**BRAF**<sup>K601E</sup> Mutation in a Follicular Thyroid Adenoma: A Case Report

Elisabetta Macerola, PhD<sup>1</sup>, Liborio Torregrossa, MD, PhD<sup>2</sup>, Clara Ugolini, MD<sup>2</sup>, Sohail Bakkar, MD, PhD<sup>1</sup>, Paolo Vitti, MD<sup>1</sup>, Guido Fadda, MD<sup>3</sup>, and Fulvio Basolo, MD<sup>1</sup>

**Abstract**

*BRAF* mutations represent the most common genetic alteration in papillary thyroid carcinoma (PTC). The p.V600E mutation is specific for the classic and tall-cell variants of PTC and has been associated with a more aggressive biologic behavior. On the other hand, the p.K601E mutation is peculiar to the follicular variant of PTC, and seems to be a favorable prognostic indicator. A 12-year-old boy presented with a 10-mm left-sided thyroid nodule. Fine-needle aspiration cytology reported the lesion as suspicious for a follicular neoplasm (Bethesda category IV). The patient underwent lobectomy, and histopathology revealed a follicular adenoma with normal surrounding tissue. The cytological smear was found to be positive for *BRAF* p.K601E mutation, and this was later confirmed on the corresponding paraffin block. This case was independently revised by 4 expert pathologists, all of whom confirmed the benign nature of the thyroid lesion. This article describes the presence of a *BRAF* mutation in a benign thyroid lesion. To the authors’ knowledge, this is the fourth case of follicular adenoma carrying *BRAF<sup>K601E</sup>* reported in literature to date. *BRAF<sup>K601E</sup>* mutation can occur in benign thyroid lesions. This finding, in the context of the current literature and the recently proposed reclassification of the noninvasive encapsulated follicular variant of papillary thyroid carcinoma into a benign lesion, confirms the importance of preoperative *BRAF* p.K601E testing in offering patients a tailored treatment plan and avoiding overtreatment.

**Keywords**

thyroid pathology, *BRAF* mutation, *BRAF* K601E, follicular adenoma, thyroid cancer

**Introduction**

Thyroid nodules are frequently encountered in clinical practice. Their prevalence largely depends on the population being evaluated and the detection method used. A prevalence rate as high as 67% has been reported with the use of high-resolution ultrasonography. The main objective of the diagnostic evaluation of a thyroid nodule is to determine its nature, as this allows offering patients an optimal treatment modality. Cytology is the gold standard diagnostic modality for determining the nature of a thyroid nodule. However, it yields a reliable diagnosis in only 60% to 70% of cases. Accordingly, in 20% to 30% cases, distinguishing malignant nodules from benign ones is not possible based on cytology alone. At times, the need for surgery as a diagnostic means may even emerge. In order to maximize the diagnostic yield of cytology some have turned to molecular diagnostic and/or their combination with certain ultrasonographic features. *BRAF* mutations are well known for their association with malignancy. These represent the most common genetic alterations detected in papillary thyroid carcinoma (PTC). The p.V600E mutation (c.1799T>A) is highly specific for the classic and tall-cell variants of PTC, and is also common in poorly differentiated and anaplastic thyroid carcinomas. This mutation has been widely studied in thyroid cancer, and was found to be associated with an aggressive biologic behavior. On the other hand, the *BRAF* p.K601E mutation (c.1801A>G) is peculiar to the follicular variant of PTC (FVPTC), however, with a low frequency of occurrence. Recently, the prognostic significance of this mutation has been extensively investigated. It came to light that *BRAF<sup>K601E</sup>* was associated with a favorable prognosis, and that its preoperative identification could help avoid overly radical surgery; patients positive for this mutation could be

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1. University of Pisa, Pisa, Italy
2. University Hospital of Pisa, Pisa, Italy
3. Catholic University, Foundation Agostino Gemelli University Hospital, Rome, Italy

**Corresponding Author:**
Fulvio Basolo, Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, via Savi 10–56126 Pisa, Italy. Email: fulvio.basolo@med.unipi.it
offered a lobectomy rather than a total thyroidectomy. Interestingly, the p.K601E has been reported in 2 cases of follicular thyroid carcinoma (FTC)\textsuperscript{9,10} and unexpectedly, in 3 cases of follicular adenoma.\textsuperscript{6,11,12}

Patient

In June 2015, a 12-year-old boy presented to the authors’ institute with a 10-mm left-sided thyroid nodule. Fine-needle aspiration cytology (FNAC) revealed a microfollicular lesion with mild cytonuclear atypia (a TIR3B lesion according to the Italian consensus guidelines for the classification and reporting of thyroid cytology\textsuperscript{3}; this corresponds to Bethesda category IV\textsuperscript{13}). At the authors’ institute, such cytology is routinely analyzed for the BRAF oncogene. The DNA was purified from the follicular cells of the stained smear, carefully selected by a pathologist, and isolated by manual macrodissection. The molecular analysis was subsequently performed by pyrosequencing. DNA was amplified using a validated kit (anti-EGFR MoAb response: BRAF status - Diatech Pharmacogenetics, Jesi, Italy), on a Rotor-Gene TM6000 (Corbett Research, Sydney, Australia), according to the manufacturer’s instructions. The reaction products were run on a PyroMark Q96 ID system, and the results were analyzed with PyroMark Q24 1.0.9 software (Biotage AB, Uppsala, Sweden). The cytological smear was found to be positive for the p.K601E mutation.

Because of the small nodule size and the absence of any other clinical features suggestive of malignancy, the patient underwent a thyroid lobectomy. A frozen section examination was performed and was unyielding (microfollicular neoplasm). At histologic examination, the specimen was grossly found to contain a single 10-mm nodule. Microscopically, the lesion was found to be a follicular adenoma with a microfollicular architecture. Nuclei tended to be round, and no nuclear atypia was observed, as well as no overlapping nor chromatin clearing were identified (see Figure 1). The adenoma was surrounded by a thin fibrous capsule without any signs of invasion (Figure 2a).

\textit{BRAF} sequence analysis was performed on the formalin-fixed paraffin-embedded tissue sample as described earlier and the p.K601E mutation was confirmed. In light of the molecular result, the histological slides were

\begin{figure}[h]
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\includegraphics[width=\textwidth]{image1.png}
\caption{Histological specimens of the follicular thyroid adenoma carrying \textit{BRAF}\textsuperscript{K601E} mutation. (a) Partial areas with pseudopapillary structures; there is no well-developed central fibrovascular core and the stroma encloses numerous follicles; at high magnification (b), the follicular cells lining these structures are cuboidal or columnar, with basally located nuclei that tend to be round and normo- or hyperchromatic (hematoxylin and eosin stain, ×10 and ×40 original magnification, respectively). (c) Prevalent microfollicular growth of this tumor; these hypercellular areas are composed by small follicles with minimal amount of colloid; at high magnification (d), the follicles are lined by follicular cells with normo- or hyperchromatic nuclei that are round with smooth contours (hematoxylin and eosin stain, ×10 and ×40 original magnification, respectively).}
\end{figure}
independently revised by 4 expert pathologists (CU, LT, GF, and FB) to detect any potential malignant features. However, none of them did. Moreover, in order to better characterize the lesion an immunohistochemistry (IHC) analysis was performed for a routine panel of markers (HBME-1, Galectin-3, and CD56). Proper positive and negative controls were included for each marker (data not shown). The lesion showed a strong positivity for HBME-1, a focal and weak immunoreactivity for CD56, and complete negativity for Galectin-3 (Figure 2). Since the immunoreactivity for HBME-1 alone is not sensitive enough to render a diagnosis of malignancy,\textsuperscript{14,15} the diagnosis of follicular adenoma was maintained exclusively on the basis of morphological appearance.

**Discussion**

Over the past decade, \textit{BRAF} mutations have been widely studied across thyroid cancer types. These mutations have been almost always identified in malignant tumors. The only exceptions were three cases of follicular adenoma, all carrying the p.K601E substitution.\textsuperscript{6,11,12} The p.V600E mutation has been strongly associated with poor clinical outcome, whereas the p.K601E mutation has been linked to tumors with peculiar phenotypic features, the follicular variant of PTC, in particular.

The 2015 study by Afkhami et al\textsuperscript{6} focusing on the significance of the \textit{BRAF}\textsuperscript{p.K601E} mutation in thyroid cancer, confirmed the strong association between this mutation and the follicular variant of PTC. In their study, the p.K601E mutation was found to prevail in encapsulated follicular variant PTCs, and tumors carrying this alteration showed a favorable clinical outcome. Similar results have been confirmed later by Torregrossa et al.\textsuperscript{7}

The proposed reclassification of the noninvasive encapsulated follicular variant PTC to a “noninvasive follicular neoplasm with papillary-like nuclear features” (NIFTP),\textsuperscript{16} will certainly increase the proportion of lesions considered as low risk. This in turn would reflect on the distribution of the p.K601E mutation; more benign lesions are likely to be found harboring this mutation. Despite the need for a full understanding of the molecular characteristics of NIFTP, the presence of the p.K601E has been described in 1 out of 27 cases of NIFTP analyzed in the study that proposed the new nomenclature.\textsuperscript{16} Since the diagnosis of NIFTP can be made only by excluding the presence of capsular invasion,
surgical excision of the nodule is necessary. In this context, the value of molecular testing performed on cytological specimens must not be underscored. This strongly confirms the importance of $BRAF^{K601E}$ preoperative testing in avoiding overtreatment of patients.

This study describes the presence of a $BRAF$ mutation in a benign thyroid lesion. To the authors’ knowledge, this is the fourth case of follicular adenoma carrying $BRAF^{K601E}$ reported in literature to date. Even if the presence of this alteration cannot independently determine the nature of a thyroid nodule, its presence is strongly suggestive of a neoplasm with a low malignant potential. Therefore, the detection of this mutation in presurgical cytological smears might guide clinical decision making toward a more conservative treatment strategy.

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