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Unraveling the significance of *TSHR* mutations in indeterminate thyroid cytology specimens

Stefen Andrianus, MD[1], **Maria A. Gubbiotti, MD, PhD**[1], **Susan J. Mandel, MD**[2], **Yuri E. Nikiforov, MD, PhD**[3], **Zubair Baloch, MD, PhD**[1]

[1]Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA

[2]Division of Endocrinology, Diabetes and Metabolism, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA

[3]Department of Pathology, University of Pittsburgh Medical Center, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Abstract

Objectives: We investigated the clinical significance of thyroid-stimulating hormone receptor (*TSHR*) mutations detected in thyroid fine needle aspiration (FNA) specimens.

Methods: The pathology archives at our institution were reviewed between 2018 and 2021 for indeterminate (Bethesda category III and IV) specimens with Thyroseq® analysis showing *TSHR* mutations.

Results: A total of 2184 cases diagnosed as atypia/follicular lesion of undetermined significance (AUS/FLUS), and 2625 diagnosed as follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) were identified. A total of 1735 AUS/FLUS and 2339 SFN/FN underwent Thyroseq® analysis; 69 showed TSHR mutations (1.6%, 59 female, 10 male, average age: 55 years). Ten cases showed oncocytic features. Twelve patients underwent radionuclide scans within 1 year of FNA:11 were hyperfunctioning. Nodule size and TSH levels were weakly correlated. Twenty-two different *TSHR* mutations were identified (most common: M453T). A second mutation was found in five cases (*EZH1* $n = 2$, and *EIF1AX* $n = 3$). The expression of sodium-iodide transporter (*NIS*) mRNA was in the range of 0.01%–62.43% out of all sequencing reads, and was elevated in 49 (71%) cases. Surgical pathology follow-up was available in five cases (all benign except one). On follow-up available for 38 cases (mean: 24 months; range: 7–47 months), 34 (89.5%) nodules remained stable and 3 (8%) increased in size.

Conclusions: *TSHR* mutations in thyroid FNA samples classified as indeterminate are rare, generally benign, and commonly associated with autonomy on scan if performed.

Correspondence: Stefen Andrianus, Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104, USA. stefen.andrianus@pennmedicine.upenn.edu.

AUTHOR CONTRIBUTIONS

Zubair Baloch conceived the study, provided critical feedback throughout the research, edited the manuscript, and approved the final version of this manuscript. Stefen Andrianus and Maria Gubbiotti were responsible for data collection and analysis, as well as writing the manuscript. Yuri Nikiforov and Susan Mandel also collected and analyzed data in this study. All authors contributed to the interpretation of the results and critical revisions of the manuscript, and have read and approved the final manuscript.

Keywords

Bethesda; fine needle aspiration; thyroid; ThyroSeq®; *TSHR*

1 | INTRODUCTION

Thyroid stimulating hormone receptor (TSHR), a G protein-coupled receptor, binds thyroid stimulating hormone (TSH) which consequently promotes thyrocyte growth as well as stimulates other intracellular signaling pathways to support normal thyroid function.[1,2] Also imperative for thyroid homeostasis is the sodium-iodide symporter (NIS), which allows for the accrual of iodide into thyrocytes to be used for the synthesis of thyroid hormone.[3] Unsurprisingly, given their important role in thyroid physiology, genetic alterations in *TSHR* and *NIS* have been linked to disease. For example, activating mutations in *TSHR* lead to hyperthyroidism and loss-of-function mutations contrarily result in hypothyroidism.[1,4–10] Likewise, elevated NIS levels have been reported in patients with Graves disease.[11]

Thyroid nodules that are clinically producing thyroid hormone or are hyperfunctional compared to the background thyroid on radionuclide scan tend to be benign; as such these lesions are usually not assessed via fine needle aspiration (FNA).[6–8] However, some of these do occasionally undergo FNA and, in cases where cytology is indeterminate, molecular testing is quite useful for documenting mutations in *TSHR* or *NIS* overexpression given their common association with benignity.[12,13] Previous work illustrated that *TSHR* mutations and/or *NIS* overexpression were detected in FNA specimens in 6.4% of nodules with indeterminate cytology, all of which were determined to be benign following surgical pathology analysis or were stable upon clinical follow-up if surgery was not performed.[9] All *TSHR* mutations were gain-of-function alterations and greater than 50% of cases did not have concurrent overexpression of *NIS*.[9] Moreover, few nodules showed *NIS* alterations in the absence of *TSHR* mutation; however at least one nodule with *NIS* overexpression also showed alterations in *EIF1AX* and *GNAS*.[9]

Rarely, *TSHR* mutations have been identified within nodules determined to be malignant, including follicular carcinoma, papillary thyroid carcinoma (PTC), and oncocytic carcinoma.[14–20]

Interestingly, the molecular profile of malignant neoplasms with *TSHR* alterations do not often show concurrent mutations in known oncogenes such as *BRAF*, *GNAS*, *RAS*, *TP53*, *RET*, *PAX8::PPARG*; additionally, only few cases are reported with synchronous *TSHR* mutations and *RAS* or *PAX8::PPARG* rearrangement.[16,17,21,22] Of note, TSHR mutations in thyroid nodules can occur with variable allele frequency where higher allelic frequency (i.e., >30%) in nodules classified as indeterminate for malignancy by FNA correlated with a 60% risk of follicular carcinoma, providing a paradigm shift from the classic dogma that all hyperfunctioning nodules have no increased risk of malignancy.[18] Moreover, the previous study showed that all *TSHR* mutations associated with malignancy occurred downstream of codon 453.[18]

Given this information, we sought to investigate all *TSHR* mutations identified in nodules with indeterminate cytology at our institution in order to ascertain the clinical significance of these findings. Our conclusions support those in the literature that *TSHR* mutations detected in thyroid FNA specimens are commonly associated with follicular cell hyperfunction and benign diagnoses with only rare exceptions.

2 | MATERIALS AND METHODS

This study was approved by the institutional review board at the Hospital of the University of Pennsylvania. The electronic pathology archives were searched from 2018 to 2021 for all thyroid nodules classified as indeterminate (Bethesda category III and IV) with *TSHR* mutations detected on ThyroSeqV3® targeted next-generation sequencing analysis.[23] Patient demographics, tumor characteristics and molecular information were collected for analysis. Levels of expression of sodium-iodide symporter (*NIS*) mRNA as a percentage of *NIS* reads to all RNA sequencing reads detected by ThyroSeqV3® were obtained. Levels of *NIS* > 5% were considered elevated. Cytology and surgical pathology slides were reviewed. If available and performed within 12 months of the FNA, radionuclide thyroid scans were reviewed and correlated with the ultrasound images by one of the authors (SJM) to determine if the biopsied nodule was hyperfunctioning compared to the background thyroid. Statistical analyses were performed and $p < .05$ was considered statistically significant.

3 | RESULTS

Our search revealed a total of 2184 cases diagnosed as atypia/follicular lesion of unknown significance (AUS/FLUS) and 2625 cases as follicular neoplasm/suspicious for follicular neoplasm (FN/SFN). Thyroseq® analysis was performed on 1735 cases classified as AUS/FLUS and 2339 as SFN/FN. Of this cohort, only 69 cases showed *TSHR* mutations (1.6%) in 59 female and 10 male patients with an average age of 55 years (range: 23–86 years). The frequency of *TSHR* mutations was significantly higher ($p < .001$) in the AUS/FLUS nodules (3.1%, 54/1735) than in SFN/FN nodules (0.6%, 15/2339). The average nodule size was 2.2 cm (range: 1.5–3.0 cm) with 38 nodules located in the right lobe, 29 nodules in the left lobe and 2 nodules within the isthmus. Based on radiology reports, the TIRADS scores were as follows: 1 TR1, 3 TR2, 11 TR3, 36 TR4, 10 as TR5; the scores were not available in eight cases. Radionuclide scans were performed in 12 patients within 1 year of FNA and 11 nodules were determined to be hyperfunctioning. The remaining patient underwent a scan while iodine overloaded due to long-term amiodarone use so the ability of a scan to detect nodule autonomy is limited. The average thyroid-stimulating hormone (TSH) level prior to biopsy was 1.5 mIU/mL and, when available, average TSH post-biopsy was 1.2 mIU/mL (Table 1).

Cytologic evaluation classified 54 nodules as AUS/FLUS and 15 nodules as FN/SFN. Of these, 10 cases were described as having oncocytic features (4 AUS/FLUS, 6 FN/SFN; see Table 1 for details). Surgical pathology follow-up was available in 6 cases and revealed 3 adenomatous nodules, 2 follicular adenomas, and 1 minimally invasive follicular carcinoma (Figure 1). On cytopathologic evaluation, all FNA specimens were cellular and comprised of oncocytic cells showing round nuclei with even chromatin pattern and prominent

nucleoli. None of the cases demonstrated atypical nuclear features suspicious for papillary thyroid carcinoma in both alcohol-fixed on-site smears stained with Papanicolaou stain and ThinPrep® preparation.

Fifty-four FNA specimens diagnosed as TBSRTC category III showed background watery colloid and some thick colloid with monolayer sheets and cohesive cell groups with random nuclear atypia (Figure 2). All AUS/FLUS cases also showed follicular cells arranged in loosely cohesive groups with marginal vacuoles, also known as “fire flares” (Figure 2). All 15 FNA specimens diagnosed as follicular neoplasm showed a monotonous population of follicular cells arranged in microfollicles and cohesive groups with nuclear overlapping and crowding. The background contained no or scant watery colloid (Figure 1). Of these, six cases showed mostly oncocytic cells (>70% of cell population) with granular cytoplasm, round nuclei and prominent nucleoli.

On histopathology, the only malignant case in our cohort was a minimally invasive follicular carcinoma with oncocytic features (Hürthle cell) measuring 3.4 cm. On cytology, this lesion was categorized as follicular neoplasm (Bethesda category IV). It was located within the right lobe and showed minimal invasion into the tumor capsule without extension into the surrounding thyroid parenchyma. Angioinvasion was not identified. Molecular testing of this malignant lesion revealed that it harbored the L512R *TSHR* mutation. This lesion also was found to have the highest *NIS* expression of 62.43%. On rereview, the capsular invasion was focal and limited to the tumor capsule without transcapsular extension. This case also showed nodules of tumor within the capsule not connected to the main tumor mass. Retrospectively, this case could have been classified as a follicular adenoma.

Molecular analysis by Thyroseq® identified 22 distinct *TSHR* mutations. They were found affecting codons 281–633 of the gene; the most common mutation was M453T identified in 13 patients. Allele frequencies ranged from 5.5% to 43%. A second mutation was identified in 5 cases: 2 *EZH1* mutations (identified concurrently with TSHR mutations I568T and M453T) and 3 EIF1AX mutations (identified concurrently with *TSHR* mutations A623V, F631L, and I568V) (Table 2). Molecular characterization showed a wide variation in the expression of *NIS* in these samples ranging from 0.01% to 62.43% (mean, 16.5%). Among them, 49 (71%) cases, showed an elevated level of *NIS* expression (>5% of NIS sequencing reads).

Additionally, clinical and radiologic follow-up was available in 38 cases (mean: 24 months, range: 7–47 months). Of those, 34 (89.5%) nodules remained stable and 3 (8%) increased in size. One nodule was classified as indeterminate and 25 cases did not have any clinical follow-up available for review.

4 | DISCUSSION

In this study, we report the largest to date series of thyroid nodules with *TSHR* gene mutations detected in thyroid FNA samples. The results of this study show that, if performed, virtually all of these nodules are hyperfunctioning on radionuclide scanning, and, despite presenting as ultrasonographically moderately suspicious or even highly suspicious

nodules that yielded indeterminate cytology, the vast majority of them are benign nodules or very low-risk cancers.

On fine-needle aspiration, 54 nodules were classified as AUS/FLUS and 15 as FN/SFN, with a predominance of oncocytic cells in 10 cases. All cases classified as AUS/FLUS showed background of watery colloid and follicular cells with “fire-flare” appearance and cytoplasmic paravacuolar granules. This finding has been described in some cases of hyperfunctioning nodules and those arising in a background of Graves’ disease. Interestingly, a majority of cases also showed presence of paravacuolar granules. Histochemical and ultrastructural studies have shown that these granules consist of lysosomes containing hemosiderin and lipofuscin pigments. It has been suggested that the paravacoular granules are due to increased metabolic activity. However, others have disputed these findings, and as this finding which can be encountered in a variety of benign and malignant thyroid lesions.[24–26]

Overall, the results of our study corroborate the findings in the smaller reported series of *TSHR* mutation-positive neoplasms showing that only a small percentage of thyroid nodules with indeterminate cytology harbor *TSHR* mutations.[9] A vast majority of *TSHR*-mutated lesions are either benign or extremely low-risk malignant neoplasms.[9] As reported by other investigators, the most commonly identified mutation in our cohort was seen in M453T.[9,27]

With regard to risk of malignancy, our study only detected a single case of minimally invasive follicular carcinoma with oncocytic features. However, on retrospective review it was felt that the capsular invasion was focal without a transcapsular component and could have been classified as a follicular adenoma.[28,29] It is well-known that a diagnosis of follicular carcinoma based on capsular invasion without a transcapsular component is controversial. Some authors have classified these lesions as “atypical adenoma.” While our study and those reported in the literature show that *TSHR* mutations are associated with low-risk lesions, it also brings up an important point regarding the ability to predict malignancy. Mon and colleagues reported that the allelic frequency of *TSHR* mutations positively correlates with the risk of malignancy.[18] Specifically, all carcinomas identified in this study showed >30% allele frequency with regard to their respective TSHR mutation.[18] Moreover, all malignancies had mutations downstream of codon 453.[18] Our malignant case also follows this pattern with the *TSHR* mutation found at L512R with an allele frequency of 41.4%. Of note, this lesion also had the highest percentage of *NIS* at 62.43% of all sequencing reads. Therefore, in thyroid FNA cases where a *TSHR* mutation is detected, the allele frequency along with the specific codon where the mutation occurs, may predict the clinical behavior of the lesion. However, it is important to note that this case represented a very low risk cancer with a single focus of invasion limited to tumor capsule only.

Most of the detected *TSHR* mutations are known to be activating and therefore are expected to activate, via cAMP pathway, the expression of multiple genes involved in iodine uptake and metabolism including *NIS*. However, not all *TSHR* mutations are functional and activating, and therefore not all of them are expected to lead to NIS overexpression. In this study, we observed high levels of NIS mRNA expression in 71% of these cases defined

as expression greater than 5% of all sequencing reads. This percentage is greater than that reported in the literature (71% vs. 44.5%).[9]

Regarding the clinical findings that determine the functional status of these nodules, the literature shows that nodules harboring *TSHR* mutations and/or NIS overexpression can exhibit different levels of functioning status on thyroid scintigraphy.[9] Twelve out of the 69 nodules harboring *TSHR* mutations in our cohort had a concurrent radioiodine scan result, 11 of which were hyperfunctioning compared to the background thyroid. The remaining scan was performed in a state of iodine overload limiting interpretation. These findings provide additional support that most thyroid nodules harboring *TSHR* mutation are hyperfunctioning. Out of these 12 nodules, one nodule was excised and was diagnosed as minimally invasive follicular carcinoma on surgical resection.

Second mutation was identified in 5 cases: 3 cases with *EIF1AX* mutation and 2 cases with *EZH1* mutation. Two out of three cases with *E1F1AX* mutation do not appear to have overexpressed *NIS* (0% and 2% when normalized to all epithelial cells). These findings contradict the hypothesis that concurrent *EIF1AX* mutation might lead to overexpressed *NIS* through an unknown molecular alteration.[9] The two cases of *EZH1* mutation did show an overexpressed *NIS*. Both *EZH1* and *E1F1AX* mutation have been associated with benign thyroid lesions in the majority of cases.[24,26] It is also important to note that *EZH1* mutation is strongly associated with *TSHR* mutation. This association is thought to be a 2-hit model in the pathogenesis of autonomous thyroid adenomas due to the constitutive activation of the cAMP pathway and hyperproliferation of follicular cells.[25] Unfortunately, neither surgery nor thyroid scintigraphy, were performed on these nodules in our study with second mutation. The results of our study also demonstrate concurrent *TSHR* and *EZH1* or *EIF1AX* mutations do not increase the risk of malignancy in thyroid nodules classified as indeterminate on FNA.

Autoimmune thyroid disease (AITD) was previously thought to occur more frequently in nodules harboring *TSHR* mutations and/or *NIS* overexpression with unknown pathogenesis.[9] All cases with surgical follow-up in our cohort do not show any evidence of autoimmune thyroid disease. It would be interesting to explore this association with further studies if there are any.

In summary, our study based on a large series of cases with *TSHR* mutations demonstrates that most of these nodules are associated with increased *NIS* expression and scintigraphic evidence of cell hyperfunctioning, and that these nodules are associated in the vast majority of cases with benign disease, which may be helpful to guide clinical management of these patients.

DATA AVAILABILITY STATEMENT

Research data are not shared.

REFERENCES

1. Kopp P The TSH receptor and its role in thyroid disease. Cell Mol Life Sci CMLS. 2001;58(9):1301–1322. doi:10.1007/pl00000941 [PubMed: 11577986]
2. Dumont JE, Lamy F, Roger P, Maenhaut C. Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. Physiol Rev. 1992;72(3):667–697. doi:10.1152/physrev.1992.72.3.667 [PubMed: 1320763]
3. Levy O, De la Vieja A, Carrasco N. The Na+/I− symporter (NIS): recent advances. J Bioenerg Biomembr. 1998;30(2):195–206. doi:10.1023/a:1020577426732 [PubMed: 9672241]
4. Hébrant A, van Staveren WCG, Maenhaut C, Dumont JE, Leclère J. Genetic hyperthyroidism: hyperthyroidism due to activating TSHR mutations. Eur J Endocrinol. 2011;164(1):1–9. doi:10.1530/EJE-10-0775 [PubMed: 20926595]
5. Sunthornthepvarakul T, Hayashi Y, Refetoff S. Polymorphism of a variant human thyrotropin receptor (hTSHR) gene. Thyroid Off J Am Thyroid Assoc. 1994;4(2):147–149. doi:10.1089/thy.1994.4.147
6. Russo D, Arturi F, Suarez HG, et al. Thyrotropin receptor gene alterations in thyroid hyperfunctioning adenomas. J Clin Endocrinol Metab. 1996;81(4):1548–1551. doi:10.1210/jcem.81.4.8636365 [PubMed: 8636365]
7. Parma J, Duprez L, Van Sande J, et al. Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature. 1993;365(6447):649–651. doi:10.1038/365649a0 [PubMed: 8413627]
8. Trülzsch B, Krohn K, Wonerow P, et al. Detection of thyroid-stimulating hormone receptor and Gsalpha mutations: in 75 toxic thyroid nodules by denaturing gradient gel electrophoresis. J Mol Med Berl Ger. 2001;78(12):684–691. doi:10.1007/s001090000170
9. Guan H, Matonis D, Toraldo G, Lee SL. Clinical significance of thyroid-stimulating hormone receptor gene mutations and/or sodium-iodine symporter gene overexpression in indeterminate thyroid fine needle biopsies. Front Endocrinol. 2018;9:566. doi:10.3389/fendo.2018.00566
10. Stein SA, Oates EL, Hall CR, et al. Identification of a point mutation in the thyrotropin receptor of the hyt/hyt hypothyroid mouse. Mol Endocrinol Baltim Md. 1994;8(2):129–138. doi:10.1210/mend.8.2.8170469
11. Joba W, Spitzweg C, Schriever K, Heufelder AE. Analysis of human sodium/iodide symporter, thyroid transcription factor-1, and paired-box-protein-8 gene expression in benign thyroid diseases. Thyroid Off J Am Thyroid Assoc. 1999;9(5):455–466. doi:10.1089/thy.1999.9.455
12. Nikiforov YE, Carty SE, Chiosea SI, et al. Impact of the multi-gene ThyroSeq next-generation sequencing assay on cancer diagnosis in thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance cytology. Thyroid Off J Am Thyroid Assoc. 2015;25(11):1217–1223. doi:10.1089/thy.2015.0305
13. Abdelhakam DA, Mojica RE, Huenerberg KA, Nassar A. Impact of a genomic classifier on indeterminate thyroid nodules: an institutional experience. J Am Soc Cytopathol. 2021;10(2):155–163. doi:10.1016/j.jasc.2020.09.005 [PubMed: 33067175]
14. Camacho P, Gordon D, Chiefari E, et al. A Phe 486 thyrotropin receptor mutation in an autonomously functioning follicular carcinoma that was causing hyperthyroidism. Thyroid Off J Am Thyroid Assoc. 2000; 10(11):1009–1012. doi:10.1089/thy.2000.10.1009
15. Führer D, Tannapfel A, Sabri O, Lamesch P, Paschke R. Two somatic TSH receptor mutations in a patient with toxic metastasising follicular thyroid carcinoma and non-functional lung metastases. Endocr Relat Cancer. 2003;10(4):591–600. doi:10.1677/erc.0.0100591 [PubMed: 14713269]
16. Niepomniszcze H, Suárez H, Pitoia F, et al. Follicular carcinoma presenting as autonomous functioning thyroid nodule and containing an activating mutation of the TSH receptor (T620I) and a mutation of the Ki-RAS (G12C) genes. Thyroid Off J Am Thyroid Assoc. 2006;16(5):497–503. doi:10.1089/thy.2006.16.497
17. Russo D, Arturi F, Schlumberger M, et al. Activating mutations of the TSH receptor in differentiated thyroid carcinomas. Oncogene. 1995; 11(9):1907–1911. [PubMed: 7478621]

18. Mon SY, Riedlinger G, Abbott CE, et al. Cancer risk and clinicopathological characteristics of thyroid nodules harboring thyroid-stimulating hormone receptor gene mutations. Diagn Cytopathol. 2018;46(5):369–377. doi:10.1002/dc.23915 [PubMed: 29516685]

19. Gozu H, Avsar M, Bircan R, et al. Does a Leu 512 Arg thyrotropin receptor mutation cause an autonomously functioning papillary carcinoma? Thyroid Off J Am Thyroid Assoc. 2004;14(11):975–980. doi:10.1089/thy.2004.14.975

20. Russo D, Wong MG, Costante G, et al. A Val 677 activating mutation of the thyrotropin receptor in a Hürthle cell thyroid carcinoma associated with thyrotoxicosis. Thyroid Off J Am Thyroid Assoc. 1999;9(1):13–17. doi:10.1089/thy.1999.9.13

21. Lado-Abeal J, Celestino R, Bravo SB, et al. Identification of a paired box gene 8-peroxisome proliferator-activated receptor gamma (PAX8-PPARgamma) rearrangement mosaicism in a patient with an autonomous functioning follicular thyroid carcinoma bearing an activating mutation in the TSH receptor. Endocr Relat Cancer. 2010;17(3):599–610. doi:10.1677/ERC-09-0069 [PubMed: 20427420]

22. Cross GAD, Suarez H, Pitoia F, et al. Fatal outcome of a young woman with papillary thyroid carcinoma and graves' disease: possible implication of "cross-signalling" mechanism. Arq Bras Endocrinol Metabol. 2008;52(7):1194–1200. doi:10.1590/s0004-27302008000700018 [PubMed: 19082310]

23. Nikiforova MN, Mercurio S, Wald AI, et al. Analytical performance of the ThyroSeq v3 genomic classifier for cancer diagnosis in thyroid nodules. Cancer. 2018;124(8):1682–1690. doi:10.1002/cncr.31245 [PubMed: 29345728]

24. Eimoto T, Naito H, Hamada S, Masuda M, Harada T, Kikuchi M. Papillary carcinoma of the thyroid. A histochemical, immunohistochemical and ultrastructural study with special reference to the follicular variant. Acta Pathol Jpn. 1987;37(10):1563–1579. [PubMed: 3434281]

25. Sidawy MK, Costa M. The significance of paravacuolar granules of the thyroid. A histologic, cytologic and ultrastructural study. Acta Cytol. 1989;33(6):929–933. [PubMed: 2480046]

26. Layfield LJ, Wax T, Jones C. Cytologic distinction of goiterous nodules from morphologically normal thyroid: analyses of cytomorphologic features. Cancer. 2003;99(4):217–222. doi:10.1002/cncr.11221 [PubMed: 12925983]

27. Nishihara E, Amino N, Maekawa K, et al. Prevalence of TSH receptor and Gsalpha mutations in 45 autonomously functioning thyroid nodules in Japan. Endocr J. 2009;56(6):791–798. doi:10.1507/endocrj.k09e-073 [PubMed: 19550078]

28. Mete O, Asa SL. Thyroid tumor capsular invasion: the bottom line or much ado about nothing? Endocr Pathol. 2020;31(2):141–142. doi:10.1007/s12022-020-09621-6 [PubMed: 32212065]

29. Zhu Y, Li Y, Jung CK, et al. Histopathologic assessment of capsular invasion in follicular thyroid neoplasms-an observer variation study. Endocr Pathol. 2020;31(2):132–140. doi:10.1007/s12022-020-09620-7 [PubMed: 32236857]

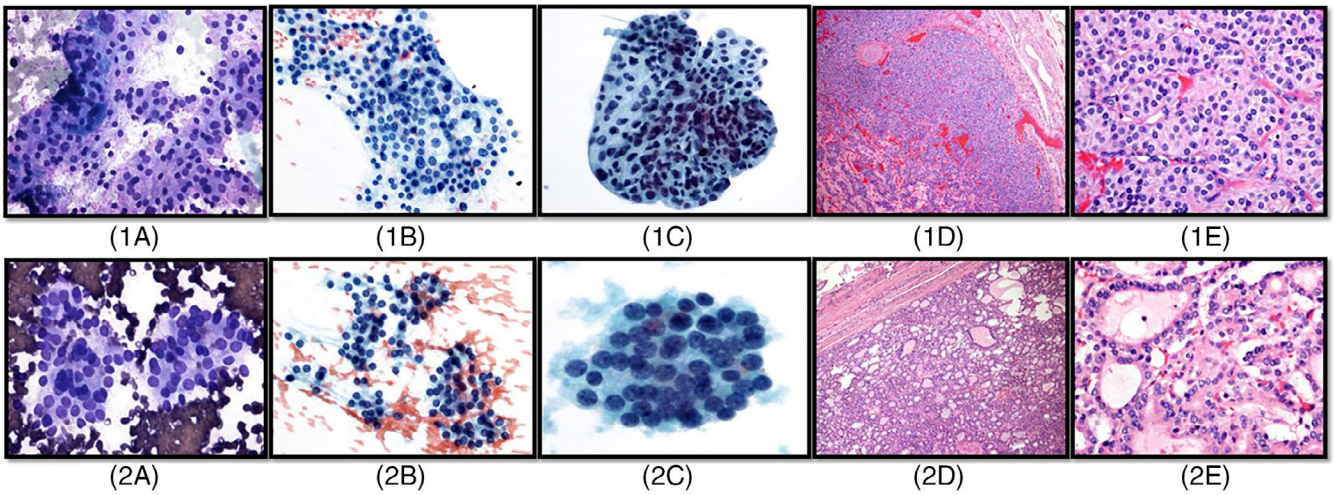

FIGURE 1.

A composite figure of two cases with *TSHR* mutation. Case 1: The FNA sample was diagnosed as "Atypia of Undetermined Significance," it was cellular and showed monolayer sheets and cohesive groups of follicular cells with oncocytic cytoplasm and random nuclear atypia. No nuclear features of papillary carcinoma were seen (A: Diff-Quik® stain. B: Papanicolaou stain. C: ThinPrep® preparation). The surgical pathology follow-up was diagnosed as follicular adenoma (D–E: hematoxylin and eosin stains at 50× and 200×). Case 2: The FNA samples from this case show monotonous populations of follicular cells arranged in cohesive groups and microfollicles with round nuclei, with evenly distributed nuclear chromatin pattern. No nuclear features of papillary carcinoma were seen 2 (A: Diff-Quik® stain. B: Papanicolaou stain. C: ThinPrep® preparation). The surgical pathology follow-up was follicular adenoma (D–E at 50× and 200× hematoxylin and eosin stains). [Color figure can be viewed at wileyonlinelibrary.com]

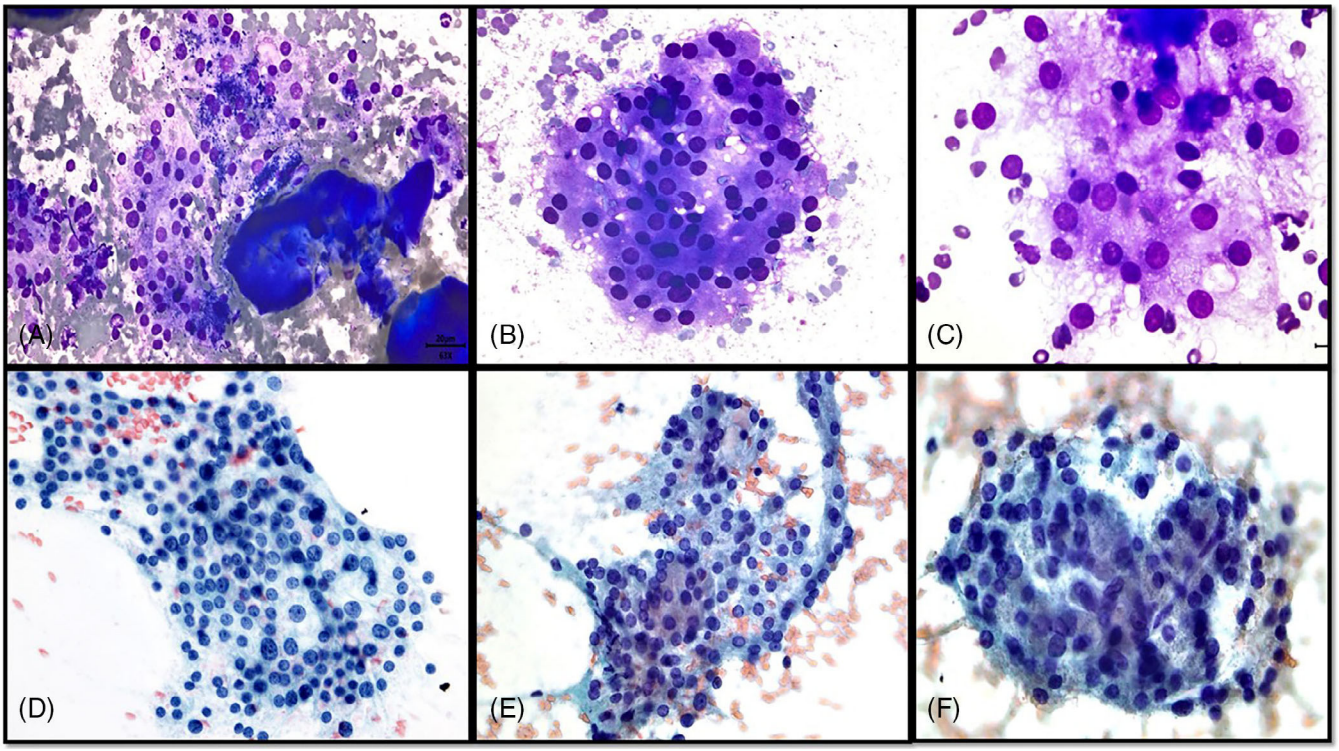

FIGURE 2.

A composite figure of cytologic features of cases with *TSHR* mutation. Follicular cells arranged in loosely cohesive groups with marginal vacuoles, also known as “fire flares” (air-dried smear stained with Diff-Quik stain A–C, 50×, 100× and 200×). The alcohol fixed smears stained with Papanicolaou stain demonstrate follicular cells with round nuclei with even chromatin pattern, moderate amount of cytoplasm containing paravacoular granules (D–F, 50× and 200×). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1

Compilation of information for all patients included in the study including demographics, pertinent laboratory studies, radiologic characteristics of the nodules and corresponding cytologic and, if resected, surgical histopathologic diagnoses.

Patient demographics	Age (years)
	55 (range: 23–86)
	Sex (Male:Female)
	10:59
	Laterality
	Right: 38, Left: 29, Isthmus: 2
	Size (cm)
	2.2 (range: 1.5–3.0)
	Pre-biopsy/pre-operative TSH level (IU/mL) (*n* = 59)
	1.5 (range: 0.008–10.51)
	Post-biopsy/post-operative TSH level (IU/mL) (*n* = 48)
	1.2 (range: 0.01–5.12)
TIRADS	1: 1
	2: 3
	3: 11
	4: 36
	5: 10
	Not available: 8
Radio-iodine uptake scan (concurrent) (*n* = 12)	Hyperfunctioning—11 patients
	Limited interpretation due to sodium overload—1 patient
Cytologic diagnosis	AUS/FLUS: 54, FN/SFN: 15
Follow-up	Surgery—6
	Stable—34
	Increased in size—3
	Indeterminate—1
	Not available—25
Surgical pathology diagnosis (*n* = 6)	Benign: Adenomatous nodules—3, follicular adenoma—2
	Malignant: Minimally invasive follicular carcinoma—1

TABLE 2

List of thyroid stimulating hormone receptor (*TSHR*) mutations in order of decreasing frequency, average percentage of sodium-iodide symporter (*NIS*), and additional mutations in other genes identified following ThyroSeq® analysis.

Mutation	Allele frequency %	NIS %	NIS normalized to all epithelial cells	Additional mutation	Mutation	Allele frequency %
A623V	14.3	15.6	45%			
A623V	7.3	1.34	3%			
A623V	13.6	0.06	0%	*EIF1AX*	G15D	17.4
D619G	11	0.31	1%			
D619G	12.4	0.14	0%			
D633E	12.5	56.58	347%			
D633E	24.7	1.09	2%			
D633H	5.7	41.05	174%			
D633Y	10.4	26.19	95%			
D633Y	8	16.34	50%			
D633Y	22.5	14.02	68%			
D633Y	9.3	11.7	35%			
D633Y	35.1	8.03	24%			
D633Y	24.3	6.28	32%			
F631I	22.4	32.62	173%			
F631L	19.8	33.82	158%			
F631L	17.5	32.28	228%			
F631L	22.2	20.49	71%			
F631L	14.5	15.53	71%	*EIF1AX*	R13P	14.3
F631L	22.7	10.71	33%			
F631V	8.6	0.38	1%			
I486F	18.5	39.63	230%			
I486F	7.4	34.39	140%			
I486F	13	21.26	71%			
I486F	20.6	15.54	51%			
I486F	12.4	13.5	37%			
I486F	39.2	8.36	23%			
I486M	17.4	35.72	135%			
I486M	25.3	3	9%			
I486M	17.1	0.06	0%			
I568F	26.1	16.96	58%			
I568F	20	13.32	65%			
I568F	7.8	11.67	34%			
I568F	7.2	6.25	16%			
I568T	16.7	42.58	294%	*EZH1*	Q571R	18.1
I568T	27.9	23.66	80%			
I568T	14.5	4.94	16%			

Mutation	Allele frequency %	NIS %	NIS normalized to all epithelial cells	Additional mutation	Mutation	Allele frequency %
I568T	11.9	4.03	12%			
I568T	6.7	3.67	11%			
I568V	6.7	0.59	2%	*EIF1AX*	G9R	7.2
I630L	16.1	0.13	0%			
L512R	41.4	62.43	692%			
L512R	23.3	52.19	296%			
L512R	43	36.47	282%			
L512R	12.7	18.14	53%			
M453T	8.4	41.2	175%			
M453T	27.5	36.49	169%			
M453T	26.2	32.81	198%			
M453T	17.4	30.48	156%			
M453T	21.7	27.65	111%			
M453T	35.4	21.9	88%			
M453T	7.1	16.57	58%			
M453T	25.9	13.6	38%			
M453T	13.5	11.85	30%	*EZH1*	Q571R	10.2
M453T	13.2	11.14	33%			
M453T	14.3	10.08	26%			
M453T	5.5	8.8	31%			
M453T	13.3	4.42	10%			
P639A	TBD	TBS	TBD			
S281T	13.2	4.3	11%			
S281T	15.4	0.54	2%			
S505R	27.2	9.94	34%			
S505R	6.2	0.11	0%			
T620N	17.1	0.01	0%			
T632A	29.7	0.76	2%			
T632I	19.7	9.05	54%			
T632I	14.8	7.96	22%			
T632I	31.1	7.32	23%			
T632I	9.3	1.67	4%			