* 47%) and less in the follicular variant of PTC (17% *vs.* 47% in BRAF– P<0.001). The age-independent summary of the TNM classification according to the actual 8$^{th}$ edition showed that 99% of BRAF– patients could be assigned to stage 1. By contrast, 14.2% of the patients were allocated of stage 2 and higher in BRAF+. However, age also played a crucial role as n=11, 32.4% of the patients older than 55 years were assigned to stage 2.

### *Tumor therapy*

A total of 173 total thyroidectomies and 13 hemithyroidectomies were performed. No BRAF dependent significant difference were detected (P>0.6). Also, with regard to the implementation and extension of lymph node dissection no significant differences between the groups could be revealed.

An ablative RIA was performed in 168 cases (90.3%) and 89 patients (47.8%) were treated with more than one therapy.

Concerning perioperative complications (laryngeal nerve palsy, hypocalcemia and re-operation due to infection or bleeding) no significant differences between the groups could be found. It should be mentioned that due to the retrospective character of this study a differentiation between persistent and transient laryngeal nerve palsy and an information to postoperative hypoparathyroidism due to lack of routinely determined parathormone values cannot be made.

### *Microcarcinoma*

A total of n=63 (n=34 BRAF+ and n=29 BRAF-)

**Table 1** patient and tumor characteristics for all patients included in the study and stratified by BRAF V600E-status

| Characteristic | All patients (n=186) | BRAF+ (n=98) | BRAF− (n=88) | P value |
|---|---|---|---|---|
| Gender, n (%) | | | | |
| Female | 140 (75.3) | 69 (70.4) | 71 (80.7) | $P_{\chi 2}$=0.105 |
| Male | 46 (24.7) | 29 (29.6) | 17 (19.3) | |
| Body mass index (BMI) | | | | |
| BMI, mean (± SD), kg/m$^2$ | 26.7±5.2 | 27.0±4.9 | 26.4±5.2 | $P_t$=0.468 |
| Normal weight n (%) | 78 (41.9) | 38 (38.8) | 40 (45.5) | |
| Overweight n (%) | 63 (33.9) | 33 (33.7) | 30 (34.1) | |
| Obese n (%) | 45 (24.2) | 27 (27.6) | 18 (20.5) | |
| Age in years, mean ± SD | 46.7 | 49.5±13.3 | 43.9±16.1 | $P_t$=0.011 |
| Follow-up in years, mean ± SD | | 5±3.0 | 5.1±3.2 | |
| Thyreotropin (TSH), n (%) | | | | |
| TSH normal | 146 (78.5) | 79 (80.6) | 67 (76.1) | $P_{\chi 2}$=0.458 |
| TSH increased | 9 (4.8) | 4 (4.1) | 5 (5.7) | |
| TSH decreased | 31 (16.7) | 15 (15.3) | 16 (18.2) | |
| Pathological variation, n (%) | | | | |
| Classical | 118 (63.4) | 77 (78.6) | 41 (46.6) | $P_{\chi 2}$<0.001 |
| Follicular | 58 (31.2) | 17 (17.3) | 41 (46.6) | |
| Other | 10 (5.4) | 4 (4.1) | 6 (6.8) | |
| T classification, n (%) | | | | |
| TX | 1 (0.5) | 0 (0) | 1 (1.1) | |
| T1a | 63 (33.9) | 34 (34.7) | 29 (33.0) | |
| T1b | 42 (22.6) | 25 (25.5) | 17 (19.3) | |
| T2 | 42 (22.6) | 16 (16.3) | 26 (29.5) | |
| T3a | 7 (3.8) | 1 (1.0) | 6 (6.8) | |
| T3b | 28 (15.1) | 20 (20.4) | 8 (9.1) | |
| T4a | 3 (1.6) | 2 (2.0) | 1 (1.1) | |
| Tumor size, mean ± SD, mm | 16.3±11.0 | 14.4±8.8 | 18.3±12.8 | $P_t$=0.018 |
| Tumor growth, n (%) | | | | |
| Monofocal | 141 (75.8) | 68 (69.4) | 73 (82.1) | $P_{\chi 2}$=0.031 |
| Multifocal | 45 (24.2) | 30 (30.6) | 15 (17.0) | |
| Tumor expansion, n (%) | | | | |
| Intrathyroidal | 155 (83.3) | 76 (77.6) | 79 (89.8) | $P_{\chi 2}$=0.026 |
| Extrathyroidal | 31 (36.0) | 22 (22.4) | 9 (10.2) | |

**Table 1** (*continued*)

**Table 1** (*continued*)

| Characteristic | All patients (n=186) | BRAF+ (n=98) | BRAF− (n=88) | P value |
|---|---|---|---|---|
| N classification, n (%) | | | | |
| N0 | 139 (74.7) | 72 (73.5) | 65(76.7) | $P_{\chi 2}$=0.073 |
| N1a | 31 (16.7) | 14 (14.3) | 18 (20.5) | |
| N1b | 17 (9.1) | 12 (12.2) | 5 (5.7) | |
| M classification, n (%) | | | | |
| M0 | 183 (98.4) | 95 (96.9) | 88 (100.0) | $P_F$=0.144 |
| M1 (PUL) | 3 (1.6) | 3 (3.1) | 0 (0.0) | |
| AJCC tumor stage, n (%) | | | | |
| Stage I | 171 (91.9) | 84 (85.7) | 87 (98.9) | $P_{RR}$<0.001 |
| Stage II | 13 (7.0) | 12 (12.2) | 0 (0.0) | $P_{RR}$=0.020 |
| Stage I (<55 years) | 126 (67.7) | 61 (62.2) | 65 (73.9) | |
| Stage II (<55 years) | 1 (0.54) | 1 (1.0) | 0 (0.0) | |
| Stage I (>55 years) | 45 (24.2) | 23 (23.5) | 22 (26.1) | |
| Stage II (>55 years) | 12 (6.5) | 11 (11.2) | 0 (0.0) | |
| Stage IVB | 2 (1.1) | 2 (2.0) | 0 (0.0) | $P_{RR}$=0.330 |
| Tumor recurrence | | | | |
| No recurrence, n (%) | 169 (90.9) | 89 (90.9) | 80 (90.9) | |
| Recurrence, n (%) | 17 (9.1) | 9 (9.1) | 8 (9.1) | $P_{\chi 2}$=0.810 |
| Recurrence at T1a (microcarcinoma), n (%) | 4 (2.2) | 2 (2.0) | 2 (2.3) | |
| Re-operation after months, mean ± SD | 29.3±29.9 | 35.80±29.6 | 22.30 ± 30.0 | |
| Operative therapy, n (%) | | | | |
| Hemithyroidectomy | 13 (7.0) | 6 (6.1) | 7(8.0) | |
| Total thyroidectomy | 173 (93.0) | 92 (93.9) | 81 (92.0) | |
| Lymph node dissection, n (%) | 139 (74.7) | 72 (73.5) | 67 (76.1) | |
| No dissection | 47 (25.2) | 26 (16.5) | 21 (23.9) | |
| Cent. compart. dissect. | 114 (61.3) | 56 (57.1) | 58 (65.9) | |
| Cent. & lat. compart. dissect. | 25 (13.4) | 16 (16.3) | 9 (10.2) | |
| Radio iodine ablative therapy (RIA), n (%) | | | | |
| No RIA | 18 (9.7) | 7 (7.1) | 11 (12.5) | |
| RIA | 168 (90.3) | 91 (92.9) | 77 (87.5) | |
| Perioperative complications, n (%) | | | | |
| Laryngeal nerve palsy | 22 (11.8) | 14 (14.3) | 8 (9.1) | |
| Hypocalcemia | 57 (30.1) | 27 (27.6) | 30 (34.1) | |
| Reoperation (bleeding/infection) | 5 (2.7) | 3 (3.1) | 2 (2.3) | |

$P_t$ = *t*-test; $P_{\chi 2}$ = $Chi^2$-test; $P_F$ = Fisher's test; $P_{RR}$ = risk ratio.

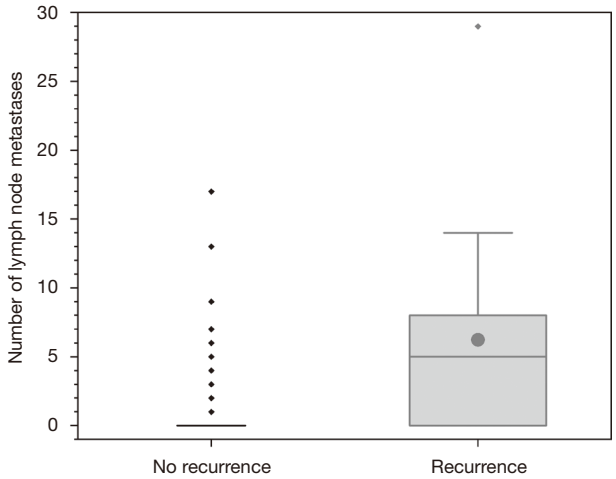


**Figure 2** Number of lymph node metastases at the time of primary surgery for patients who later develop a relapse and for patients who do not develop a relapse in the follow-up period. The box depicts the upper and lower quartile, the vertical lines the median and the filled dots the mean. Whiskers indicate 1.5 times the interquartile range.

microcarcinoma (pT1acN0) were removed in the examined period of which n=9 (n=3 BRAF+, n=6 BRAF–) were resected during hemithyroidectomy. A total of n=4 (2 in each group BRAF+) were re-operated because of lymph node metastasis. None of the patients who previously underwent hemithyroidectomy required reoperation.

### *Tumor recurrence*

Tumor recurrence was defined as any positive disease found on the whole-body scan in a previously negative area with a correlating positive finding seen on clinical imaging (e.g., neck ultrasound or CT scan) that could be approached surgically. Metastatic patients were also coded as recurrent at time of diagnosis. In 17 individuals (n=9 BRAF+ and n=8 BRAF–; n.s.) a recurrence of the PTC was detected in a mean follow-up period of five years. Of the individuals without lymph node metastasis (n=139) at time of primary treatment, n=5 (5.1%) patients developed a recurrence. In patients with initial central lymph node metastasis (n=31) and in individuals with lateral lymph node metastasis (n=17) tumor recurrence occurred in 5 (16.1%) and 4 (23.5%) cases, respectively. In addition, the number of lymph node metastasis at time of initial operation was higher in patients who developed tumor recurrence (mean 6.2) later, than in patients without tumor recurrence (mean 1.3; *Figure 2*).

Out of the patients with distant metastasis (n=3) which all occurred in the lung, n=2 patients also developed a local recurrence. In one patient only the lung metastasis could be detected on the body scan without local neck recurrence. Regarding the recurrence rate and the recurrence time-point defined as time of re-operation after primary diagnosis no significant difference between BRAF+ and BRAF– patients was found (P>0.05).

To further determine variables effecting tumor recurrence, a multiple logistic regression model with iterative backward and forward selection containing the following parameters was performed: age at surgery, body mass index, sex, tumor variant, tumor focality, tumor extension, pathologic tumor size, information about the number of lymph node metastasis (0, <6, or ≥6), distant metastasis, and BRAF mutation status. A model containing the information about the number of lymph node metastases, and distant metastasis can best explain tumor recurrence according to the area under the curve (AUC =0.796) with a sensitivity of 0.111, and a specificity of 0.994 [$Chi^2$(183) =27.992, P<0.001]. Adding the BRAF mutation to this model, the occurrence of relapse increases sensitivity to 0.389, and decreases specificity slightly to 0.976, while keeping the AUC nearly constant [AUC =0.792; $Chi^2$(182) =28.267, P<0.001]. However, BRAF status alone is not a significant influencing factor in this model (see *Table 2*).

## Discussion

Due to the excellent course of PTC, the prognosis is influenced by disease-free survival rather than the overall survival unlike other types of cancer with higher mortality. Approximately 30% of patients with differentiated thyroid carcinoma suffer from tumor persistence or recurrence (27). Therefore, the focus is on precise detection of these patient groups for an individualized therapy and follow-up. In recent years, the BRAF V600E mutation is regarded as a surrogate marker for a more aggressive tumor behavior, but its influence on tumor recurrence remains controversial as many studies have failed to prove correlations of BRAF status and tumor outcome (22,28,29).

In our study, 52.7% of the examined tumors were BRAF+. This patient group was significantly older than the BRAF wild-type group irrespective of tumor stage. In fact, age has been already described as a risk factor in thyroid cancer in general (6). Regarding the cut-off point of 55 years which demarks the age associated risk in thyroid cancer, BRAF status significantly influences tumor progression in

**Table 2** Results of the multiple logistic regression: (I) best predictive model, (II) model including BRAF status

| Variables | Estimate | Standard error | Odds ratio | z-value | Wald test | | 95% confidence interval (odds ratio scale) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Wald statistic | P value | Lower bound | Upper bound |
| (I) Coefficients without BRAF status | | | | | | | | |
| (Intercept) | –3.384 | 0.441 | 0.034 | –7.669 | 58.810 | <0.001 | 0.014 | 0.081 |
| LN information* | 1.629 | 0.358 | 5.098 | 4.552 | 20.720 | <0.001 | 2.528 | 10.281 |
| pM status** | 3.647 | 1.344 | 38.373 | 2.714 | 7.364 | 0.007 | 2.754 | 534.673 |
| (II) Coefficients if BRAF status is included | | | | | | | | |
| (Intercept) | –3.230 | 0.518 | 0.040 | –6.237 | 38.905 | <0.001 | 0.014 | 0.109 |
| LN information* | 1.621 | 0.358 | 5.059 | 4.531 | 20.529 | <0.001 | 2.509 | 10.202 |
| pM status** | 3.798 | 1.378 | 44.618 | 2.756 | 7.594 | 0.006 | 2.994 | 664.827 |
| BRAF status*** | –0.303 | 0.579 | 0.738 | –0.523 | 0.274 | 0.601 | 0.237 | 2.298 |

*, LN information coded as: number of lymph node metastasis (0, <6, or ≥6). **, PM status: distant metastasis present (yes/no). ***, BRAF status: BRAF positive/negative.

this patient group. Recently, it has been demonstrated that age-associated mortality risk is dependent on BRAF status, and therefore it has been suggested to differentiate between patients with BRAF V600E mutation and wild-type when applying age to risk stratification and management of PTC (30).

Despite of smaller tumor size, a multifocal, and a more extensive extrathyroidal tumor extension were detected in BRAF+ patients. Although the amount of lymph node metastases did not differ between the two groups, BRAF+ tumors affected the lateral lymph node compartment twice as often, and distant metastases were only seen in the BRAF+ group. These results are in line with the mentioned studies suggesting a more aggressive tumor behavior of BRAF+ PTC (see *Table 3*).

A major weakness of retrospective analyses relates to the definition of the term "tumor recurrence" as the differentiation between persistent and recurrent disease is difficult due to the fact that routine follow-ups occur every 6 to 12 months, and a differentiation between recurrence and persistence as defined in the classic oncological definition is not always possible. Moreover, it is assumed that in most cases tumor recurrence in thyroid cancer is rather tumor persistence than true tumor recurrence due to incomplete preoperative staging and/or incomplete surgery (40).

Therefore, we used the term tumor recurrence defined as "the reappearance of tumor (either locally in thyroid bed, in neck nodes, or as distant metastatic disease) after a well-documented disease-free period" as proposed by Elisei *et al.* (41). Hence, we could exclude persistent tumor diseases and exclusively analyze patients with "real" recurrence. Irrespective of BRAF-status, we observed a tumor recurrence rate of 9.1% which did not differ between the groups, and we also did not observe differences regarding microcarcinoma incidences.

The largest cohort study to date showed that BRAF mutation is an independent risk factor for tumor recurrence over a median follow-up of 36 months (17). But in this study the overall recurrence rate was fairly high, regardless of the mutation status (20.9% in BRAF+, 11.6% in BRAF–). This is probably due to the fact that biochemical and macroscopic recurrences have been summarized. Niederer-Wüst *et al.* detected a 10-year recurrence-free survival in their retrospective single-center cohort of patients with PTC larger than 1 cm of 94% which fits with our data. Interestingly, in their analysis BRAF V600E mutation status was not associated with clinicopathologic characteristics of aggressive behavior such as extrathyroidal extension, lymph node metastases, higher T-categories, male sex, and older age (42). Due to our study design, we could not calculate survival time or disease-free survival, as we could only include patients who were still alive due to the consent required to analyze the pathological samples. This increases the uncertainty of the respective analysis to such an extent that it would be essentially meaningless. However, since the median follow-up of both groups is similar, and since

**Table 3** Relationship between BRAF V600E mutation and potential prognostic factors in selected studies

| Study | Number | BRAF+ | Age | Sex | Tumor size | Lymph node metastasis | Distant metastasis | Histological variant | Extension (extrathyroidal) | Tumor growth (multifocal) |
|---|---|---|---|---|---|---|---|---|---|---|
| Tabriz *et al.* | 186 | 52.70% | √ | × | √[a] | × | × | √ | √ | √ |
| Basolo *et al.* (31)* | 1,060 | 44.60% | √[b] | × | √ | √ | | | √ | √ |
| Elisei *et al.* (32)** | 319 | 33.20% | √[b] | × | × | | | √ | | √ |
| Frasca *et al.* (33) | 323 | 38.60% | × | × | √ | √ | | √ | √ | × |
| Joo *et al.* (18) | 148 | 53.40% | × | × | ×[c] | √[d] | | | √ | × |
| Kebebew *et al.* (34) | 347 | 38.60% | √ | × | ×[c,e] | √[e] | √[e] | √ | | × |
| Kim *et al.* (35)[M] | 5,655 | 49.40% | | | ×[c] | √ | | | √ | |
| Li *et al.* (36)[M] | 6,372 | 50.90% | × | √ | √ | √ | | √ | √ | √ |
| Lim *et al.* (37) | 3,130 | 73.90% | × | × | √[e] | √[e] | | √ | √[e] | √[e] |
| Tufano *et al.* (38)[M] | 2,470 | 45.30% | | | | √ | × | | √ | |
| Xing *et al.* (39) | | 38.40% | × | × | × | √ | | | √ | × |

Special features of proven significant: [a], significant for small tumor size; [b], significant for younger age; [c], significant for advanced tumor stadium (TNM); [d], significant for centrocervical lymph node metastasis; [e], significant only for papillary thyroid carcinomas of classical variant. Special features in study design: *, limited to tumor size <20 mm. **, limited to low-risk intrathyroidal tumors. [M], meta-analysis.

the size of the BRAF+ and BRAF– group are approximately equal, it can be assumed that in our cohort the BRAF+ mutation has at the very least no major impact on survival time.

Even if the BRAF-status alone is not associated with tumor recurrence, our results demonstrate the aggressive tumor behavior of BRAF+ PTC. The question arises whether BRAF mutation plays a role as a prognostic marker, and how we should deal with BRAF+ tumors. This issue is not new since many different prognostic scales have been established, among which the AGES, AMES and MACIS scale are the most commonly used (43). They all have in common that the BRAF mutation status is not considered. Due to the inconsistent role of BRAF mutation on the increased risk of relapse, the ATA recommendations do not recommend the routine use of BRAF mutation analysis on initial risk stratification, but they present an additional continuous risk scale including the BRAF status for disease recurrence (6). This shows that risk stratification in thyroid carcinoma should not be dependent on single parameters such as BRAF mutation. It is much more a combination of several factors and "an ongoing process" as outlined by Omry-Orbach *et al.* (27), which is highlighted by our results. BRAF mutation status could be considered as one component of a basket full of risk factors in the assessment of tumor recurrence for an individualized tailored diagnostic and therapeutic strategy.

Regarding PTC subtypes such as microcarcinoma (pT1a), which might have an indolent prognosis, it is often discussed how BRAF+ microcarcinoma should be handled, i.e. watchful waiting *vs.* hemithyroidectomy or total thyroidectomy and/or prophylactic central node lymphadenectomy. There is also disagreement on this issue as the influence of BRAF V600E mutation is controversial (36,44-46). Due to the retrospective design, the small amount of microcarcinoma, and the postoperative determination of BRAF status in our study, a general recommendation for a more aggressive surgical approach cannot be given. But the potential multifocal growth of BRAF+ tumors should be considered in the decision-making process for resection of the contralateral side and lymph node dissection after an initial hemithyroidectomy. This procedure presupposes that BRAF V600E mutation status is routinely performed in cases of postoperatively detected PTC which is not clinically widespread yet.

## Conclusions

Based on our results, more aggressive tumor behavior of BRAF+ PTC in terms of multifocal tumor growth and extrathyroidal expansion may be concluded. However, it is important to note that this tumor behavior was not

associated with higher tumor relapse in a median follow-up of five years. It seems that BRAF V600E status alone does not influence patient outcome and its routine postoperative determination remains currently without therapeutic consequences, and therefore does not need to be performed routinely. It should however be considered together with further risk factors such lymph node status for an individualized tumor follow-up. The role of BRAF status in papillary microcarcinoma remains debatable, and it should be explored in further studies, for instance with the focus on survival time, as it may possibly change the therapeutic procedure.

## Acknowledgments

We thank Belinda Verdoodt and Rolf-Peter Henke for the assistance in preparation and analysis of the tumor specimen.
*Funding:* None.

## Footnote

*Reporting Checklist:* The authors have completed the STROBE reporting checklist. Available at http://dx.doi.org/10.21037/gs-20-244

*Data Sharing Statement:* Available at http://dx.doi.org/10.21037/gs-20-244

*Peer Review File:* Available at http://dx.doi.org/10.21037/gs-20-244

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/gs-20-244). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional/regional/national ethics/committee/ethics board of the Carl von University of Oldenburg (No. 005/2017) and informed consent was taken from all the patients.



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**Cite this article as:** Tabriz N, Grone J, Uslar V, Tannapfel A, Weyhe D. BRAF V600E mutation correlates with aggressive clinico-pathological features but does not influence tumor recurrence in papillary thyroid carcinoma—10-year single-center results. Gland Surg 2020;9(6):1902-1913. doi: 10.21037/gs-20-244