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■ CASE STUDY ■

A Case of Multifocal Papillary Thyroid Carcinoma Consisting of One Encapsulated Follicular Variant with *BRAF* K601E Mutation and Three Conventional Types with *BRAF* V600E Mutation

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Multifocal papillary thyroid carcinoma (mPTC) comprises about 20-30% of PTC. In mPTC, individual tumor foci can be identical or frequently composed of different histological types including follicular, solid, tall-cell or conventional patterns. We report a case of mPTC consisting of one encapsulated follicular variant of papillary thyroid carcinoma (FVPTC) and three conventional PTCs in a 44-year-old woman. This case genetically demonstrates unique features including the simultaneous presence of the *BRAF* V600E (T1799A) mutation and the *BRAF* K601E (A1801G) mutation in conventional PTC and FVPTC, respectively.

Key Words: Thyroid gland; Carcinoma, papillary; *BRAF* V600E; *BRAF* K601E

Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer. There are several histopathological variants other than the conventional type, and among these variants the follicular variant (FVPTC) is the most common.[1] Although FVPTC has been recognized for more than 50 years, its diagnostic features, molecular mechanisms involved and patient prognosis remain unclear.[1] FVPTC has a wide range of observer variation in diagnosis and its actual proportion in PTC varies depending on the study.

The *BRAF* V600E mutation has been established as a reliable genetic marker of PTC with a prevalence of 45% to 91.1% of all conventional PTCs.[2,3] However, this mutation is absent or less commonly detected in FVPTC. FVPTC is characterized and identified by another distinct *BRAF* mutation, K601E, which has been detected with a relatively low frequency of 7%.[4] In addition to these differences in *BRAF* mutations, FVPTC also exhibits different genetic alterations, which include increased rates of allelic imbalance and aneuploidy as compared to conventional PTCs.[5-7]

Multifocal PTCs (mPTCs) are found in about 20% to 30% of all PTCs according to previously published reports.[8] In mPTC, individual tumor foci can be identical or frequently composed of different histological types including follicular, solid, tall-cell or conventional patterns. These mPTC foci may occur from intraglandular metastases from a single dominant tumor, or arise independently as unrelated neoplastic clones from a distinct progenitor cell.[9,10] There have been several previous studies about the origin of mPTC foci with evidence supporting both arguments, i.e., *de novo* carcinogenesis or intrathyroidal metastasis. By determining the presence of a polymorphism in the X-linked androgen-receptor gene or heterogeneous distribution of *BRAF* mutations among noncontiguous, discrete tumor foci of mPTC,

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some researchers have previously suggested that mPTC often arises independently.[9,10] In contrast, Jovanovic *et al.*[11] recently showed that the majority of mPTCs demonstrate the features of monoclonal derivation in spite of morphological diversity of each tumor foci in analysis based on allelic imbalances and the *BRAF* V600E mutation, suggesting intrathyroidal spread from a primary tumor. In another recent report by Jovanovic *et al.*,[5] FVPTC foci of mPTCs had different patterns of loss of heterozygocity and *BRAF* V600E from other foci in spite of monoclonality and this was similar to the above-mentioned results comparing solitary FVPTC and other PTCs. The complexity of arguments about the origin of mPTC suggests that mPTC may arise through diverse and heterogeneous genetic mechanisms.

Here, we describe a genetically unique case of mPTC consisting of one encapsulated follicular variant harboring the *BRAF* K601E mutation and three conventional types harboring the *BRAF* V600E mutation.

CASE REPORT

A 44-year-old female was referred from a local clinic for a papillary carcinoma of the thyroid that was diagnosed by fine needle aspiration. The patient did not have a past history of neck radiation or a family history of thyroid cancer. Thyroid ultrasonography revealed a spiculated, hypoechoic solid nodule measuring 0.8 cm in diameter with a capsular invasion at the left paraisthmic area of the thyroid. The ultrasonography also revealed three smooth, oval, and slightly hypoechoic solid nodules measuring 2.3 cm, 1.3 cm, and 0.8 cm in diameter at the right upper, middle, and lower lobes, respectively. The lymph nodes at the central and lateral neck were not significantly enlarged. Total thyroidectomy with central node dissection was performed.

Gross findings

The specimen consisted of a reddish brown total thyroidectomy tissue. The right thyroid gland measured 4.5×2.3×2.0 cm, and the left thyroid gland measured 4.5×2.3×1.7 cm. Both glands weighed 20 g in total. On the serial cut sections of the right thyroid, there were two ill-defined grayish white solid masses measuring 1.2×0.9×0.6 cm and 2.1×1.9×1.5 cm at the upper and lower poles, respectively. At the right mid pole, there also was a well-defined tan, yellow, solid mass measuring 1.3×1.1×0.8 cm. On the serial cut sections of the left lobe, there was an ill-defined grayish white nodule measuring 0.8×0.6×0.5 cm at the paraisthmic area.

Microscopic findings

Histological examination revealed three conventional PTCs in the right upper and lower poles and also in the left paraisthmic area. All the conventional PTCs revealed an infiltrative growth pattern with typical histological features. The mass at the right mid pole was characterized by total encapsulation without papilla formation or psammoma bodies and corresponded to the encapsulated variant of FVPTC. Characteristic nuclear features, particularly nuclear enlargement, chromatin clearing and irregular nuclear contours were present. Nuclear grooves were present, but not frequently. Deeply eosinophilic colloid with peripheral scalloping was also present (Fig. 1). Three perithyroidal lymph nodes were involved by conventional PTC.

Immunohistochemical findings

Immunohistochemical staining was performed on the 5-μm-thick sectioned slides obtained from the formalin-fixed, paraffin embedded tissue using an autoimmunostainer (Ventana, Tucson, AZ, USA) with monoclonal mouse anti-cytokeratin 19 (CK19) antibody (clone A53-B/A2.26, NeoMarkers, Fremont, CA, USA) and polyclonal rabbit anti-galectin-3 antibody (Zymed, San Francisco, CA, USA). The tumor cells of the conventional PTC exhibited a strong, diffusely positive immunoreactivity to CK19 (1:1,500) and galectin 3 (1:100), whereas the tumor cells of the follicular variant revealed no reactivity to galectin 3, and showed only focal and weak staining to CK19 (Fig. 2).

Molecular analysis

The tumor cells were micro-dissected from both the conventional and follicular variant areas of the hematoxylin and eosin stained 10 μm-thick sections of the formalin-fixed, paraffin embedded tissue. The genomic DNA was extracted using the extraction buffer solution (50 mM Tris buffer, pH 8.3; 1 mM EDTA, pH 8.0; 5% Tween 20 and 100 μg/mL proteinase K). Mutations of V600E (T1799A) and K601E (A1801G) in exon 15 of the *BRAF* gene and the codons 12, 13, and 61 of the *KRAS* and *NRAS* genes were evaluated by the pyrosequencing method. The DNA extracted from the FVPTC foci revealed a *BRAF* K601E (A1801G) mutation. The conventional PTCs revealed *BRAF* V600E (T1799A) mutations in all 3 tumors (Fig. 3). Neither the conventional nor follicular variant revealed *KRAS* or *NRAS* mutations.

DISCUSSION

The most characteristic growth pattern of PTC is papillary,

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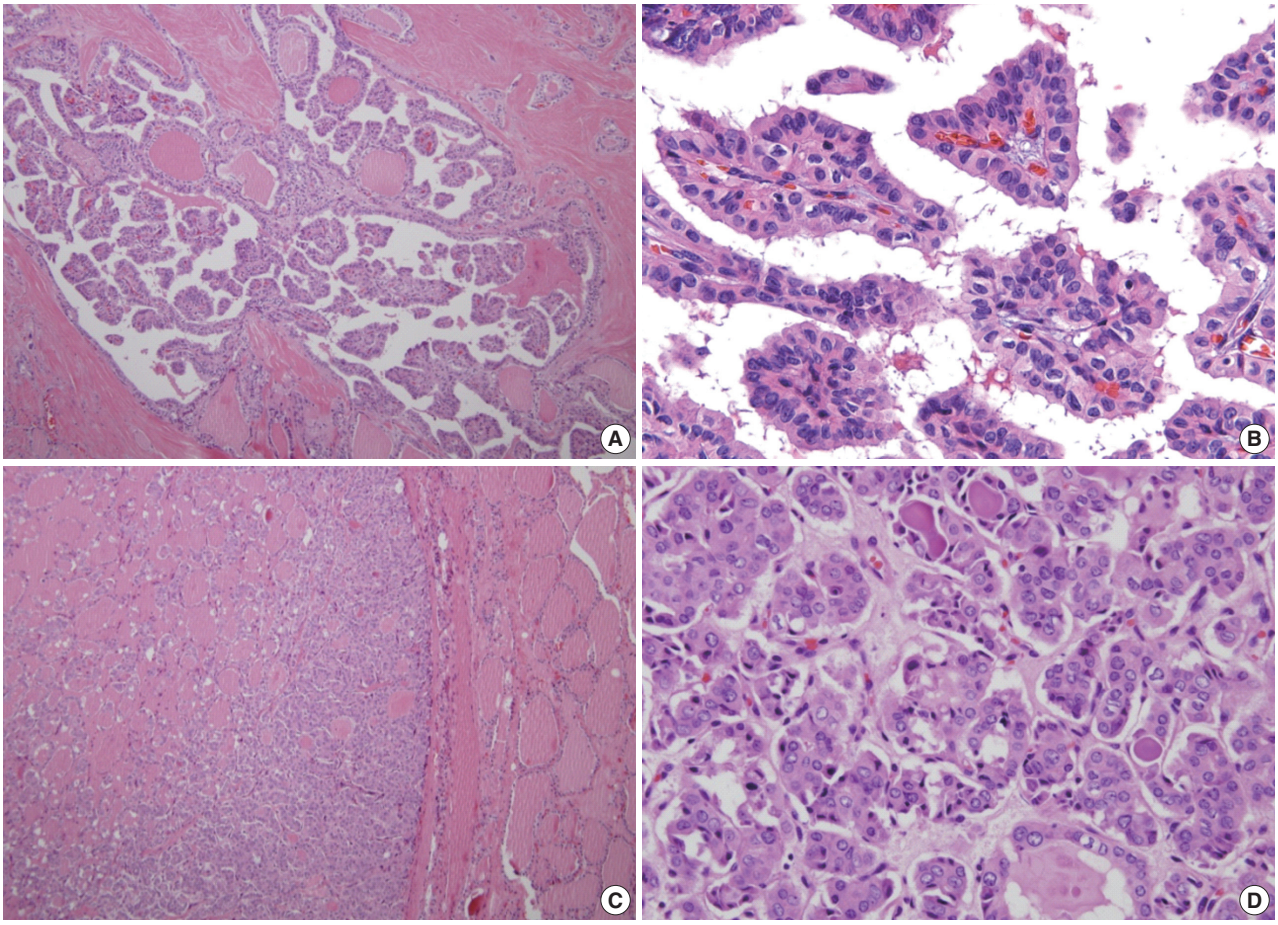

Fig. 1. (A, B) The conventional types in multifocal papillary thyroid carcinoma reveals typical complex and branching papillae with characteristic nuclear features. (C) The follicular variant is encapsulated and composed of small to medium sized, irregularly shaped follicles without papilla formation. (D) Tumor cells of the follicular variant show enlarged, clear nuclei with infrequent nuclear grooves and less irregular nuclear contours compared to conventional foci. Also deeply eosinophilic colloid showing peripheral scalloping is seen within some follicles.

although it is rarely pure and is typically mixed together with a variable proportion of neoplastic follicles. Papillary growth predominates in approximately two thirds of these tumors, whereas another one third is predominantly follicular in characterization. Other histological patterns include solid and trabecular patterns, which are observed in about 20% of cases, but are rarely described as predominate.[12] The present case revealed three conventional PTCs and one FVPTC that were distinguished by a complete lack of well-formed papillae, an exclusively microfollicular growth pattern and the characteristic nuclear features of conventional PTC.

As a mPTC, this case was genetically very unique in that different kinds of *BRAF* mutations were found simultaneously in accordance with the different histological foci of mPTC. Three conventional PTCs of mPTC had the *BRAF* V600E (T1799A) mutation and the discrete, noncontiguous FVPTC exhibited the *BRAF* K601E (A1801G) mutation. To our knowledge, it has never been reported that two different *BRAF* mutations were noted together in the mPTC of an individual patient at the same time. In previous studies regarding mPTC and its genetic alterations, some cases of mPTC revealed a discordant and mixed pattern of *BRAF* V600E mutation with a combination of *BRAF* V600E-positive *BRAF* V600E-negative PTC foci, although most mPTC exhibited a concordant pattern of either all positive or negative with regards to its *BRAF* V600E mutation status.[5] Since PTC foci that do not harbor the *BRAF* V600E mutation often show different histopathological types other than the conventional type, *BRAF*-discordant mPTC tend to show different morphotypes between tumor foci.[5] The present case corresponds to the small population of mPTC exhibiting the different morphotype and the discordance of *BRAF* V600E mutations among discrete tumor foci of mPTC. Moreover, our case was characterized by the presence of the *BRAF* K601E (A1801G) mutation in *BRAF* V600E-negative FVPTC foci. If

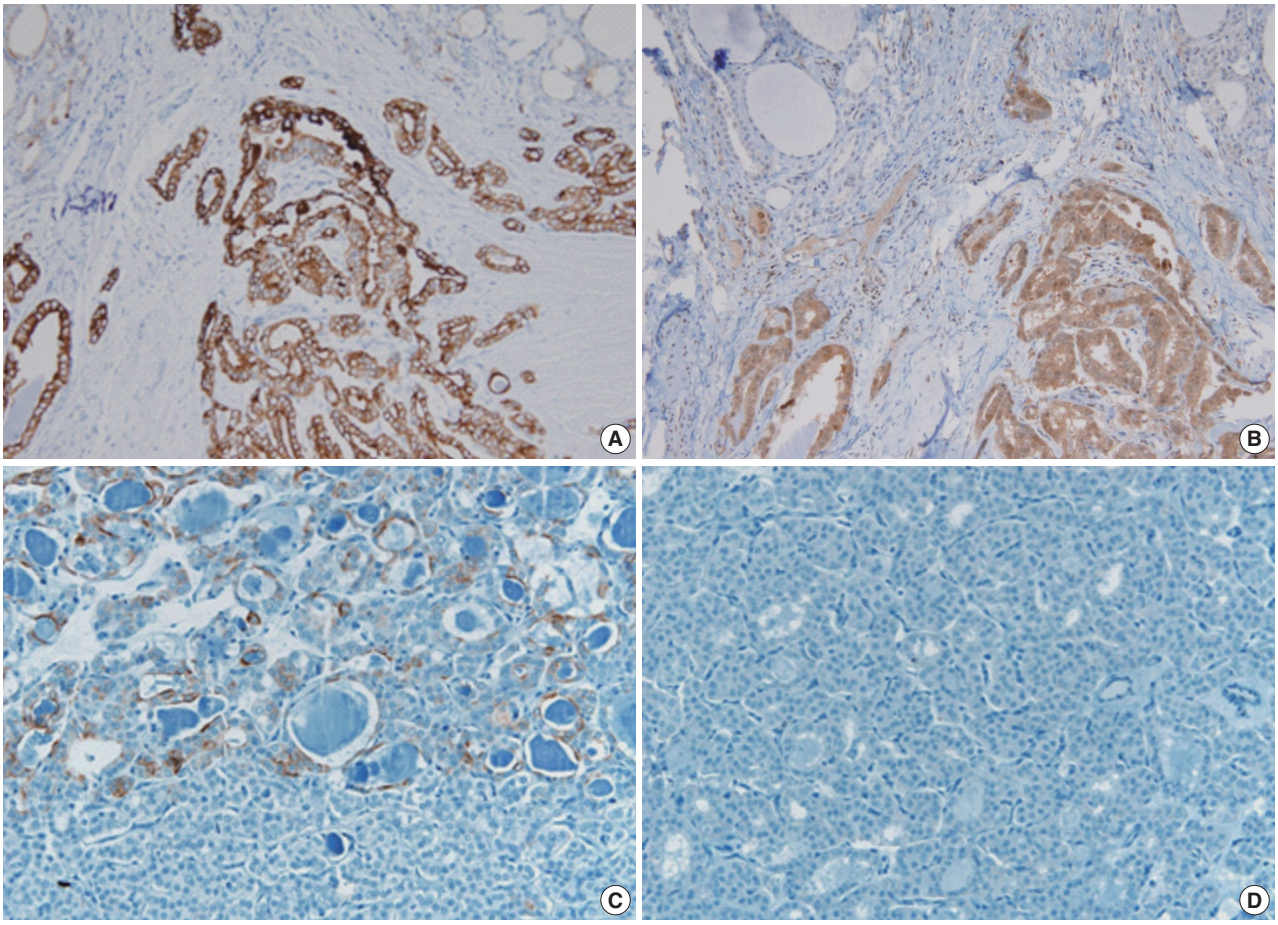

Fig. 2. The tumor cells of the conventional papillary thyroid carcinoma (A, B) show strong, diffusely positive immunoreactivity to cytokeratin 19 (A) and galectin 3 (B), whereas the tumor cells of the follicular variant reveal focal, weak positivity to cytokeratin 19 (C), and no reactivity to galectin 3 (D).

we consider a very low incidence of 7% of *BRAF* K601E mutations in solitary FVPTC,[4] this simultaneous presence of *BRAF* K601E and V600E mutations must be a very rare event with regards to genetic alterations observed in mPTC. In comparing the frequency of the *BRAF* K601E mutation with that of the *BRAF* V600E mutation in FVPTC, the *BRAF* V600E mutation is more commonly detected in FVPTC due to its high prevalence in PTC. However, the *BRAF* K601E mutation has special diagnostic value for the detection of FVPTC in that most of this mutation has been encountered and described in FVPTC, although it has been reported in a few cases of follicular adenoma and carcinoma.[4,13]

The discordant pattern of *BRAF* mutations in the present case might be associated with the question whether mPTC arise de novo or from intrathyroidal metastasis. The observed discordant pattern of *BRAF* mutations in our case seems to support the concept of *de novo* development of mPTC, suggesting an independent origin of the different tumor foci. However, because there was a previous study that reported that mPTC showing a discordant pattern of *BRAF* mutations usually has monoclonal derivation when considering the result of genome-wide allelic imbalances,[5,11] it remains uncertain whether our case resulted from *de novo* tumorigenesis or intraglandular spread.

Immunohistochemical staining showed focal weak positivity to CK19 and no reactivity to galectin 3 in FVPTC, in contrast to diffuse strong reactivity of both makers in conventional PTC. The difference of galectin 3 expression between the conventional type and the follicular variant has been suggested in the previous studies. A large scale comprehensive study reported that the incidence of galectin 3 expression in FVPTC (79.7%) was significantly lower than that of conventional PTC (98.3%),[14] although some previous studies on small numbers of FVPTC reported that galectin 3 was expressed in almost all cases like the conventional types.[15,16] In terms of CK19 expression, there was no significant difference between FVPTC and the conventional PTC in previous studies.[16,17] However, the numbers of

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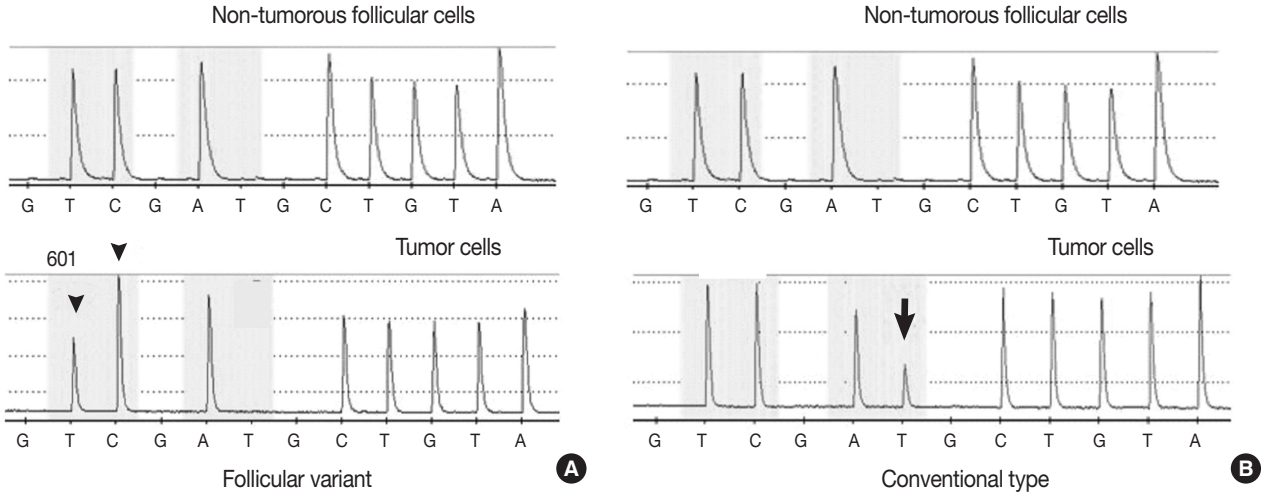

Fig. 3. The pyrosequencing analysis of the *BRAF* mutation shows the *BRAF* K601E (A1801G) mutation in one follicular variant (A) and the *BRAF* V600E (T1799A) mutation in three conventional types (B) of multifocal papillary thyroid carcinoma. (A) When the substitution of thymine to cytosine occurs at nucleotide 1801 of exon 15 in the reverse strand, an additional cytosine peak is fused to the next cytosine peak (arrowhead) at nucleotide 1800 with a decrease of the thymine peak at nucleotide 1801 in mutational analysis of the follicular variant. (B) In the three conventional types, the additional thymine peak (arrow) is shown with a decrease of the adenine peak at nucleotide 1799 in reading along the reverse strand.

FVPTC cases analyzed in these studies on CK19 expression was too small to be generalized. Decreased expression of CK19 and galectin 3 in FVPTC of the present mPTC may reflect the immnohistochemical features of the encapsulated variant of FVPTC.

Whether the prognosis of mPTC is worse than that of solitary PTC is controversial. Several previous studies have demonstrated that mPTC was associated with a higher rate of recurrence, whereas Schindler *et al.*[18] suggested the opposite.[12] The present case consisted of three conventional PTC foci with *BRAF* V600E mutations and one encapsulated FVPTC foci with the *BRAF* K601E mutation. Considering that the behavior of encapsulated FVPTC was reported to be indolent in most cases,[19] the conventional PTC foci are more likely to affect the clinical course of this patient. In addition, the predominant role of conventional PTC foci in reference to the prognosis might be associated with the fact that *BRAF* V600E has about 2.5 times the kinase activity of *BRAF* K601E.[20]

In summary, we report a genetically unique case of mPTC having the *BRAF* V600E (T1799A) mutation in conventional PTC foci and the *BRAF* K601E (A1801G) mutation in noncontiguous, discrete follicular variant foci, simultaneously.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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