Endocrinology, Diabetes & Metabolism CASE REPORTS

J Nguyen and D Joseph | Invasive PTC with TSHR I568T mutation

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Locally invasive classical papillary thyroid carcinoma with TSH receptor I568T mutation: case report

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Summary

Autonomous thyroid adenomas are caused by activating mutations in the genes encoding the thyroid-stimulating hormone receptor (TSHR) or mutations in the Gas subunit of the TSHR. Nodules with suspicious sonographic features should be submitted to fine-needle aspiration. Additional molecular testing may be performed to characterize the thyroid nodule's malignant potential further. We present a patient who underwent whole-transcriptome RNA-sequencing that indicated a *TSHR* I568T mutation after an ultrasound showed suspicious sonographic features and fine-needle aspiration was 'suspicious for malignancy'. The patient underwent thyroid resection and was found to have a locally invasive classical papillary thyroid carcinoma. Most reports of *TSHR* I568T mutation have been seen in patients with benign thyroid conditions. While there is insufficient data to suggest that the *TSHR* I568T mutation causes aggressive thyroid malignancy, we believe clinicians who identify the presence of this mutation on genome sequencing should be cautious about the possibility of locally invasive thyroid malignancy, especially when associated with Bethesda V cytopathology.

Learning points:

- Germline and somatic activating mutations in the genes coding for the thyroid-stimulating hormone receptor (TSHR) have been frequently reported in familial and sporadic autonomous thyroid adenomas and non-autoimmune hyperthyroidism.
- Most reports of *TSHR* I568T mutation have been detected in patients with benign thyroid conditions.
- We present a patient who underwent whole-transcriptome RNA-sequencing that indicated a *TSHR* I568T mutation and subsequently underwent thyroid resection and was found to have a locally invasive classical papillary thyroid carcinoma.
- Clinicians who identify the presence of *TSHR* I568T mutation on genome sequencing should be cautious about the possibility of locally invasive thyroid malignancy, especially when associated with Bethesda V cytopathology.

Background

Thyroid nodules are common and usually benign. Most are not hyper-functioning and are usually discovered incidentally on routine physical examinations or radiological procedures. Rarely, adenomas can autonomously produce thyroid hormones without thyrotropin stimulation or antibodies to the thyroid-stimulating hormone (TSH) receptor (TSHR) and are referred to as 'autonomous thyroid adenomas' (ATNs) or toxic adenomas. ATNs are caused by activating mutations in the genes encoding the TSHR or mutations in the Gas subunit of the TSHR, which is a G-protein-coupled receptor (1). These mutations usually occur

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sporadically, but ATNs can be due to germline mutations (2). ATNs are more common in adults than children and cause clinical and laboratory findings consistent with hyperthyroidism (1).

Ultrasound (US) is usually performed in the initial thyroid nodule assessment. Thyroid scintigraphy is used to determine the functional status of a nodule when thyrotropin levels are low. Since hyper-functioning nodules are rarely malignant, such nodules usually do not require a fine-needle aspiration (FNA) (3). Nodules with sonographic features concerning for malignancy are often followed up with an FNA. Cytopathologic findings that were neither clearly benign nor clearly malignant according to the Bethesda System for Reporting Thyroid Cytopathology – this includes atypia of undetermined significance, follicular lesion of undetermined significance, and follicular neoplasm – historically were followed up with repeat FNA or resection of the thyroid gland together with confirmatory histopathologic assessment despite nearly 75% of such thyroid nodules being benign (4).

Molecular testing (mutational analysis or mRNA genome expression) may be paired with initial FNA studies to further characterize a nodule's malignant potential. The Afirma® Xpression Atlas (XA) (Veracyte, South San Francisco, CA, USA) detects gene variants and fusions in thyroid nodule FNA samples from a curated panel of 593 genes using whole-transcriptome RNA sequencing in Bethesda III–IV Gene Sequencing Classifier Suspicious or Bethesda V and VI nodules. Afirma Xpression Atlas can supplement clinical decision-making, including the risk of malignancy, *BRAF*^{V600E}-like vs *RAS*-like (or non-*BRAF*-non-*RAS*) pathway signaling, iodine metabolism, neoplasm histology, risk of lymph node metastasis, actionable intraoperative management, risk of recurrence, and risk of mortality (5, 6). While *BRAF*^{V600E}, *RET/PTC*, and *PAX8/PPARG* mutations are strongly associated with thyroid cancer, the significance of many other mutations is less recognized (7). Histopathologic assessment of resected thyroid tissue is the gold standard for determining the potentially malignant nature of a thyroid nodule with otherwise indeterminate cytopathologic findings (8).

We present a patient who underwent Afirma® whole-transcriptome RNA-sequencing after an FNA with Bethesda V (suspicious for malignancy) cytopathologic findings showed a *TSHR* mutation not previously reported to be associated with thyroid carcinoma. The patient underwent thyroid resection and was found to have a locally invasive classical papillary thyroid carcinoma.

Case presentation

The patient is a 53-year-old female referred to the endocrinology clinic to evaluate a thyroid nodule discovered on US. She had a remote history of a benign thyroid cyst more than 2 decades ago but had recently reported dysphagia and occasional globus sensation. Past medical history was negative for hyperthyroidism, radioactive iodine treatment, and external radiation to the neck. Family history was negative for thyroid cancer. Physical examination (including that of the neck) was normal.

Investigation

Thyrotropin level was normal (1.69 uIU/mL (reference 0.27–4.20 uIU/mL)). An in-office US revealed a right-sided solid, densely hypoechoic 1.3 cm nodule with irregular margins, intra-nodular vascularity, and microcalcifications. The central and lateral neck nodal basins were unremarkable. Due to high-suspicion sonographic features, she underwent an US-guided FNA. Cytopathology revealed scattered follicular cells with crowded, enlarged round-to-ovoid nuclei, a few of which contained apparent intranuclear inclusions. The final report was 'Bethesda Category V: Suspicious for Malignancy'. Afirma whole-transcriptome RNA-sequencing revealed a *TSHR*:p.I568T c.1703T>C mutation with a notation that there is 'insufficient published literature regarding risk of malignancy from being Bethesda V and *TSHR* p.I568T positive'. *BRAF* p.V600E c.1799T>A, *RET/PTC1*, and *RET/PTC3* mutations were negative.

Treatment

Subsequently, she underwent total thyroidectomy, which revealed a right-sided 2 cm classical papillary thyroid carcinoma. The tumor was present focally at the posterior-inferior soft tissue margin. Removal of the tumor revealed a 1 × 3 mm concavity present in the space between the second and third tracheal cartilages on the right anterior portion of the trachea. Frozen section performed on soft tissue from this hole was also consistent with PTC (Fig. 1). She also underwent resection of the anterior two-thirds of the second and third tracheal rings. Angioinvasion, lymphatic invasion, and peri-neural invasion were absent. Immunohistochemical staining of the right anterior tracheal margin was performed to confirm that the malignant cells observed around the trachea were of thyroid origin. Staining was positive for TTF1, PAX8, and

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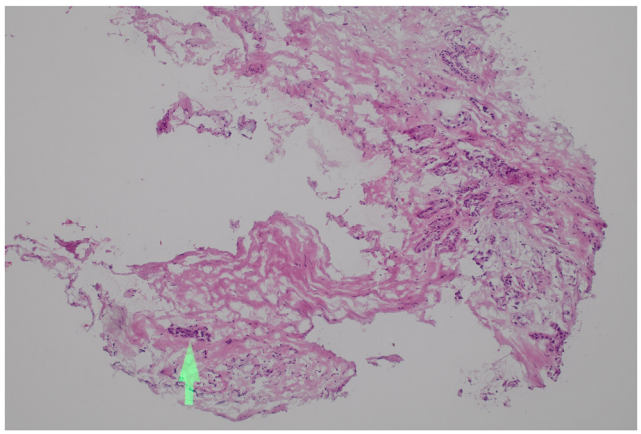

Figure 1
Biopsy of soft tissue located between the second and third tracheal cartilages on the right anterior portion of the trachea revealed histopathologic findings consistent with locally invasive classical papillary thyroid carcinoma, hematoxylin and eosin, 10×.

thyroglobulin (Tg) (Fig. 2). Prophylactic nodal dissection was not performed since the nodal basins appeared normal on the pre-operative ultrasound. The final staging was pT4a pNx Mx (TNM Stage I based on the AJCC UICC 8th edition). According to the American Thyroid Association (ATA) classification system, this tumor is considered a 'high risk for recurrence' due to gross extra-thyroidal extension.

Outcome and follow-up

After levothyroxine withdrawal, the patient underwent radioactive iodine ablation with 160 mCi of I-131. Stimulated Tg was 6.1 ng/mL, and a whole-body scan showed only remnant thyroid tissue without regional iodine avid metastases.

At a follow-up visit 4 months after surgery, Tg and Tg antibodies were undetectable. At a 12-month follow-up visit, no remnant thyroid tissue or recurrence was noted on thyroid US, but the Tg was weakly positive at 0.3 ng/mL.

Discussion

Germline and somatic activating mutations in the genes coding for the TSHR have been frequently reported in familial and sporadic ATNs and non-autoimmune hyperthyroidism. The published literature rarely describes thyroid nodules with *TSHR* mutations associated with thyroid cancer.

In a study of 388 FNAs followed by genome sequencing, *TSHR* mutations alone were found in ten nodules (2.6%), and *TSHR* mutations together with sodium-iodine

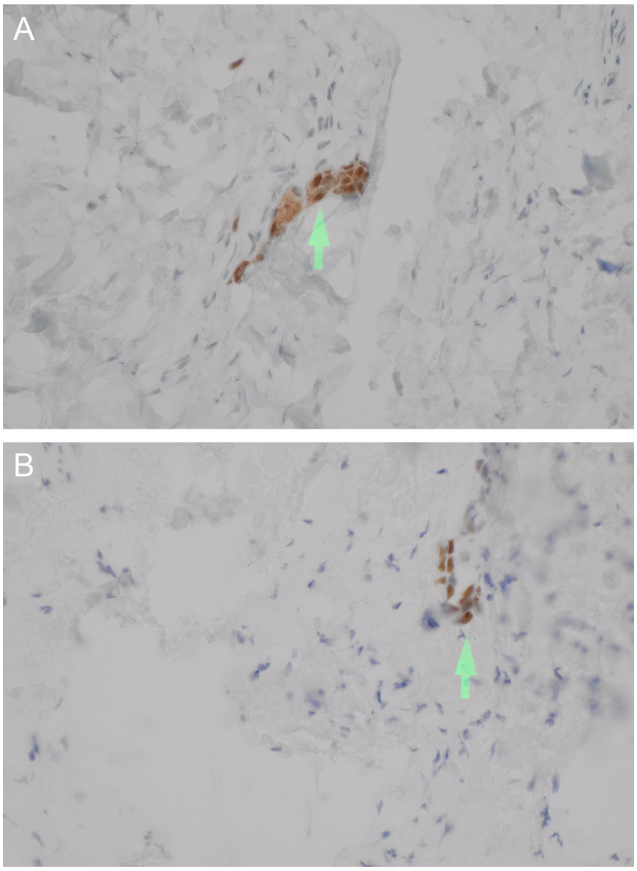

Figure 2
Immunohistochemical staining of the same sample was positive for PAX8 (A) and TTF1 (B), 40×.

symporter gene over-expression were seen in eight nodules (2.1%). Of the nodules with *TSHR* mutations, two were associated with I568T mutations. Thyroid tissue was resected in both patients and was histopathologically determined to be benign (8).

The prevalence of *TSHR* mutations in nodules with indeterminate cytology has been estimated at approximately 5%. In a study of 703 thyroid nodules with indeterminate cytopathology on FNA, molecular testing revealed *TSHR* mutation in 31 of them. Surgical resection and subsequent histopathologic assessment of 15 of them revealed 12 (80%) to be benign and 3 (20%) to be follicular thyroid carcinoma (FTC). Histopathologic assessment could not be performed on the 16 thyroid nodules that were not resected. Therefore, the actual prevalence of FTC in this sample of 31 thyroid nodules could range from ~10 (3 of 31) to 20% (3 out of 15). There was one case of PTC, but it also had a co-mutation with *BRAF* V600E, which was more likely the oncogenic variant (9).

According to a database of 638 published reports of *TSHR* mutations last updated in 2018, there are 20 published

cases of *TSHR* I568T mutations. These cases include two benign nodules caused by germline I568T mutations, 17 benign nodules caused by somatic mutations, and 1 malignant nodule determined to be a FTC. There were only four cases of PTC, and these were associated with *TSHR* mutations - A623S, M453T, and L512R. Other mutations described in this database associated with other forms of thyroid cancer were I486P (FTC), I568F (FTC), T620I (FTC), I630L (FTC), F631I (FTC), F631L (FTC), T632A (FTC), T632I (FTC), D633H (insular thyroid carcinoma), D633Y (FTC), and L677V (hurthle cell carcinoma). According to the database, when associated with malignancy, TSHR mutations have been associated with FTC more frequently than PTC. Furthermore, when associated with malignancy, TSHR I568T mutations have only been associated with FTC, not PTC.

In a study of 28 toxic adenomas, *TSHR* mutations were seen in 11 nodules, 2 of which were due to I568T mutations. One resected sample revealed hyperplastic histopathology, while the other revealed adenomatous histopathology. Both had a final diagnosis of ATN (10).

In one case report, a 12-year-old girl diagnosed with follicular thyroid carcinoma was found to have a somatic *TSHR* I568T activating mutation (11).

In nodules with *TSHR* mutations, EZH1 mutation Q571R has been described as a 'second-hit' that may induce tumorigenesis. This mutation was detected in two of four malignant nodules carrying *TSHR* mutations (9). Our patient did not have this mutation.

TSHR mutations may be associated with an increased cancer risk when present at a high allelic frequency (9). The allelic frequency could not be determined in our patient using the Afirma whole-transcriptome RNA-sequencing platform.

Our patient had classical papillary thyroid cancer with a *TSHR* I568T mutation. Despite the small tumor size, there was a significant extra-thyroidal extension and tracheal invasion, which classified the patient as 'high risk' according to the ATA recurrence staging system. Previous reports of *TSHR* I568T mutation have been seen primarily in thyroid nodules later determined to be benign. We believe that clinicians who identify the presence of this mutation on genome sequencing in non-hyper-functioning nodules, especially in association with Bethesda V cytopathology, should be cautious about the possibility of a locally advanced thyroid malignancy like the one seen in our patient. Clinicians should also be cautious not to assume this mutation is exclusively associated with benign thyroid disease, as the current literature might otherwise suggest.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Patient consent
Written informed consent for publication of their clinical details and/or clinical images were obtained from the patient/parent/guardian/relative of the patient.

Author contribution statement
Both J N and D J substantially contributed to the drafting of this manuscript, including surveying the literature, identifying relevant articles, and incorporating supporting comments. D J provided details about the patient's clinical presentation and course of treatment. J N and D J have read and approve the final manuscript and agree to the journal's submission policies. We would like to thank Dr. Virgilius Cornea, MD for providing the figures used in this case report, and we would like to thank Dr. Joshua Klopper, MD for offering constructive feedback.

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