The *BRAF*^{V600E} Mutation Is an Independent, Poor Prognostic Factor for the Outcome of Patients with Low-Risk Intrathyroid Papillary Thyroid Carcinoma: Single-Institution Results from a Large Cohort Study

Rossella Elisei, David Viola, Liborio Torregrossa, Riccardo Giannini, Cristina Romei, Clara Ugolini, Eleonora Molinaro, Laura Agate, Agnese Biagini, Cristiana Lupi, Laura Valerio, Gabriele Materazzi, Paolo Miccoli, Paolo Piaggi, Aldo Pinchera, Paolo Vitti, and Fulvio Basolo

Departments of Endocrinology and Metabolism (R.E., D.V., C.R., E.M., L.A., A.B., L.V., A.P., P.V.) and Surgery (L.T., R.G., C.U., C.L., G.M., P.M., F.B.), University of Pisa 56100 Pisa, Italy; Department of Electrical Systems and Automation (P.P.), University of Pisa, 56100 Pisa, Italy; and World Health Organization Collaborating Center for the Study and Treatment of Thyroid Diseases and Other Endocrine and Metabolic Disorders (R.E., D.V., C.R., E.M., L.A., A.B., A.P., P.V.), University of Pisa, 56124 Pisa, Italy

Background: The *BRAF*^{V600E} mutation, the most frequent genetic alteration in papillary thyroid carcinoma (PTC), was demonstrated to be a poor prognostic factor. The aim of this study was to evaluate its prognostic significance in a large cohort of low-risk intrathyroid PTC.

Methods: Among the 431 consecutive PTC patients, we selected 319 patients with an intrathyroid tumor and no metastases (T1-T2, N0, M0). The *BRAF*^{V600E} mutation was analyzed by PCR-single-strand conformation polymorphism analysis and direct genomic sequencing. The correlation between the presence/absence of the mutation, the clinical-pathological features, and the outcome of the PTC patients was investigated.

Results: The *BRAF*^{V600E} mutation was present in 106 of 319 PTC patients (33.2%). Its prevalence was also the same in subgroups identified according to the level of risk. The *BRAF*^{V600E} mutation correlated with multifocality, aggressive variant, absence, or infiltration of the tumoral capsule. *BRAF*^{V600E}-mutated PTC also required a higher number of radioiodine courses to obtain disease-free status. The *BRAF*^{V600E} mutation was the only prognostic factor predicting the persistence of the disease in these patients after 5 yr of follow-up.

Conclusions: The *BRAF*^{V600E} mutation was demonstrated to be a poor prognostic factor for the persistence of the disease independent from other clinical-pathological features in low-risk intrathyroid PTC patients. It could be useful to search for the *BRAF*^{V600E} mutation in the workup of low-risk PTC patients to distinguish those who require less or more aggressive treatments. In particular, the high negative predictive value of the *BRAF*^{V600E} mutation could be useful to identify, among low-risk PTC patients, those who could avoid 131-I treatment. **(*J Clin Endocrinol Metab* 97: 4390–4398, 2012)**

Although rare, papillary thyroid carcinoma (PTC) is the most common endocrine malignancy (1), and the incidence of this human tumor type has been growing faster over the last few decades. The most increased PTCs are those measuring less than 1 cm (49%) or less than 2 cm (87%). The increased incidence of small PTCs is attributed

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Abbreviations: PTC, Papillary thyroid carcinoma; SSCP, single-strand conformation polymorphism analysis; Tg, thyroglobulin; TgAb, Tg autoantibody; TNM, tumor node metastasis.

to improved diagnostic techniques for thyroid cancers that are not clinically evident and that do not affect overall mortality (2, 3). However, a real increase of PTC cannot be completely excluded (4, 5). The possibility to discriminate a small PTC with biological behavior that appears more aggressive compared with others with an indolent course would be of great relevance in planning therapeutic strategies.

Alterations of several oncogenes (*i.e. RET/PTC*, *PAX8-PPARγ*, *Ras*, *p53*, and *TRK*) involved in the pathogenesis of thyroid tumors have been identified (6). Recently a thymidine-to-adenine transversion at nucleotide 1799 resulting in a valine to glutamic acid substitution at amino acid 600 ($BRAF^{V600E}$) has been found to be the most common mutation in PTC (7, 8).

The $BRAF^{V600E}$ mutation shows a high specificity for PTC, especially the classic variant, whereas it was never found in follicular and medullary thyroid carcinoma or in benign thyroid neoplasms (9). It is also present in 20–25% of anaplastic thyroid carcinomas, most likely originating from the dedifferentiation of PTC (9). Many authors demonstrated a relationship between the presence of the $BRAF^{V600E}$ mutation and more aggressive clinical and pathological features of PTC not only in large but also in small tumors (8, 10–12). The presence of the $BRAF^{V600E}$ mutation has also been associated with a worse outcome (13, 14) and thyroid cancer-related death (15).

Even if some of the previously mentioned studies analyzed small subgroups of low-risk PTCs, to our knowledge, the correlation between the presence of the $BRAF^{V600E}$ mutation and the outcome of low-risk PTC has not been investigated. The aim of the present study was to evaluate the prognostic significance of the $BRAF^{V600E}$ mutation in a large series of consecutive low-risk intrathyroid PTC patients with a mean follow-up of at least 5 yr.

Patients and Methods

Study group

Between January 2005 and January 2006, we collected tumoral thyroid tissue from 431 patients affected by PTC who consecutively underwent total thyroidectomy at the Department of Surgery and then followed up at the Department of Endocrinology of Pisa University. Cases with a presurgical or intrasurgical suspicion of lymph node metastases were also treated with lymphadenectomy.

The clinical and pathological data of these 431 PTC patients were collected in a computerized database. According to their pathological tumor node metastasis (16) stage, we distinguished our patients into different groups: group A (n = 37) and group B (n = 97) including T1aN0M0 cases, not ablated or ablated with radioiodine (131-I), respectively; group C (n = 185), including T1bN0M0 or T2N0M0 cases; group D (n = 108) including any T N1M0 and T3N0M0 and group E, any T any N M1 (n = 4). All patients included in the last three groups were treated with 131-I for remnant ablation and for metastatic disease when indicated.

For the purpose of this study, we selected 319 of 431 (74%) PTC patients with an intrathyroid tumor and no evidence of lymph node or distant metastases at diagnosis who were included in group A, group B, and group C.

According to the standard procedures used in our institution since 1991, the majority of these patients (280 of 282, 99.3%) were treated with a low activity (30 mCi) of 131-I for thyroid remnant ablation; 100–150 mCi activities of 131-I were administered, one or several times, to those who were not ablated with the first 131-I treatment (22 of 282, 7.8%). Patients were followed up to the present time with a mean follow-up of 5.3 ± 0.8 yr.

Patients signed an informed consent for the study which was approved by the Internal Reviewing Board.

Clinical-pathological features of the study group and outcome

Clinical and pathological data were retrieved from a computerized database, in which all patients followed at the Department of Endocrinology of Pisa are recorded.

Currently all patients included in the present study are still actively followed up with controls every 12–18 months. During the controls, patients are usually submitted to a physical examination, a basal serum thyroglobulin (Tg) determination, serum anti-Tg autoantibody (TgAb) measurement, neck ultrasound, and/or other imaging procedures according to their disease status.

In agreement with the recent guidelines for the diagnosis and management of thyroid cancer (17, 18), patients were considered as cured (*i.e.* free of disease) when neck ultrasound and diagnostic whole-body scan were negative, basal and TSH-stimulated serum Tg levels were less than 1 ng/ml, and circulating antithyroglobulin antibodies were undetectable. Because the 37 patients of group A did not undergo 131-I remnant ablation, the above-mentioned criteria for the definition of disease-free status was not applicable; thus, they were considered free of disease when the neck ultrasound was negative and the serum Tg or TgAbs did not increase during the follow-up.

Pathology

Two pathologists (L.T. and F.B.) of the Department of Oncology of the University Hospital of Pisa performed the histological diagnosis. The sections obtained from tissues were fixed in 10% neutral buffered formalin, embedded in paraffin blocks, and stained with hematoxylin and eosin for histological examination. The histological diagnosis was made according to the standard classification (19). Several common pathological parameters including the intrathyroidal vascular invasion were analyzed.

DNA extraction

Serial 5-μm-thick sections were taken from paraffin blocks for DNA extraction from the primary tumor. The presence of tumor tissue was confirmed in the first and last section for each section series. Tumor tissue was manually microdissected from one to two sections and samples were submitted to xylene deparaffination and then lysed and digested with proteinase K. DNA extraction was performed using the spin column procedure

(QUIamp minikit; QIAGEN, Valencia, CA) and finally reconstituted in 30 μl of AE buffer.

Detection of *BRAF*^{V600E} mutations by PCR-single-strand conformation polymorphism analysis (SSCP) and DNA sequencing

PCR-SSCP screening of *BRAF*^{V600E} mutation was performed with regard to exon 15 using the following primers: 5′(F)-tca taa tgc ttg ctc tga tag ga-3′, 5′(R)-ggc caa aaa ttt aat cag tgg a-3′ (resulting amplicon for exon 15 is 215 bp). Previously reported conditions were used for both PCR and SSCP (15). Altered migration patterns in two or three independent PCR-SSCP runs were considered to be indicative of DNA mutations. Purified PCR products were then sequenced on an ALF II automated sequencer (Amersham Biosciences, Freiberg, Germany) using Thermo Sequenase Cy5 Dye Terminator cycle sequencing kit (Amersham Biosciences). DNA sequences were compared with those of the normal *BRAF* gene exon 15 in the GenBank database using the Basic Alignment Search Tool software available at the National Center for Biotechnology Information (Bethesda, MD).

DNA extracted from ARO and TPC, two human cell lines (kindly gifted by Dr. James Fagin from Memorial Sloan Kettering Cancer Center, New York, NY) known to be heterozygous and negative for the *BRAF*^{V600E} mutation, respectively, was used as positive and negative controls, respectively.

Statistical analysis

Statistical analysis was performed with a χ^2 test and a Mann-Whitney test to analyze the clinical and pathological data of the patients with and without the *BRAF*^{V600E} mutation. The multiple logistic regression test was used to determine the independent effect of the *BRAF*^{V600E} mutation and the other clinical and pathological features on the outcome of PTC patients. Data analysis was performed using StatView 4.5 software (Abacus Concepts Inc., Berkeley, CA). $P < 0.05$ was regarded to be statistically significant.

Results

Clinical and pathological features and *BRAF*^{V600E} status

Of the 319 low-risk intrathyroid PTC patients, 238 were females and 81 were males. The mean age at diagnosis was 43.4 ± 13.6 yr (median 43 yr, range 13–84 yr) and the mean tumor size was 1.5 ± 0.9 cm (median 1.3 cm, range 0.1–4.0 cm). The absence of the tumor capsule or its infiltration, a more aggressive variant, and multifocality were present in 128 of 268 (47.8%), 42 of 319 (13.2%), and 84 of 319 (26.3%) cases, respectively. According to tumor node metastasis (TNM) classification (16), 33 patients were classified as stage II (10.3%) and 286 patients as stage I (89.7%). The *BRAF*^{V600E} mutation was present in 106 of 319 PTC tumoral tissues (33.2%). As shown in Table 1, the clinical and pathological features of the three subgroups were similar with the exception of the infiltration/absence of tumoral capsule that was unexpectedly more frequent in group B and group A than in group C ($P < 0.0001$).

Relationship between *BRAF*^{V600E} status and clinical and pathological features

Among the 106 *BRAF*^{V600E}-positive cases, 81 were females (76.4%) and 25 were males (23.6%), with a mean age of 41.0 ± 13.9 yr (median 40.5 yr, range 16–84 yr). The

TABLE 1. Clinical-pathological features and *BRAF*^{V600E} status in a series of low-risk intrathyroid PTC patients

Clinical-pathological features	All (n = 319)	Group C (n = 185)[a]	Group B (n = 97)[a]	Group A (n = 37)[a]	*P* value[b]
Age, mean ± SD, median; range (yr)	43.4 ± 13.6 43; 13–84	42.7 ± 13.3 43; 13–84	42.9 ± 14.0 42; 17–79	48.2 ± 13.6 46; 21–70	NS[c]
Age ≥ 45 (yr)	143/319 (44.8%)	79/185 (42.7%)	41/97 (42.3%)	23/37 (62.2%)	NS[c]
Male gender	81/319 (25.4%)	43/185 (23.2%)	25/97 (25.7%)	13/37 (35.1%)	NS
Tumor size, mean ± SD	1.5 ± 0.9	2.1 ± 0.8	0.7 ± 0.2	0.5 ± 0.2	
Median; range (cm)	1.3; 0.1–4.0	2; 1.1–4.0	0.7; 0.2–1.0	0.5; 0.1–0.8	
Tumoral capsule infiltration	128/268 (47.8%)[d]	59/161 (36.6%)[d]	56/83 (67.4%)[d]	13/24 (54.1%)[d]	**<0.0001**
Intrathyroidal vascular invasion	19/264 (7.2%)[d]	14/157 (8.9%)[d]	5/81 (6.2%)[d]	0/26[d]	NS
Aggressive variant[e]	42/319 (13.2%)	24/185 (12.9%)	16/97 (16.5%)	2/37 (5.4%)	
Multifocality	84/319 (26.3%)	46/185 (24.8%)	38/97 (39.2%)	0/37	**0.01**
Stage II	33/319 (10.3%)	33/185 (17.8%)	0/97	0/37	
BRAF^{V600E} mutation	106/319 (33.2%)	58/185 (31.4%)	39/97 (40.2%)	9/37 (24.3%)	NS

NS, Not significant.

[a] Groups A and B: T1aN0M0, not treated or treated with radioiodine (131-I), respectively; group C: T1b-T2N0M0, all treated with 131-I.

[b] Statistically significant *P* values deriving from the analysis of the three groups are reported in *bold*.

[c] The statistical analysis showed a significant younger age in groups C and B with respect to group A, both when the analysis was performed with the Mann-Whitney test and when performed with χ^2 test (<45 *vs.* ≥45).

[d] In a few cases, some clinical-pathological features were unknown.

[e] Aggressive variants: solid, tall cells, trabecular, and columnar; nonaggressive variants: classical, follicular, and oxyphilic.

mean tumor size was 1.4 ± 0.8 cm (median 1.2 cm). Multifocality, a more aggressive variant, the absence of the tumoral capsule or its infiltration, and the intrathyroidal vascular invasion were present in 37 of 106 (34.9%), 24 of 106 (22.6%), 63 of 98 (64.3%), and nine of 96 (9.4%) cases, respectively. According to the TNM classification, six patients were classified as stage II (5.7%) and 100 as stage I (94.3%). Fourteen of 97 PTC patients (14.4%) were required to be treated with further courses of 131-I.

Among the 213 patients without the $BRAF^{V600E}$ mutation, 157 were females (73.7%) and 56 were males (26.3%), with a mean age of 44.6 ± 13.4 yr (median 44 yr). The mean tumor size was 1.6 ± 1.0 cm (median 1.3 cm). Multifocality, a more aggressive variant, the absence of the tumoral capsule or its infiltration, and the intrathyroidal vascular invasion were present in 47 of 213 (22.1%), 18 of 213 (8.5%), 65 of 170 (38.2%), and 10 of 168 (6.0%) cases, respectively. According to the TNM classification, 27 patients were classified as stage II (12.7%) and 186 as stage I (87.3%). Eight of 185 PTC patients (4.3%) were required to be treated with further courses of 131-I.

As shown in Table 2, when the epidemiological and pathological features of the 319 low-risk intrathyroid PTCs were compared according to the presence or absence of $BRAF^{V600E}$ mutation, the univariate analysis revealed no correlation between the $BRAF^{V600E}$ mutation with gender ($P = 0.6$), tumor size ($P = 0.2$), stage ($P = 0.052$), and intrathyroidal vascular invasion ($P = 0.3$). Conversely, the presence of the $BRAF^{V600E}$ mutation was significantly associated with a younger age ($P = 0.03$) when the analysis was performed with the Mann-Whitney test, multifocality ($P = 0.02$), a more aggressive variant ($P = 0.0004$), and the absence of tumoral capsule or its infiltration ($P < 0.0001$). The presence of the $BRAF^{V600E}$ mutation was also associated with a higher number of 131-radioiodine administered courses ($P = 0.003$). As shown in Table 3, similar results were obtained in group C, whereas only the correlation between the absence of tumoral capsule or its infiltration and the $BRAF^{V600E}$ mutation was confirmed in groups B and A.

According to the outcome, after 5 yr of follow-up, 24 of 319 patients (7.5%) showed persistent disease. The details of the type of persistence and the relationship with the $BRAF^{V600E}$ mutation are reported in Table 4. In particular, among patients with a macroscopic persistent disease, the eight *BRAF*-positive cases were all positive at neck ultrasound for local disease, but only one showed neck 131-I uptake. The total body computerized tomography was positive for mediastinal lymph nodes in one case and completely negative in all the other cases. Conversely, the two *BRAF*-negative patients with macroscopic persistent disease showed extrathyroid 131-I uptake (*i.e.* lung and bone, respectively).

According to the correlation of the presence of $BRAF^{V600E}$ mutation and the disease status at the end of this median term follow-up, we found that the negative predictive value of $BRAF^{V600E}$ mutation (*i.e.* patients disease free and without $BRAF^{V600E}$ mutation) was 96.7%, whereas the positive predictive value (*i.e.* patients with persistent disease and with $BRAF^{V600E}$ mutation) was only 16% when calculated in the entire group (Fig. 1, panel 1). Similarly, the negative predictive value and the positive predictive value were 96.1% and 19% in group C (Fig. 1, panel 2) and 96.6% and 15.4% in group B (Fig. 1, panel 3).

Correlation of $BRAF^{V600E}$ and other clinical-pathological features of PTC with the outcome

As shown in Table 5, the univariate analysis of clinical and pathological features potentially influencing the outcome of our PTC patients revealed that, in this series of

TABLE 2. Correlation between clinical-pathological features and $BRAF^{V600E}$ mutation

Clinical-pathological features	*BRAF*+ (n = 106)	*BRAF*− (n = 213)	*P* value[a]
Age, mean ± SD	41.0 ± 13.9	44.6 ± 13.4	**0.03**
Median (yr)	40.5	44	
Age ≥ 45 yr	41/106 (38.7%)	102/213 (47.9)	0.2
Male gender	25/106 (23.6%)	56/213 (26.3%)	0.6
Tumor size, mean ± SD	1.4 ± 0.8	1.6 ± 1.0	0.2
Median (cm)	1.2	1.3	
Multifocality	37/106 (34.9%)	47/213 (22.1%)	**0.02**
Aggressive variant[b]	24/106 (22.6%)	18/213 (8.5%)	**0.0004**
Tumoral capsule infiltration	63/98 (64.3%)[c]	65/170 (38.2%)[c]	**<0.0001**
Intrathyroidal vascular invasion	9/96 (9.4%)[c]	10/168 (6.0%)[c]	0.3
Stage II	6/106 (5.7%)	27/213 (12.7%)	0.052
Treatments $^{131}I > 1$, n	14/97 (14.4%)[d]	8/185 (4.3%)[d]	**0.003**

[a] Statistically significant *P* values are reported in *bold*.

[b] Aggressive variants: solid, tall cells, trabecular, and columnar; nonaggressive variants: classical, follicular, and oxyphilic.

[c] In a few cases, some clinical-pathological features were unknown.

[d] Nine *BRAF*+ and 28 *BRAF*− patients belonging to group A were not submitted to 131-I ablation.

TABLE 3. Correlation between clinical-pathological features and *BRAF*^{V600E} mutation in the three subgroups of low-risk intrathyroid PTC patients

Clinical-pathological features	Group C (n = 185)[a]			Group B (n = 97)[a]			Group A (n = 37)[a]		
	BRAF+ (n = 58)	*BRAF*− (n = 127)	*P* value[b]	*BRAF*+ (n = 39)	*BRAF*− (n = 58)	*P* value[b]	*BRAF*+ (n = 9)	*BRAF*− (n = 28)	*P* value[b]
Age, mean ± SD	41.6 ± 14.3	43.3 ± 12.9	0.4	39.5 ± 13.2	45.3 ± 14.3	0.07	44.0 ± 15.1	49.5 ± 13.1	0.2
Median (yr)	41.5	43		40	43		40	46	
Age ≥ 45 yr	21/58 (36.2%)	58/127 (45.7%)	0.2	16/39 (41.0%)	25/58 (43.1%)	0.8	4/9 (44.4%)	19/28 (67.9%)	0.2
Male gender	14/58 (24.1%)	29/127 (22.8%)	0.8	9/39 (23.1%)	16/58 (27.6%)	0.6	2/9 (22.2%)	11/28 (39.3%)	0.4
Tumor size, mean ± SD	1.9 ± 0.7	2.2 ± 0.8	0.053	0.7 ± 0.2	0.7 ± 0.2	0.6	0.5 ± 0.2	0.4 ± 0.2	0.6
Median (cm)	1.9	2.0		0.7	0.7		0.5	0.4	
Tumoral capsule infiltration	27/53 (50.9%)[c]	32/108 (29.6%)[c]	**0.008**	30/38 (78.9%)[c]	26/45 (57.8%)[c]	**0.04**	6/7 (85.7%)[c]	7/17 (41.2%)[c]	**0.046**
Intrathyroidal vascular invasion	6/51 (11.8%)[c]	8/106 (7.5%)[c]	0.4	3/37 (8.1%)[c]	2/44 (4.5%)[c]	0.5	0/8[c]	0/18[c]	ND
Aggressive variant[d]	13/58 (22.4%)	11/127 (8.7%)	**0.01**	9/39 (23.1%)	7/58 (12.1%)	0.1	2/9 (22.2%)	0/28	**0.01**
Multifocality	22/58 (37.9%)	24/127 (18.9%)	**0.006**	15/39 (38.5%)	23/58 (39.7%)	0.9	0/9	0/28	ND
Stage II	6/58 (10.3%)	27/127 (21.2%)	0.09	0/39	0/58	ND	0/9	0/28	ND
Treatments $^{131}I > 1$, n	11/58 (19.0%)	7/127 (5.5%)	**0.004**	3/39 (7.7%)	1/58 (1.7%)	0.14	0/9	0/28	ND

ND, Not determined.

[a] Groups A and B: T1aN0M0, not treated or treated with radioiodine (131-I), respectively; group C: T1b-T2N0M0, all treated with 131-I.

[b] Statistically significant *P* values are reported in *bold*.

[c] In a few cases, some clinical-pathological features were unknown.

[d] Aggressive variants: solid, tall cells, trabecular, and columnar; nonaggressive variants: classical, follicular, and oxyphilic.

intrathyroidal PTC, none of the classical poor prognostic features (*i.e.* age, gender, tumor size, aggressive variant, multifocality, tumoral capsule infiltration, and TNM stage) were able to predict the persistence of disease except for the presence of the *BRAF*^{V600E} mutation ($P < 0.0001$) and intrathyroidal vascular invasion ($P = 0.03$). However, although the *BRAF*^{V600E} mutation strongly correlated with the outcome when the analysis was restricted to the two C and B subgroups, this correlation was lost for the intrathyroidal vascular invasion (Table 5).

The multivariate logistic regression analysis confirmed that *BRAF*^{V600E} mutation was the only poor prognostic factor for the persistence of the disease both in the entire group ($P = 0.0014$) and in group C ($P = 0.014$) but not in group B ($P = 0.11$) (data not shown).

Discussion

Due to the increasing incidence of small PTC in the last decades (2, 20), there is a question of how aggressively these patients need to be treated. This dilemma is even more important, considering that the increased incidence is the result of detecting small tumors, which will likely never become clinically relevant (2). For this reason, the appropriate treatment of small tumors is largely disputed between American experts (17), who treat these patients with minimal surgical treatment (*i.e.* lobectomy alone), and European experts (18), who suggest the more aggressive total thyroidectomy followed by the administration of 131-I anytime a presurgical diagnosis of PTC is performed. The only exception to 131-I ablation treatment occurs when small PTCs (≤1 cm) that are unifocal and nonaggressive variants are detected. The question of the aggressiveness of the initial treatment is difficult to answer because, even if the mortality of patients with small PTC is rare, a low percentage of these patients can show lymph nodes metastases or a persistent/recurrent disease after surgery and, in some cases, an unfavorable outcome (21, 22). For these reasons, there is a need to distinguish small, low-risk intrathyroid PTC with an indolent course from those that are more difficult to be cured.

TABLE 4. Low-risk intrathyroid PTC patients with persistent disease after 5 yr of follow-up

	All (n = 319)		Group C (n = 185)[a]		Group B (n = 97)[a]		Group A (n = 37)[a]	
	BRAF+ (n = 106)	*BRAF*− (n = 213)	*BRAF*+ (n = 58)	*BRAF*− (n = 127)	*BRAF*+ (n = 39)	*BRAF*− (n = 58)	*BRAF*+ (n = 9)	*BRAF*− (n = 28)
Persistent disease								
All	17 (16%)	7 (3.3%)	11 (19%)	5 (3.9%)	6 (15.4%)	2 (3.4%)	0	0
Biochemical[b]	9 (8.5%)	5 (2.3%)	4 (6.9%)	3 (2.4%)	5 (12.8%)	1 (1.7%)	0	0
Macroscopic[c]	8 (7.5%)	2 (1.0%)	7 (12.1%)	2 (1.5%)	1 (2.6%)	1 (1.7%)	0	0

[a] Groups A and B: T1aN0M0, not treated or treated with radioiodine (131-I), respectively; group C: T1b-T2N0M0, all treated with 131-I.

[b] Basal or stimulated Tg greater than 1 ng/ml and/or detectable levels of TgAb in low and very low-risk PTC; increasing serum Tg values in very very low-risk PTC.

[c] Positive neck ultrasound and/or posttherapeutic whole-body scan.

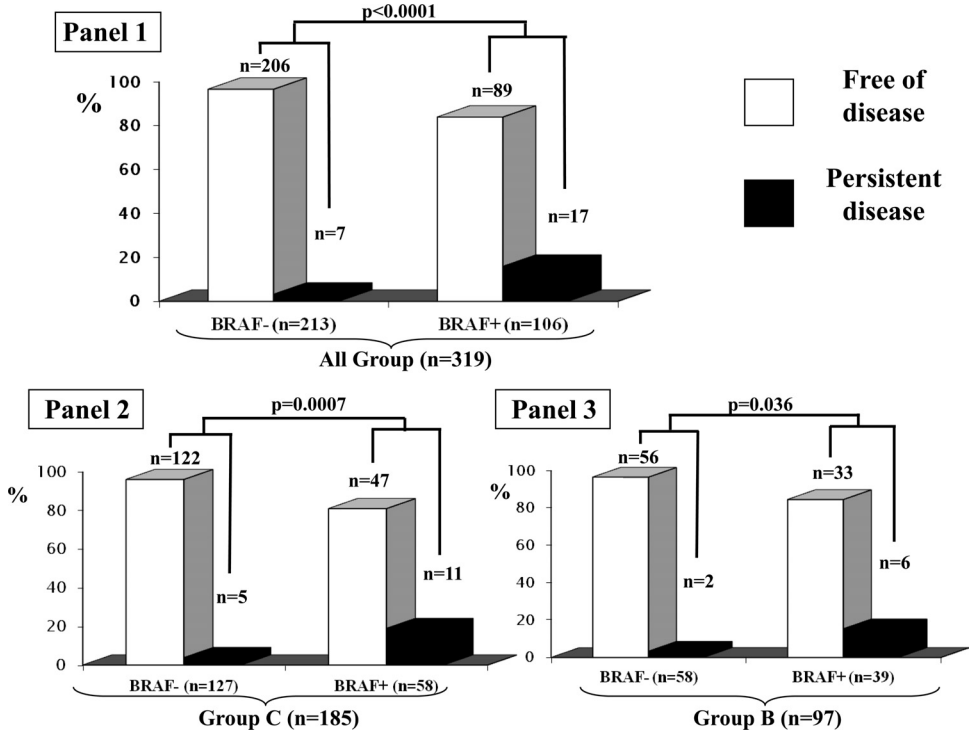

FIG. 1. Correlation between the disease status and the *BRAF*^{V600E} mutation in 319 low intrathyroid PTC. A statistically significant higher prevalence of persistent disease was found in cases with the *BRAF*^{V600E} mutation with respect to those without the *BRAF*^{V600E} mutation in the entire study group (panel 1) as well as in group B (T1aN0M0) (panel 2), and in group C (T1b-T2N0M0) (panel 3). In group A (T1aN0M0, not treated with 131-I), this analysis was meaningless because no persistent disease cases were observed. The statistical analysis was performed with a χ^2 test.

Recently the *BRAF*^{V600E} mutation was confirmed to be one of the most relevant prognostic factors influencing the persistence/recurrence of the disease and the survival of PTC patients (13–15). Thus, it could be useful to preoperatively evaluate its presence in the tumoral tissue to identify patients that are more likely to develop a persistent/recurrent disease (23) or those with a higher risk to have extrathyroidal 131-I uptake, which determines a change in the risk level (24). Our group recently demonstrated that the *BRAF*^{V600E} mutation also is present in small PTCs and

TABLE 5. Correlation of *BRAF*^{V600E} mutation and other clinical-pathological features of low-risk intrathyroid PTC with the outcome

Clinical-pathological features	All (n = 319)			Group C (n = 185)[a]			Group B (n = 97)[a]		
	Persistent disease (n = 24)	Free of disease (n = 295)	*P* value[b]	Persistent disease (n = 16)	Free of disease (n = 169)	*P* value[b]	Persistent disease (n = 8)	Free of disease (n = 89)	*P* value[b]
Age, mean ± SD	38.7 ± 15.3	43.8 ± 13.5	0.09	40.0 ± 17.1	43.0 ± 13.0	0.4	36.1 ± 11.5	43.6 ± 14.2	0.2
Median	36.5	43		37.5	43		36.5	42	
Age ≥ 45 yr	8/24 (33.3%)	135/295 (45.8%)	0.2	6/16 (37.5%)	73/169 (43.2%)	0.7	2/8 (25.0%)	39/89 (43.8%)	0.3
Male gender	7/24 (29.2%)	74/295 (25.1%)	0.7	6/16 (37.5%)	37/169 (21.9%)	0.2	1/8 (12.5%)	24/89 (27.0%)	0.4
Tumor size, mean ± SD	1.7 ± 0.9	1.5 ± 1.0	0.09	2.1 ± 0.7	2.1 ± 0.8	0.7	0.7 ± 0.2	0.7 ± 0.2	0.8
Median (cm)	1.7	1.3		2.0	2.0		0.8	0.7	
Tumoral capsule infiltration	12/22 (54.5%)[c]	116/246 (47.2%)[c]	0.5	7/14 (50.0%)[c]	52/147 (35.4%)[c]	0.3	5/8 (62.5%)	51/75 (68.0%)[c]	0.8
Intrathyroidal vascular invasion	4/21 (19.0%)[c]	15/243 (6.2%)[c]	**0.03**	3/13 (23.0%)[c]	11/144 (7.6%)[c]	0.06	1/8 (12.5%)	4/73 (5.4%)[c]	0.4
Aggressive variant[d]	3/24 (12.5%)	39/295 (13.2%)	0.9	2/16 (12.5%)	22/169 (13.0%)	0.9	1/8 (12.5%)	15/89 (16.9%)	0.8
Multifocality	7/24 (29.2%)	77/295 (26.1%)	0.8	5/16 (31.3%)	41/169 (24.3%)	0.5	2/8 (25.0%)	36/89 (40.4%)	0.4
Stage II	1/24 (4.2%)	32/295 (10.8%)	0.3	1/16 (6.3%)	32/169 (18.9%)	0.2			
BRAF^{V600E} mutation	17/24 (70.8%)	89/295 (30.2%)	**<0.0001**	11/16 (68.8%)	47/169 (27.8%)	**0.0007**	6/8 (75.0%)	33/89 (37.1%)	**0.036**

[a] Group B: T1aN0M0, treated with radioiodine (131-I); group C: T1b-T2N0M0, all treated with 131-I. The total group includes group A patients who were all disease free at the time of the present study; no correlation analysis was performed in this subgroup because no cases with persistent disease were present.

[b] Statistically significant *P* values are reported in *bold.*

[c] In a few cases, some clinical-pathological features were unknown.

[d] Aggressive variants: solid, tall cells, trabecular, and columnar; nonaggressive variants: classical, follicular, and oxyphilic.

that in this subgroup of PTCs, it correlates with the presence of lymph node metastases, tumoral capsular invasion, and other pathological features of aggressiveness (25). To our knowledge, no studies on the prognostic value of the *BRAF*^{V600E} mutation in low-risk intrathyroid PTC with a medium long-term follow-up have been reported so far.

In this study, we evaluated the prognostic significance of the *BRAF*^{V600E} mutation in a large series of consecutive low-risk intrathyroid PTC patients accurately selected for not having node metastases or extracapsular invasion at diagnosis, and we further distinguished these patients into three levels of risk to verify whether the presence of the mutation could be useful for distinguishing, among them, those with a greater difficulty in being cured.

Our study confirms that the prevalence of *BRAF*^{V600E} mutation in intrathyroid PTC (33.2%) is similar to that reported in the literature in unselected PTCs. In particular, we observed that this prevalence was similar when the entire study group was divided into the three groups: C (31.4%), B (40.2%), and A (24.3%). These findings confirm that the *BRAF*^{V600E} mutation is an early event in PTC and, very likely, a leading transforming event (26).

When we correlated the presence of the *BRAF*^{V600E} mutation with the clinical and pathological features of intrathyroid PTC, we found that, as previously found by us and other authors (10, 25, 28) in unselected PTC series, *BRAF*^{V600E} mutation correlated with the presence of multifocality, the more aggressive variant, and the absence or the infiltration of the tumoral capsule. The presence of *BRAF*^{V600E} mutation correlated with the absence or the infiltration of the tumoral capsule also in the subgroups B and A. It is known that the absence or the infiltration of the tumoral capsule is one of the most important negative prognostic factors (29, 30) for the cure of PTC patients. This fact is particularly true for the PTC follicular variant, in which the integrity or the infiltration of the tumoral capsule correlates with the presence/absence of the *BRAF*^{V600E} mutation and the biological behavior of the tumors (31). However, the tumoral capsular invasion is a histological finding that we can only determine after the surgical treatment, whereas the *BRAF* mutation can be detected presurgically.

We also found that in this series of low-risk intrathyroid PTC patients, those harboring the *BRAF*^{V600E} mutation were submitted to a higher number of 131-I courses. The interpretation of this finding is not intuitive because the rationale of the radioiodine ablation treatment is mainly represented by the need to eliminate normal thyroid tissue that should not harbor the mutation. A possible explanation is that in the mutated cases, tumoral foci may be more frequently present in the postsurgical remnant. As matter of fact, the *BRAF*^{V600E} mutation correlates with multifocality both in the present study and also in others (15).

As far as the relationship between the presence of *BRAF*^{V600E} mutation and the lower ability to take up 131-I (32, 33) is concerned, we observed that among patients with macroscopic persistent disease, only one of eight of *BRAF*^{V600E}-mutated and two of two of *BRAF*^{V600E}-nonmutated cases were positive at the posttherapeutic 131-I whole-body scan. Although the number of our cases with persistent disease is relatively low, these results are consistent with previous observations (33, 34).

Because many other clinical and pathological features can play a role as prognostic factors (27), we investigated all features that may influence the outcome of our group of intrathyroid PTC patients (*i.e.* age at diagnosis, gender, tumor size, absence or infiltration of tumoral capsule, aggressive variant, multifocality, and TNM stage). With the exception of the intrathyroidal vascular invasion, which was significantly correlated with a worse outcome in the entire group, none of these features were recognized as an important prognostic factor for the persistence of the disease in this restricted group of low-risk intrathyroid PTC. This finding was unexpected; however, because no studies on conventional prognostic factors in low-risk PTC patients have been reported so far, we hypothesize that in this subgroup of PTC, there are no clinical or pathological prognostic factors. Indeed, at the multivariate analysis, the only significant predictor of persistent disease both in the entire group and in the patients of groups C and B was the presence of the *BRAF*^{V600E} mutation.

In conclusion, this study demonstrated that *BRAF*^{V600E} mutation correlated with a persistent disease after 5 yr of follow-up and that the patients with the *BRAF*^{V600E} mutation in the primary tissue are usually submitted to a greater number of radioiodine courses, thus confirming the poor prognostic role of the *BRAF*^{V600E} mutation in low-risk intrathyroid PTC. On the basis of our finding, it is conceivable to include a search of the *BRAF* mutation in the workup of low-risk intrathyroid PTC to distinguish those requiring more aggressive treatments from those who can be treated with a more conservative approach, as suggested by American Thyroid Association guidelines (17). In particular, both the high negative predictive value of *BRAF*^{V600E} mutation for the persistence of disease and the fact that in the present study, a relatively aggressive treatment was used in the majority of cases suggest to use a less aggressive treatment for *BRAF*^{V600E}-negative other than a more aggressive treatment for *BRAF*^{V600E}-positive intrathyroid PTC.

Acknowledgments

Address all correspondence and requests for reprints to: R. Elisei, M.D., Department of Endocrinology, University of Pisa, Via Paradisa 2, 56124 Pisa, Italy. E-mail: relisei@endoc.med.unipi.it.

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