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The DOI for this manuscript is doi: 10.5858/arpa.2020-0816-OA

The final published version of this manuscript will replace the Early Online Release version at the above DOI once it is available.
Solid Thyroid Follicular Nodules With Longitudinal Nuclear Grooves

Clinicopathologic, Immunohistochemical, and Molecular Genetic Study of 18 Cases

David Suster, MD; A. Craig Mackinnon, MD, PhD; Vania Nosé, MD, PhD; Saul Suster, MD

- **Context.**—Follicular thyroid nodules can be a source of diagnostic difficulties, particularly when they display atypical features commonly associated with malignancy, such as nuclear grooves.

  - **Objective.**—To differentiate lesions with atypical features from similar-appearing benign and malignant lesions.

  - **Design.**—Eighteen cases of atypical follicular thyroid nodules characterized by a solid growth pattern and prominent longitudinal nuclear grooves were studied and examined for clinicopathologic characteristics.

  - **Results.**—The lesions occurred in 16 women and 2 men aged 36 to 88 years and measured from 0.2 to 1.5 cm. The tumors were well circumscribed and noninvasive, and histologically characterized by a predominantly solid growth pattern with rare scattered follicles or a combination of solid growth pattern with minor follicular areas. A striking feature seen in all cases was the occurrence of longitudinal nuclear grooves. Immunohistochemical stains showed negativity for cytokeratin 19 (CK19) and HBME-1 in 8 cases; in the other 10, there was focal positivity for HBME-1 in 4 cases and diffuse positivity in 6. All cases were negative for galectin-3 and for CK19, with the exception of 1 case, which was CK19+/HBME-1+. Next-generation sequencing of 16 cases with a 161-gene panel detected 14 single nucleotide variants in 12 cases, predominantly NRAS and HRAS mutations. Clinical follow-up ranging from 18 to 72 months (median, 43.7 months) did not disclose any evidence of recurrence or metastases.

  - **Conclusions.**—We interpret these lesions as low-grade, indolent follicular proliferations that need to be distinguished from papillary thyroid carcinoma, follicular adenoma, and noninvasive follicular thyroid neoplasms with papillary-like nuclear features.

  (Arch Pathol Lab Med. doi: 10.5858/arpa.2020-0816-OA)

Follicular thyroid nodules can often be a source of diagnostic difficulties owing to the wide variability in their architectural and cytologic features, particularly when they display nuclear features that are closely associated with papillary thyroid carcinoma (PTC). The occurrence of longitudinal nuclear grooves represents one of the features that has been traditionally regarded as a highly distinctive feature of PTC.1–4 and their presence in thyroid nodules has predictably triggered a diagnosis of carcinoma. In recent years, however, there has been an increasing realization that some thyroid lesions bearing the nuclear features of PTC may not warrant a diagnosis of malignancy and may be treated in a more conservative manner, as demonstrated by the recently introduced category of noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP), which were found in a recent study to behave in an indolent fashion despite the presence of nuclear features of PTC.5

We have studied a series of 18 cases of solid follicular thyroid nodules displaying distinctive histologic features that distinguished them from papillary thyroid carcinoma, follicular adenoma, and NIFTP and which appear to follow an indolent clinical behavior. Most cases in this study were seen in consultation with the question of whether the histopathologic features were sufficient for a diagnosis of papillary thyroid carcinoma. These thyroid nodules were defined by a solid growth pattern in combination with some degree of cytologic atypia and the presence of longitudinal nuclear grooves. The predominantly solid pattern of growth essentially eliminated the consideration of NIFTP and of the follicular variant of PTC in the differential diagnosis for these lesions. The lesions also showed good circumscription and absence of invasion, lacked clearing of the nuclear chromatin, and were mostly unencapsulated. Currently, there is no established category for lesions bearing such features in the World Health Organization classification.1

None of the patients showed evidence of invasion or regional lymph node involvement at the time of diagnosis, and clinical follow-up showed that they were all free of disease and without evidence of recurrence or metastasis up
to 72 months following diagnosis. The clinicopathologic, immunohistochemical, and molecular genetic features of these cases are detailed herein.

MATERIALS AND METHODS

The cases were retrieved from the surgical pathology files of the Medical College of Wisconsin (Milwaukee) and the Massachusetts General Hospital (Boston) or from the personal consultation files of one of the authors (S.S.). From 5 to 12 hematoxylin-eosin–stained glass slides were available for review for each case. Representative paraffin blocks or unstained tissue sections on positive-coated slides were available for immunohistochemical studies in all cases. For molecular studies and DNA extraction, 10-μm-thick sections were cut from representative paraffin blocks or retrieved from unstained tissue sections. Clinical information was extracted from the medical records and surgical pathology reports for all cases. Clinical follow-up information was obtained from the institutional medical records or by contacting the referring physicians. This study was conducted under institutional review board approval.

Immunohistochemical Studies

Immunohistochemical study was performed with reagents from the Dako Envision FLEX kit and the Dako Autostainer Plus stainer (Agilent, Santa Clara, California). Stains were performed by using antibodies to HBME-1 (1:50; clone HBME-1, DAKO), cytokeratin 19 (CK19, ready-to-use; clone RCK108, DAKO), gaelectin-3 (1:50; clone 9C4, Cell Marque), and Ki-67 proliferation marker (ready-to-use; clone MIB-1, DAKO). Following pretreatment with Target Retrieval Solution, tissue was blocked with peroxidase-blocking reagent for 5 minutes and incubated with the primary antibody at room temperature. Signals were detected with the Dako FLEX detection kit. Counterstaining was performed with Envision FLEX hematoxylin for 7 minutes at room temperature. Appropriate positive and negative controls were run concurrently for all antibodies tested. The immunohistochemical reaction was graded as positive as based on nuclear, cytoplasmic, or membrane reactivity for the various antibodies.

Next-Generation Sequencing

Targeted next-generation sequencing using the Ion Torrent Oncomine Comprehensive Assay v3 (OCAv3, Thermo Fisher Scientific, Waltham, Massachusetts) was used to detect hotspot mutations in 87 genes, focal copy number variations in 43 genes, sequence analysis of the full coding region for 48 genes, and gene-fusion drivers in 51 genes. OCAv3 targets the following RET fusion partners: ACD5, AFAPI, AKAP13, CCD6, CLUX1, ERC1, FKBP15, GOLGA5, HOOK3, KIAA1468, KIF5B, KTN1, MYH13, NCOA4, PCE1, PKRKA1A, RUFY2, SQSTM1, SPEC11, TBL1XR1, TRIM24, TRIM27, and TRIM33. The assay also targets the following PPARG fusion partners: PAX8, CREBL2, and TSEN2. In all, 161 genes are analyzed with this panel. To verify the findings, the Ion AmpliSeq Cancer Hotspot Panel v2 was used to confirm the variants identified by the Oncomine panel in 5 cases (cases 9, 14, 15, 16, and 17). DNA was isolated by using the Qagen Allprep formalin-fixed, paraffin-embedded DNA/RNA kit and RNA was isolated by using the Pinpoint Slide RNA Isolation System (Zymo Research). Both DNA and RNA were quantified by using Qubit Fluorometric Quantification systems. Library and templating preparation were performed by using the Ion Chef (Thermo Fisher Scientific) following manufacturer’s recommendations. Sequencing was completed on the Ion Torrent S5 XL sequencer. Analysis of sequencing data was performed with the Ion Torrent Suite 5.8.0 and Ion Reporter Software (Thermo Fisher Scientific).

Mutation Detection by the Idylla Molecular Diagnostics System

The Idylla system uses ready-to-use test cartridges that target specific hotspot mutations within 4 genes. We analyzed 4 cases (13, 14, 15, and 17), using a combined NRAS/BRAF cartridge obtained from Biocartis (Mechelen, Belgium). Testing was performed according to the manufacturer’s recommended protocol. Briefly, a tumor-rich, 1-mm core portion of tissue was punched out of a formalin-fixed, paraffin-embedded block and inserted into the Idylla cartridge. A combination of chemical reagents, enzymes, heat, and high-intensity focused ultrasound induced deparaffinization, disruption of the tissue, and lysis of the cells inside the cartridge. The nucleic acids were liberated and ready for subsequent polymerase chain reaction (PCR) amplification. Real-time PCR was then performed by using allele-specific primers targeting codons 12, 13, 59, 61, 117, and 146 of NRAS and codon 600 of BRAF. In addition, the simultaneous detection of an endogenous sample processing control was performed. Detection of these specific targets was performed with fluorescent labeled probes. The process in the cartridge was performed automatically. Vendor-supplied software automatically analyzed the collected fluorescent signals. Results were presented on the Idylla Console. The obtained fluorescent signals were evaluated for PCR curve validity. A cycle of quantification value (Cq) was calculated for every valid curve. The presence of a mutant genotype was determined by calculating the difference between the Cq of the sample processing control and the Cq of the mutant signal(s); the difference between the control signal and the mutant signal was defined as the ACq. The mutant signal was considered valid if the ACq value was within a predefined range established by the vendor, and the detected variant was reported. At the end of the run, a final report indicated the presence or absence of a specific codon mutation in the targeted gene.

RESULTS

Clinical Findings

The main clinical findings for our patients are presented in the Table. There were 16 women and 2 men, aged 36 to 88 years (mean, 54 years). The lesions were found incidentally in 14 patients in total thyroidectomy specimens for other causes; in 4 patients, lobectomy was performed for a suspicious nodule. Two patients presented with Hashimoto thyroiditis with superimposed nodular hyperplasia. In 3 patients (cases 11, 13, and 18), preoperative fine-needle aspiration (FNA) showed findings suggestive of malignancy (Bethesda category V). In these 3 cases, the FNA was taken from the index nodule. In case 17, an FNA was from the larger nodule corresponding to a PTC. All FNAs were performed at outside institutions and slides from the cytology specimens were not available for review. Twelve of 18 cases were received in consultation to rule out papillary thyroid carcinoma. Clinical follow-up was available for 14 patients; all patients were alive and well without evidence of recurrence or metastasis for a follow-up period ranging from 18 to 72 months (median, 43.7 months); 4 patients were lost to follow-up or were recent cases.

Pathologic Findings

All cases were characterized by relatively small, well-circumscribed nodules that stood out from the surrounding thyroid parenchyma owing to their increased cellularity (Figure 1, A). Most represented small incidental findings in thyroids removed for multinodular goiter (83.3% were <1.0 cm in diameter). The nodules in all cases were sharply demarcated from the surrounding tissue without evidence of invasion; in 4 cases they showed an incomplete, thin partial capsule and in 14 cases they were unencapsulated. On low-power magnification, the nodules were predominantly composed of solid and compact microfollicular structures with collapsed lumens (Figure 1, B and C). Isolated, scattered, well-developed small follicles with...
Figure 1. A, Scanning magnification (case 3) shows a well-circumscribed, unencapsulated follicular thyroid nodule with a solid growth pattern containing a few entrapped follicles at the periphery. B, Higher magnification from preceding field shows a sharply circumscribed and unencapsulated tumor with predominantly solid growth pattern. C, Closer view showing the interface of the nodule with the surrounding thyroid parenchyma. Notice the almost total collapse of follicle lumens and sheetlike growth pattern. D, Higher magnification shows a cell population with anisocytosis that contains slightly enlarged nuclei and easily visualized, sometimes multiple nucleoli. E, High-power view shows cells with round to oval and hyperchromatic nuclei, with scant amphophilic cytoplasm and with infoldings of the nuclear membrane, resulting in longitudinal nuclear grooves (hematoxylin-eosin, original magnifications ×2 [A], ×4 [B], ×40 [C], ×60 [D], and ×100 [E]).
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<td>12</td>
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<td>Total thyroidectomy for multinodular goiter</td>
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<td>CK19/HBME⁻</td>
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### Immunohistochemical Findings

Immunohistochemical stains were done in all cases. Staining for HBME-1 was completely negative in 8 cases (Figure 3, A; Table). Six cases showed strong and diffuse cytoplasmic and membrane positivity for HBME-1 in the tumor cells (Figure 3, B). In 4 cases, focal positivity for HBME-1 was seen only in a minor subset of the tumor cells (2%–15% of the cells) (Figure 3, C). Overall, only 6 of 18 cases (33%) in our study showed strong and diffuse cytoplasmic and membrane staining for HBME-1. All cases were negative for CK19, except for 1 case (case 10) that was negative for HBME-1 and positive for CK19. Galectin-3 staining was negative in all cases. Despite the high cellularity of the lesions, stains for Ki-67 showed very low proliferative activity (<1% nuclear positivity) in all cases.
Figure 2. A, Scanning magnification (case 5) shows a well-circumscribed and unencapsulated follicular nodule sharply demarcated from the surrounding thyroid parenchyma. B, On higher magnification, the tumor is composed of solid sheets of cells lacking follicular lumens. C, On high power, the cells show oval nuclei with finely dispersed chromatin, small nucleoli, and numerous longitudinal nuclear grooves (hematoxylin-eosin, original magnifications ×2 [A], ×10 [B], and ×100 [C]).

Figure 3. A, HBME-1 stain for case 1 is negative in the small nodule (center) as well as in the surrounding thyroid parenchyma. B, Immunohistochemical stain for HBME-1 (case 8) shows focal, patchy staining of the tumor cells within the nodule. C, HBME-1 (case 5) shows diffuse membrane staining of the tumor cells throughout the entire nodule (original magnifications ×2 [A], ×4 [B], and ×60 [C]).
Molecular Genetic Findings

Sixteen of 18 cases were successfully sequenced by next-generation sequencing. Single nucleotide variants were detected in 12 cases (Table). No genomic alterations—including gene fusion events involving RET or PPARGamma and their common fusion partners described above—were identified. No copy number alterations were identified in any of the cases. BRAF V600E was not identified in any case tested. RAS/RAF/MEK pathway mutations were the most commonly observed alterations and occurred in 8 of 16 cases (50%). The most common variants observed were NRAS p.Q61R and p.Q61K (6 of 16 cases; 38%). Two cases (2 of 16; 13%) showed variants in the HRAS gene: p.Q61R (case 9) and p.Q61K (case 15). BRAF K601E was detected in 4 of 16 cases (25%). There were no unique histologic features identified in the cases with the BRAF mutations compared with the other cases. Case 2, which had an NRAS p.Q61R mutation, also harbored a HNF1A p.R114H variant. This position does not lie within any known functional domains of the HNF1A protein, and the p.R114H variant does not result in a substantial decrease in either HNF1A nuclear localization or transactivation activity in humans when compared to wild-type HNF1A. Case 10 with BRAF p.K601E also harbored an ATM p.R337H variant of uncertain significance. Although described in human cancer (COSV53729310, COSMIC), amino acid R337 does not lie within any known functional domain of the ATM protein, and the effects of this variant on ATM protein function are unknown.

To test whether the atypical solid thyroid follicular nodules with grooves shared molecular alterations with the concomitant papillary microcarcinomas present in some of our cases, additional molecular testing was performed. The foci of papillary microcarcinomas tested in 4 cases for NRAS and BRAF were from cases 13, 14, 15, and 17. In cases in which there were multifocal microcarcinomas, the largest focus was chosen for microdissection. These microcarcinomas underwent targeted molecular testing for either NRAS or BRAF variants, using real-time PCR on the Idylla system. BRAF V600E was identified within the foci of papillary microcarcinoma in 3 of 4 cases (cases 13, 14, and 17), and no genetic variants were identified in the fourth case (case 15). Importantly, we did not detect the most common mutation identified in our cohort, NRAS p.Q61, in any of the papillary microcarcinomas assayed.

DISCUSSION

Papillary thyroid carcinoma represents the most common type of thyroid cancer in the United States and developed countries. The diagnostic criteria for PTC have evolved slowly over the years. Although initially based on the papillary architecture of the lesion, the nuclear features of the tumor cells eventually acquired a greater significance to the point of overshadowing the papillary architecture for the diagnosis of PTC. This realization led to the identification of additional histologic variants of PTC that were devoid of papillary architecture, such as the follicular variant of PTC (FVPTC), the cribriform-morular variant, and the solid/trabecular variant. More recently, this trend has been reversed and newer studies have assigned once again a significant role to the architecture of the lesion for diagnosis. An example of this is the recent introduction of a new category of noninvasive follicular tumors with papillary-like features (NIFTP) for lesions that were formerly regarded as encapsulated variants of FVPTC. However, despite architecture playing once more an important role for the diagnosis of PTC, the identification of the characteristic nuclear features of PTC continues to play a central role in the diagnosis of these lesions.

The nuclear features associated with PTC have been adequately reviewed previously in the literature and principally include enlargement of nuclei with clearing of the nuclear chromatin, intranuclear cytoplasmic pseudoinclusions, and longitudinal nuclear grooves, among others. The presence of longitudinal nuclear grooves has long been regarded as a reliable indicator for the diagnosis of PTC, as they have not been described in association with many other thyroid conditions. Thyroid nodules displaying cells with longitudinal nuclear grooves are thus very likely to be regarded as highly suggestive of PTC.

Herein, a series of 18 patients who presented with small, well-circumscribed, largely unencapsulated and uninvasive, predominantly solid follicular thyroid nodules that contained longitudinal nuclear grooves, was examined. They were almost all found incidentally on thyroidectomy specimens removed for multinodular goiter or other causes, except in 4 patients in which the glands were removed for the suspicion of malignancy and in whom these lesions were found incidentally. In addition to the longitudinal nuclear grooves, the cases displayed a solid growth pattern with compressed follicles and scant or absent colloid, mild enlargement of the nuclei, dense nuclear chromatin, minimal irregularities of the nuclear envelope, and conspicuous nucleoli. All the lesions except for one arose in a background of nodular hyperplasia. The combination of the nuclear atypia, solid pattern of growth, and longitudinal nuclear grooves raised the possibility of an unusual variant of PTC. Twelve of the 18 cases were initially diagnosed as suggestive of PTC in the resection specimen and submitted in consultation for additional review. The lesions showed an indolent clinical course; none of them were associated clinically with lymph node metastases and on clinical follow-up none of the patients experienced recurrences or metastases (Table).

Immunohistochemical stains showed that most of the cases in our study were negative for markers that have been associated with PTC, including HBME-1, CK19, and galectin-3. Six cases (33%) in this study showed cytoplasmic staining for HBME-1, a marker that has been found to be associated in a significant number of cases with PTC. However, those cases were negative for CK19 and galectin-3, markers that have also been closely associated with PTC. In addition, while HBME-1 has been extensively studied in PTC and found to be commonly expressed in this tumor, several studies have also documented expression of this marker in other thyroid conditions such as hyperplastic nodules, NIFTP, follicular adenomas, and follicular carcinomas. HBME-1, therefore, is not specific or restricted to PTC and cannot be unequivocally regarded as evidence to support this diagnosis. The presence of staining for HBME-1 as seen in some of our cases can represent a pitfall for diagnosis and, in conjunction with the longitudinal nuclear grooves, may unduly influence a diagnosis of PTC. As has been repeatedly emphasized in the literature, the diagnosis of PTC cannot be reliably established on the basis of the results of immunohistochemical stains alone and depends on the identification of a constellation of findings that include the characteristic...
nuclear features, architecture, stromal changes, and invasion.\textsuperscript{20,30,32}

Molecular evaluation of thyroid neoplasms is an evolving field that has helped elucidate the multistep oncogenic mechanisms underlying carcinogenesis in thyroid neoplasms. In general, the evaluation of thyroid nodules for prognosis and risk stratification by molecular methods continues to be based on a process of elimination by identifying genetic expression patterns that help to rule out malignant disease in indeterminate thyroid nodules, rather than by positive identification of specific markers of malignancy.\textsuperscript{35–38} Unfortunately, few diagnostic molecular alterations have been identified so far that are exclusive to BRAF mutation found in thyroid neoplasms and it occurs primarily in follicular-patterned lesions, especially NIFTP (formerly classified as the encapsulated variant of FVPTC).\textsuperscript{46} BRAF V600E has also been described in overtly benign follicular lesions, such as thyroid adenomas, and has been reported in \textasciitilde1\% of PTC of the follicular variant.\textsuperscript{48–50} The broad spectrum of thyroid lesions in which this mutation has been observed, however, precludes assigning it any specific role for the various types of lesions associated with it, and is in keeping with previous observations demonstrating its expression across a spectrum of benign and malignant thyroid nodules. The HNF1A and ATM variants identified in our cases were interpreted as variants of uncertain significance and have not yet been well characterized in thyroid neoplasms.\textsuperscript{51,52}

The most common genetic alterations identified in our cohort involved NRAS and HRAS, which occurred in 8 of 16 cases (50\%), with NRAS Q61R being the most frequently observed (6 cases). The Ras gene codes for 3 homologous isoforms that include NRAS, HRAS, and KRAS. The protein product of these genes plays a role in signal transduction within the MAPK and PI3K–AKT signaling pathways, which control cell proliferation and survival.\textsuperscript{53} Upregulation of the Ras family mutations have been found to occur in both benign and malignant follicular-patterned tumors of the thyroid, including benign hyperplastic follicular nodules, follicular adenoma, follicular thyroid carcinoma, PTC, FVPTC, and NIFTP.\textsuperscript{54–58} Ras mutations are thus not diagnostic for any particular type of thyroid tumor given that they are not restricted to PTC, FVPTC, or NIFTP, but can occur over a broad spectrum of follicular lesions including benign ones. However, given that Ras mutations have been frequently encountered in FVPTC and NIFTP, the question arises as to whether the Ras variants in our cases could be related in some manner to these 2 conditions. While the lesions in this study can be conceptualized as being comparable to NIFTP, based on the combination of their sharp circumscription and for having one of the nuclear features of PTC (ie, longitudinal nuclear grooves), NIFTP was introduced to specifically address the issue of tumors showing a combination of nuclear features of PTC with a well-developed follicular growth pattern that were well-circumscribed or encapsulated and noninvasive. The current cases were mostly unencapsulated, showed a predominantly solid growth pattern, and displayed only nuclear grooves rather than the striking clearing of the nuclear chromatin and predominant follicular growth pattern observed in NIFTP.\textsuperscript{5,59,60} Moreover, current criteria for NIFTP disqualify any cases with greater than 30\% solid, trabecular, or insular growth pattern. Our lesions, therefore, do not qualify for the designation of NIFTP according to its current definition; likewise, the lack of invasion, the predominantly solid growth with absence of a well-developed follicular pattern, and the absence of the characteristic clearing of the nuclear chromatin prevent any of these lesions from being classified as FVPTC or as a papillary microcarcinoma of the follicular variant. Further supporting the notion that the lesions presented in this study are distinct from papillary microcarcinomas is the identification of BRAF V600E in 3 of 4 papillary microcarcinomas co-occurring in 4 of our cases. We also failed to detect NRAS p.Q61 in any of the papillary microcarcinomas associated with our tumors. The molecular profile in our series, which is notable for BRAF K601E and NRAS/HRAS Q61 variants, is unique from papillary microcarcinoma, where BRAF V600E is detected in approximately 60\% of cases.\textsuperscript{51} The recurrent Ras-family mutations, non-V600E BRAF mutations, and additional unique alterations identified in our cohort indicate that most, if not all, of these lesions represent clonal proliferations of thyroid follicular cells (ie, neoplastic lesions) adopting an unusual morphology. The heterogeneous genetic makeup of these lesions is a common feature of thyroid follicular nodules in general; however, the absence of genetic changes such as TP53 or TERT mutations observed in aggressive thyroid neoplasms supports a benign or indolent lesion rather than a more aggressive thyroid cancer. The possibility that some of these lesions may represent precursor lesions with a potential for subsequent development of carcinoma, however, cannot be entirely discounted. Whether they represent an early stage of a more aggressive thyroid neoplasm, or are simply benign neoplastic lesions that harbor Ras and other mutations, requires study of additional cases with longer clinical follow-up. Given the small size of the lesions, lack of invasion, indolent clinical course, and the fact that Ras family mutations have been described in other benign thyroid follicular lesions, these are favored to represent low-grade neoplastic lesions that are amenable to treatment by simple excision and can safely be followed up clinically.

In summary, we have described 18 cases of well-circumscribed, noninvasive thyroid follicular nodules that displayed varying degrees of cytologic atypia, predominantly solid growth, and most significantly, prominent longitudinal nuclear grooves, a feature that may lead to confusion with papillary thyroid carcinoma. We believe these lesions likely
Solid Follicular Nodules With Nuclear Grooves

represent low-grade, indolent follicular nodules with atypical features that can easily be mistaken for PTC owing to the presence of the longitudinal nuclear grooves. The clinical follow-up in our patients indicates that the lesions posed no immediate threat for the patients and can be treated conservatively, similar to NIFTP. Despite the traditionally accepted wisdom that longitudinal nuclear grooves represent one of the most distinctive features of PTC, such grooves have also been rarely observed in benign thyroid follicular nodules. Longitudinal nuclear grooves, in the absence of the constellation of other nuclear and architectural features of PTC, therefore, cannot be unequivocally accepted as synonymous with malignancy. The clinical and molecular features and the clinical follow-up in our cases support a benign nature for these lesions; however, the results of our study do not exclude the possibility that these lesions may represent a novel premalignant follicular process akin to something such as NIFTP. The descriptive designation of “solid thyroid follicular nodules with longitudinal nuclear grooves” is proposed for these lesions.

The authors are grateful to the following pathologists who contributed cases in consultation and follow-up information on their patients: Hector Colon, MD, Miami, Florida; John W. Bishop, MD, Sacramento, California; John Vago, MD, Cincinnati, Ohio; Sharon Tomberlin, MD, Covington, Louisiana; Robin Bideau, MD, Louisville, Kentucky; Manuela Soalta, MD, Jupiter, Florida; John Groth, MD, Chicago, Illinois; Michael Nowacki, MD, Louisville, Kentucky; Ray Franklin, MD, PhD, Orlando, Florida; George Kuntz, MD, Louisville, Kentucky; Christopher Hornsby, MD, Orlando, Florida; and Thomas H. Dudley Jr, MD, Charlottesville, Virginia.

References