

BRIEF REPORT

Bilateral follicular variant of papillary thyroid cancer with different *RAS* mutations detected with next-generation sequencing: Report of an unusual case and literature review

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Abstract

Multifocality in papillary thyroid carcinoma (PTC) is a common finding, but the clonal relationship between individual tumors remains uncertain. While multiple synchronous tumor foci of PTC may develop through permeation of intraglandular lymph vessels of a single malignant clone, they can also arise from independent progenitor clones sustained by different genetic events. We report the case of a 37-year-old man who underwent total thyroidectomy after fine-needle aspiration of two bilateral thyroid nodules that yielded cytological findings consistent with atypia of undetermined significance/follicular lesion of undetermined significance. By next-generation sequencing of a large panel of thyroid carcinoma related genes, we found that the larger tumor harbored a mutation of the *NRAS* gene, while the contralateral tumor harbored a different mutation in the *HRAS* gene. Final pathology of the surgical specimen showed two encapsulated follicular variant papillary thyroid carcinomas of 16 and 6 mm in the right and the left lobes, respectively. To the best of our knowledge, this is the fourth case of multifocal PTC showing different *HRAS* and *NRAS* mutations, and highlights that mutational heterogeneity is also present in non-*BRAF*, non-*RET* genes, supporting the hypothesis that independent progenitor clones may explain multifocality in papillary thyroid carcinoma.

KEYWORDS

HRAS, molecular diagnosis, multifocality, *NRAS*, papillary thyroid cancer

1 | INTRODUCTION

Papillary thyroid carcinoma (PTC) accounts for approximately 80%–90% of all thyroid malignancies.¹ PTC often presents with two or more anatomically separate foci, with the prevalence of multifocality ranging from 18%–87%,^{2–4} in most cases as microscopic disseminations discovered during examination of the surgical pathology sample.

Bilaterality—a specific subtype of multifocality—is discovered in 13%–56% of thyroidectomy specimens.^{5,6}

Multifocal tumors may result from a common clonal origin, arising from intrathyroidal spread of a clonal population of malignant cells. They may also develop from independent clonal foci that arise separately in a background of field cancerization, resulting in a high degree of genetic heterogeneity. Distinguishing between these two

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mechanisms of multicentricity may be important in treatment decisions and prognoses because, if a given multifocal PTC resulted from intrathyroidal metastasis, there is likely to be an increased risk of further metastases and disease recurrence.⁷

Most studies on clonality in multicentric PTC have analyzed *BRAF* and *RET/PTC* mutations.⁸⁻¹³ Information of the presence of *RAS* mutations in multifocal PTC is scarce.

We report an unusual case of a bilateral follicular variant PTC harboring two different *RAS* mutations.

2 | CASE REPORT

A 37-year-old male attended our center for evaluation of thyroid nodules. He was euthyroid and denied having any local compressive

symptoms. Physical examination was normal. There was a positive family history of gastric and PTC in his father, but no other risk factors for thyroid cancer. Antithyroid antibodies were negative.

Five months before the visit, a thyroid ultrasound performed at another center, showed two thyroid nodules that measured 14 mm (right lobe) and 10 mm (left lobe). Cytology diagnosis was of atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) (Bethesda category III) in the right thyroid nodule, and of unsatisfactory sample for cytological diagnosis (Bethesda category I) in the contralateral nodule.

Six months later, repeated fine-needle aspiration biopsies (FNAs) were performed at our center. Cytology showed AUS/FLUS in both nodules, which measured $10.6 \times 11.4 \times 16.5$ mm (right nodule) and $5.4 \times 10.2 \times 10.1$ mm (left nodule) in their maximal anteroposterior, transverse, and craniocaudal dimensions, respectively. The ultrasound examination of both nodules did not show any features suspicious of malignancy (Figure 1). A third FNA performed three months later revealed a follicular neoplasm/suspicious for follicular neoplasm (Bethesda category IV) in the right thyroid nodule, and AUS/FLUS (Bethesda category III) in the contralateral nodule (Figure 2).

DNA was extracted from FNA samples. A custom-based capture next-generation sequencing (NGS) library [SureSelect XT HS kit

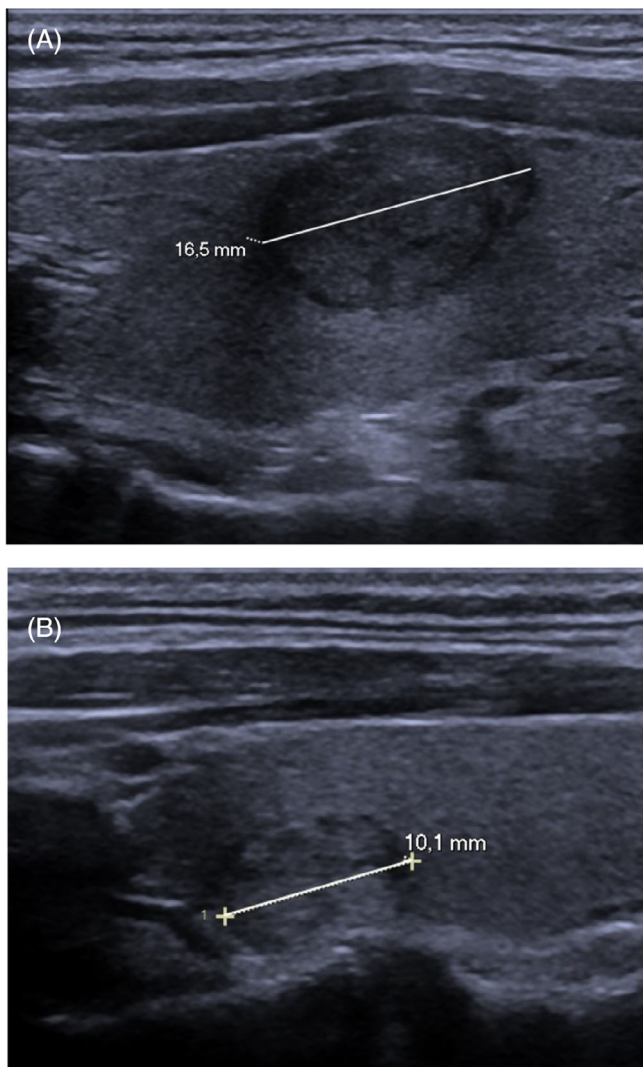


FIGURE 1 Longitudinal ultrasound scan images of the two nodules in the right (A) and left (B) thyroid lobes. Isoechoic, solid nodules with well-defined margins with low echoic halos, absence of microcalcifications or other signs suspicious of malignancy, including no evidence of cervical lymph node enlargement (not shown)

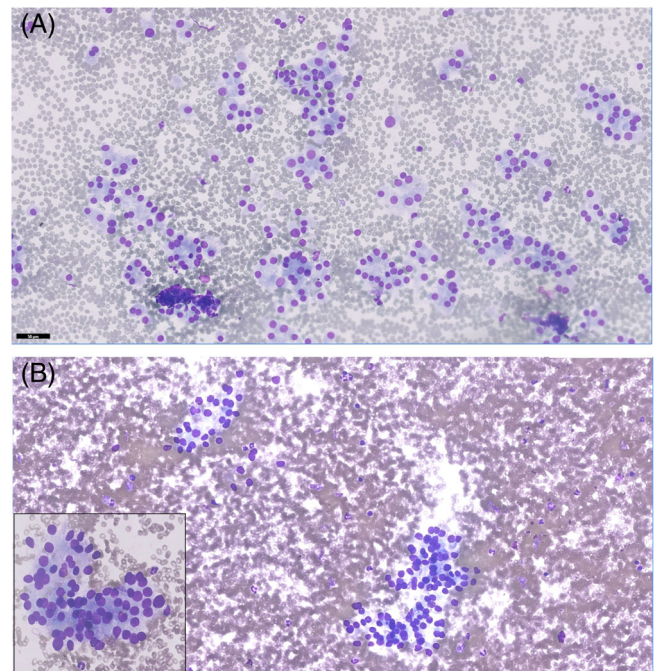


FIGURE 2 (A) Fine needle aspiration (FNA) of the right thyroid nodule showing a cellular smear consisting of cells arranged in loosely cohesive clusters, forming microfollicles, and as single cells with no colloid in the background and varying nuclear atypia and cellular pleomorphism suspicious for a follicular neoplasm. (Romanowsky stain, original magnification $\times 20$). (B) FNA of the left thyroid lobe nodule showing a paucicellular smear with very scant colloid, overlapping nuclei, and alterations in nuclear contour and shape classified as AUS/FLUS (Romanowsky stain, original magnification $\times 20$, insert: $\times 40$)

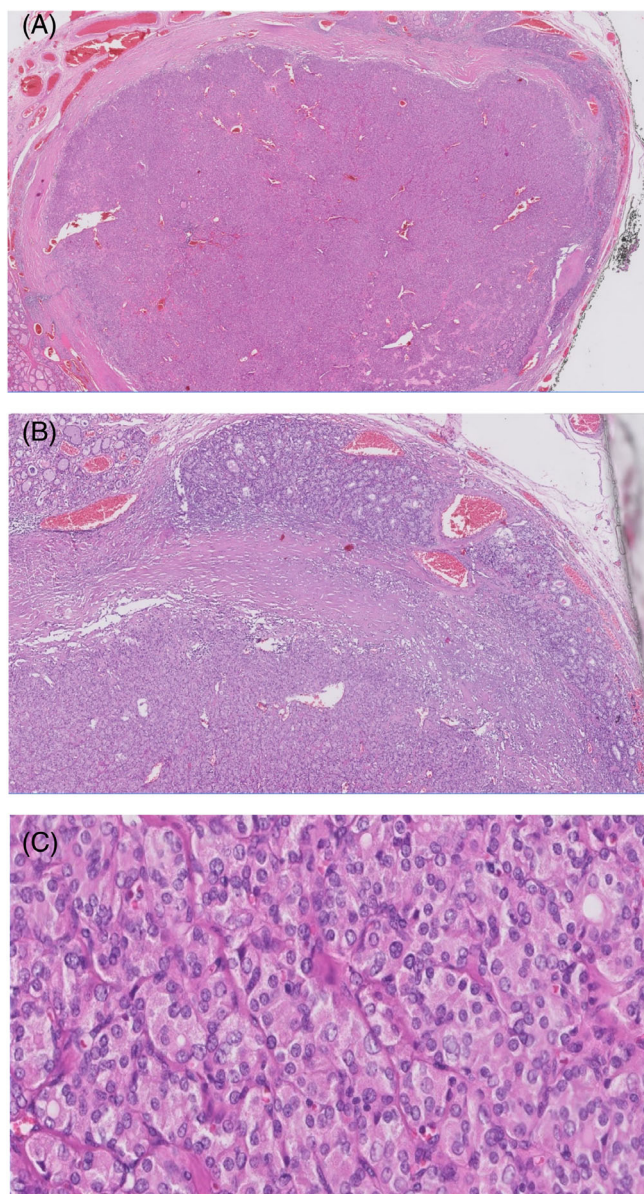


FIGURE 3 Microphotographs of the right thyroid tumor. Hematoxylin & eosin-stained sections. (A) Low-power view of the encapsulated follicular variant papillary thyroid carcinoma composed of tightly packed follicles (original magnification $\times 1$). (B) Nodule composed predominantly of variable-shaped follicles with solid growth but no well-formed papillary structures, displaying capsular invasion (original magnification $\times 5$). (C). Tumor cells with characteristic cytologic features of papillary carcinoma: nuclear enlargement and overlapping, clear appearance of nuclei, oval shape and irregularity of nuclear contours, and visible nucleoli (original magnification $\times 40$)

(Agilent Technologies)] was prepared and then sequenced in Illumina platform (Illumina Inc) for both FNA samples. Bioinformatic analysis for somatic mutations of seven genes often altered in thyroid carcinoma (*BRAF*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *RET*, and *TERT* promoter) in addition to analysis for *RET* rearrangements were performed.

High throughput sequencing by Illumina platform detects point mutations and small insertions/deletions throughout the whole coding

sequence and flanking intronic regions of the six targeted genes and *TERT* promoter, as well as *RET* rearrangements. The variants were filtered using an in-house pipeline among other bioinformatics tools.

The mutation NM_002524.4:c.182A > G p.(Gln61Arg) in *NRAS* was detected in the 16-mm right thyroid nodule. This mutation was classified as Tier I, a variant with strong clinical significance.^{14,15} The mutation was detected with a variant allele frequency (VAF) of 40%. The 10-mm left thyroid nodule harbored the mutation NM_005343.2:c.182A > G p.(Gln61Arg) (Q61R) in *HRAS* with a VAF of 38%. This mutation was classified as Tier II, a variant with potential clinical significance.^{14,16}

The patient underwent total thyroidectomy without lymph node dissection. The maximum diameters of the right and left nodules were 16 and 6 mm, respectively. Final pathology of the surgical specimen reported encapsulated follicular variant papillary carcinomas with focal invasion through the capsule in both nodules, which presented indistinguishable histopathological features (Figure 3). We did not find perineural or lympho-vascular invasions, evidence of lymphocytic infiltrate or extrathyroidal extension.

The patient was administered an ablative dose of 102 mCi of I-131 radioactive iodine. No regional or distant metastases were found.

3 | DISCUSSION/CONCLUSION

PTCs occasionally form multiple, discrete tumor nodules in the thyroid gland. It is often uncertain whether these synchronous tumor foci of PTC arise from independent progenitor clones or develop through intraglandular spread of a single malignant clone. Several techniques have been applied to determine the clonal origin of multifocal PTCs, including X-chromosome inactivation pattern analysis, loss of heterozygosity markers, and—more recently—mutational screening performed for a single mutation or *RET/PTC* rearrangement analysis, predominantly from paraffin-embedded micro-dissected tissues.^{8–12,17}

Since *BRAF* V600E mutations account for approximately 60% of the mutations found in PTC,¹ most studies assessing clonality in multifocal PTC have focused on its genetic alterations. The rate of independent clonality of multiple PTC based on *BRAF* point mutations analysis is approximately 40%.^{8,9,11,12} This prevalence is higher when NGS is applied. Thus, Lu et al.¹⁷ observed a 75% incidence of nonsynonymous mutations of *BRAF* in a series of patients with multiple PTC using this technique. Most discrepancies in *BRAF* mutational status in multicentric PTC consist in either the presence or absence of the mutation in the different tumors, not of different point mutations of *BRAF*. However, there are some cases of discordant mutations affecting *BRAF* and another different gene, more commonly *RAS*, in the same patient.¹¹

Based on *RET/PTC* gene rearrangements, Sugg et al.¹³ also reported a high frequency of diverse rearrangements within multiple PTC, with 15 out of 17 cases having multiple different co-existing *RET/PTC* transcripts within the multiple tumors, suggesting that individual tumors arise independently.

After *BRAF* mutations, activating point mutations that involve *RAS* are the next most common genetic alteration noted in thyroid carcinomas. These mutations are mainly associated with follicular-patterned thyroid neoplasms. Thus, *RAS* mutations are found in 20%–25% of follicular adenomas, 30%–45% of—both—follicular variant PTCs and follicular carcinomas, 20%–40% of poorly differentiated carcinomas, and 20%–30% of anaplastic carcinomas.^{18–20} Noninvasive follicular thyroid neoplasm with papillary-like nuclear features can also harbor *RAS* mutations,²¹ although with a lower frequency than in the follicular variant PTC.²²

In a series of 60 cases of multifocal PTC, *RAS* mutations were identified in 33 of 124 discrete tumor nodules.¹¹ Six patients (10%) with two tumors showed different types of *RAS* mutation: three cases had one tumor carrying *HRAS* and another tumor carrying *NRAS* mutations, as we have found in our case, whereas in another three cases both tumors had *NRAS* mutations with different types of nucleotide changes in codon 61. To our knowledge, ours would be the fourth documented case of discordant mutational status involving *RAS* in multifocal PTC. Both tumors corresponded to the invasive encapsulated follicular variant of PTC, which has a molecular profile comparable to follicular adenomas and carcinomas (a high rate of *RAS* and absence of *BRAF* mutations). By contrast, the infiltrative follicular variant of PTC has a divergent molecular profile, being like classical PTC (*BRAF* > *RAS* mutations).¹⁹ The mutations we found in our case are the most common *RAS* mutations described in encapsulated follicular variant of PTCs.¹⁹

The clonality results in multifocal PTC may have prognostic relevance. Multifocal PTC arising from an intrathyroidal metastasis from a single malignant clone had a significantly increased risk of extrathyroidal extension, nodal metastasis and disease recurrence compared with multifocal PTC from independent primary foci,^{2,3,5,7,23} suggesting a more aggressive treatment would be required for the former. In our case, given the total foci size of 22 mm, the paternal history of thyroid cancer and the postoperative stimulated thyroglobulin concentration (>1.0 ng/mL), we performed a near-total thyroidectomy followed by radioiodine ablation therapy.

In conclusion, we describe a patient with different *HRAS* and *NRAS* mutations in follicular variant PTC with bilateral foci. This finding is consistent with the presence of an independent clonal origin, supporting previous reports of discordant clonal status involving the *BRAF* mutation and *RET*/*PTC* rearrangements.

AUTHOR CONTRIBUTIONS

Fernando Marín prepared the manuscript and was directly involved in the management of the patient. Esther del Nuevo and Alberta Belinchón revised the manuscript and sequencing data analysis. Agustín Acevedo undertook the pathological analysis, provided corresponding images, and reviewed the manuscript. All authors contributed and approved the manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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