CME/SAM

Thyroid Pathology Findings in Cowden Syndrome

A Clue for the Diagnosis of the PTEN Hamartoma Tumor Syndrome

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ABSTRACT

Objectives: *PTEN hamartoma tumor syndrome (PHTS) is a hereditary disorder caused by germline inactivating mutations of the* PTEN *gene. PHTS includes Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome. We describe how the peculiar pathologic and immunohistochemical thyroid features lead pathologists to suggest PHTS.*

Methods: *A 28-year-old white Spanish woman had a multinodular goiter. Total thyroidectomy was performed after fine-needle aspiration biopsy. Microscopic, immunohistochemical, and molecular analyses of the thyroid lesions were realized.*

Results: *The thyroid was multinodular, showing one papillary microcarcinoma, five follicular adenomas, three adenolipomas, 46 tiny adenomatous nodules (microadenomas), scattered foci of adipose tissue, and lymphocytic thyroiditis. Tumors were positive for thyroglobulin, thyroperoxidase, pendrin, cyclin D1, and p27 but negative for calcitonin and PTEN. A germline heterozygous deletion of one adenine at nucleotide 827 in exon 8 of the* PTEN *gene was confirmed. No* BRAF, NRAS, *or* KRAS *somatic mutations were detected in the papillary microcarcinoma, follicular adenoma, adenolipomas, or microadenomas. Negativity for PTEN was also found in the colonic tubulovillous adenoma and the storiform collagenoma.*

Conclusions: *Pathologists play a crucial role in recognizing pathologic thyroid findings associated with PHTS for selecting patients for genetic testing.*

Upon completion of this activity you will be able to:
- recognize the characteristic pathologic thyroid findings of patients with PTEN hamartoma tumor syndrome (PHTS).
- apply immunohistochemistry for PTEN protein in the thyroid gland to confirm the possibility of PHTS.
- list the malignant tumors with elevated incidence in individuals with PHTS.

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Phosphatase and tensin homolog (*PTEN*) hamartoma tumor syndrome (PHTS) is a rare, autosomal dominant spectrum of disorders caused by germline inactivating mutations in the *PTEN* tumor suppressor gene located on 10q23.3. PHTS includes Cowden syndrome (CS), presenting in adulthood; Bannayan-Riley-Ruvalcaba syndrome (BRRS) in children; adult Lhermitte-Duclos disease; and autism spectrum disorders associated with macrocephaly.[1,2]

Individuals with germline *PTEN* mutations have an increased risk of breast, thyroid, endometrium, colorectum, and kidney cancer and melanoma.[3] Diagnostic criteria for CS were initially proposed in 1983.[4] The National Comprehensive Cancer Network developed testing criteria to establish when *PTEN* testing is indicated, based on the clinical profile,[5] and a new set of PHTS diagnostic criteria[1] has been recently proposed, with molecular testing recommended, to confirm a clinical diagnosis and facilitate testing of at-risk relatives.

Thyroid pathologic findings in patients with CS are distinctive and characteristic,[6,7] and the same peculiar thyroid pathology findings have been detected in patients with BRRS.[7,8] Interestingly, because negativity for PTEN protein immunostaining seems to be sensitive and specific for CS,[9] thyroid pathologists are strategically placed to alert clinicians to the possibility of PHTS.

This present study describes the peculiar pathologic and immunohistochemical thyroid features that have led us to suggest a diagnosis of PHTS, which was eventually confirmed genetically.

Clinical History

A 28-year-old white Spanish woman was admitted to the hospital with a multinodular goiter. She has a history of acral keratoses and lipoma in the neck; two melanocytic nevi in the skin of the neck and abdominal wall were also removed at 5 and 20 years of age, respectively. No history of radiation exposure or family history of goiter or other disorders was noted. Routine laboratory test results were within normal limits. Total thyroidectomy was performed after fine-needle aspiration biopsy of the main nodule in the right thyroid lobe indicated follicular neoplasm (class IV, Bethesda system).

Following the pathologic thyroid findings and the molecular genetic analysis, a workup was carried out, revealing gingival papillomatosis, one oncocytoma in the left kidney, a calcified right breast fibroadenoma, three hepatic hemangiomas, and cholelithiasis. Esophageal glycogenic acanthosis, one gastric hamartomatous polyp, one ileal lipoma, and two ganglioneuromatous polyps as well as two juvenile polyps in the large bowel were also detected. In the following years, subsequent endoscopic screening and polypectomies yielded one endometrial polyp, one esophageal squamous papilloma, and multiple gastric and duodenal hamartomatous polyps; in the large bowel, multiple ganglioneuromatous polyps, one inflammatory polyp, one serrated adenoma, and one tubulovillous adenoma were found as well. Two cutaneous storiform collagenomas in the wrist and right thigh were also diagnosed, along with two peculiar unencapsulated vascular lesions in both legs, admixtured with adipocytic and fibrous tissues, which have recently been referred to as PTEN hamartoma of soft tissue.[10] At present, 7 years after the thyroidectomy, the patient is alive and well.

Materials and Methods

The thyroidectomy specimen was fixed in neutral, phosphate-buffered, 10% formalin and included in paraffin blocks. Paraffin-embedded sections were stained with H&E, and the immunohistochemical studies were also performed on 4-µm–thick paraffin sections using a peroxidase-conjugated labeled dextran polymer (EnVision FLEX/HRP; Dako, Glostrup, Denmark), with 3,3′-diaminobenzidine as the chromogen. The primary antibodies were used as follows: thyroglobulin (polyclonal, ready to use, low pH; Dako), thyroperoxidase (MoAb47, dilution 1:50, high pH; Dako), pendrin (UIRF 01065, 1:500; MBL, Nana-ku Nagoya, Japan), calcitonin (polyclonal, ready to use, high pH; Dako), cyclin D1 (EP12, ready to use, high pH; Dako), p27 (1B4, prediluted, high pH; Novocastra, Newcastle Upon Tyne, UK), and PTEN protein (clone 6H2.1, 1:50, high pH; Dako). Nonimmune mouse and rabbit serum samples were substituted for the primary antibodies as negative control samples. As noted in other studies,[9] the vascular endothelium within the tumors served as an internal positive control for PTEN.

For molecular genetic analysis, genomic DNA was extracted from peripheral blood of the patient; polymerase chain reaction (PCR) direct sequencing of the *PTEN* gene with primers targeting all nine exons and flanking introns was performed as previously reported.[11] DNA samples from paraffin sections of one papillary microcarcinoma (mPTC), one follicular adenoma, two adenolipomas, and two adenomatous nodules (defined as nodules with a microfollicular pattern of growth that lack capsules) were also obtained by microdissection, using the Leica AS LMD system (Leica Microsystems, Wetzlar, Germany). These samples were screened for somatic *BRAF* gene mutations by real-time PCR (Cobas 4800 BRAF V600 Mutation Test; Roche Diagnostics, San Cugat del Vallés, Spain) and for *NRAS* and *KRAS* mutations by pyrosequencing (PyroMark Q24; Qiagen, Hilden, Germany).

We were also able to investigate the immunohistochemical expression of PTEN protein in the renal oncocytoma, one of the storiform collagenomas, the two hamartomatous lesions of the soft tissue, the tubulovillous adenoma, and two ganglioneuromatous polyps of the large bowel.

Results

Gross, Histologic, and Immunohistochemical Findings

The surgical specimen weighed 37.5 g and measured 50 × 35 × 27 mm in the right lobe and 35 × 20 × 16 mm in the left lobe. The cut surface was multinodular, showing seven well-delimited, solid nodules involving both lobes. Microscopically, one mPTC, follicular variant, that was 2.5 mm in diameter, with lymphocytic infiltration, fibrosis, and psammoma bodies but no vascular invasion

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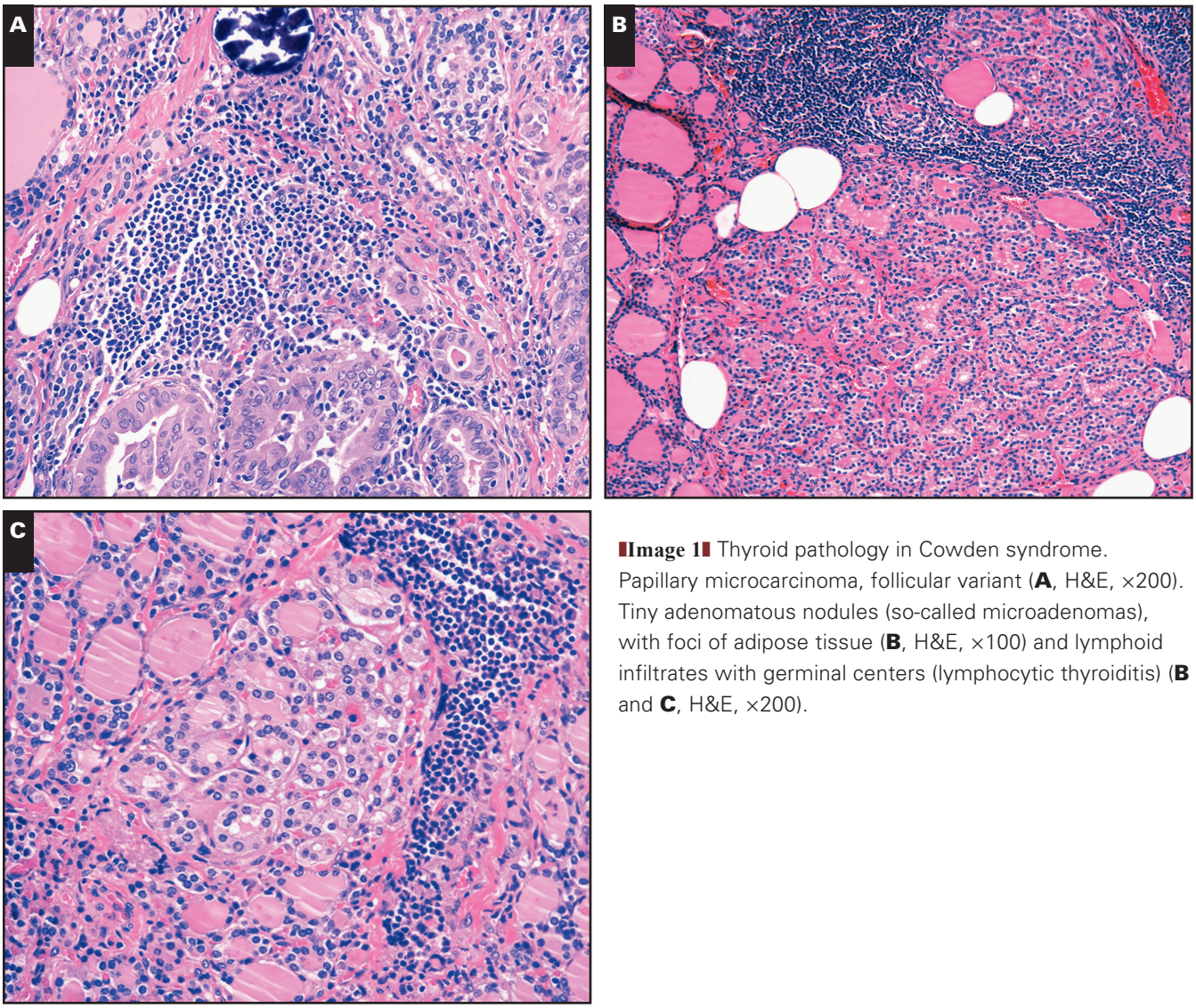

Image 1 Thyroid pathology in Cowden syndrome. Papillary microcarcinoma, follicular variant (**A**, H&E, ×200). Tiny adenomatous nodules (so-called microadenomas), with foci of adipose tissue (**B**, H&E, ×100) and lymphoid infiltrates with germinal centers (lymphocytic thyroiditis) (**B** and **C**, H&E, ×200).

or infiltration of the thyroid capsule, was found in the isthmus (stage I, pT1 N0 M0) **Image 1**. Five follicular adenomas with trabecular and/or microfollicular patterns of growth, including oncocytic (Hürthle cell) or clear cells, and three adenomas with a mixture of mature fat and follicles (adenolipomas), ranging from 2 to 30 mm in greatest diameter, were identified in both lobes (Image 1). At least 46 tiny adenomatous nodules (so-called microadenomas), one with adipose infiltration, were distributed throughout the gland. Scattered foci of adipose tissue and multiple lymphoid infiltrates with germinal centers (lymphocytic thyroiditis) distributed throughout the thyroid parenchyma were observed (Image 1). Small foci of follicular cells with oncocytic or squamoid change were also identified associated with some lymphocytic aggregates.

All thyroid tumors were positive for thyroglobulin, thyroperoxidase, pendrin, cyclin D1, and p27; no reaction was found for calcitonin and PTEN protein (Image 1). Isolated calcitonin-positive C cells were found, but no C-cell hyperplasia was detected.

Strong immunoreactivity for PTEN protein was observed in the renal oncocytoma and ganglioneuromatous polyps, very weak staining was found in the hamartomatous lesions of the soft tissues, but no reaction was detected in the storiform collagenoma or in the tubulovillous adenoma **Image 2**.

Molecular Findings

Germline mutational analysis of the *PTEN* gene showed a heterozygous deletion of one adenine at nucleotide 827 in exon 8 (NM_000314.4(PTEN): c.827delA); this mutation creates a frame shift starting at codon Asn276, and the new reading frame ends at a stop codon, 14 positions downstream (p.Asn276Ilefs*15). However, no *BRAF*, *NRAS*, or *KRAS* somatic mutations were detected in any of the tumors examined.

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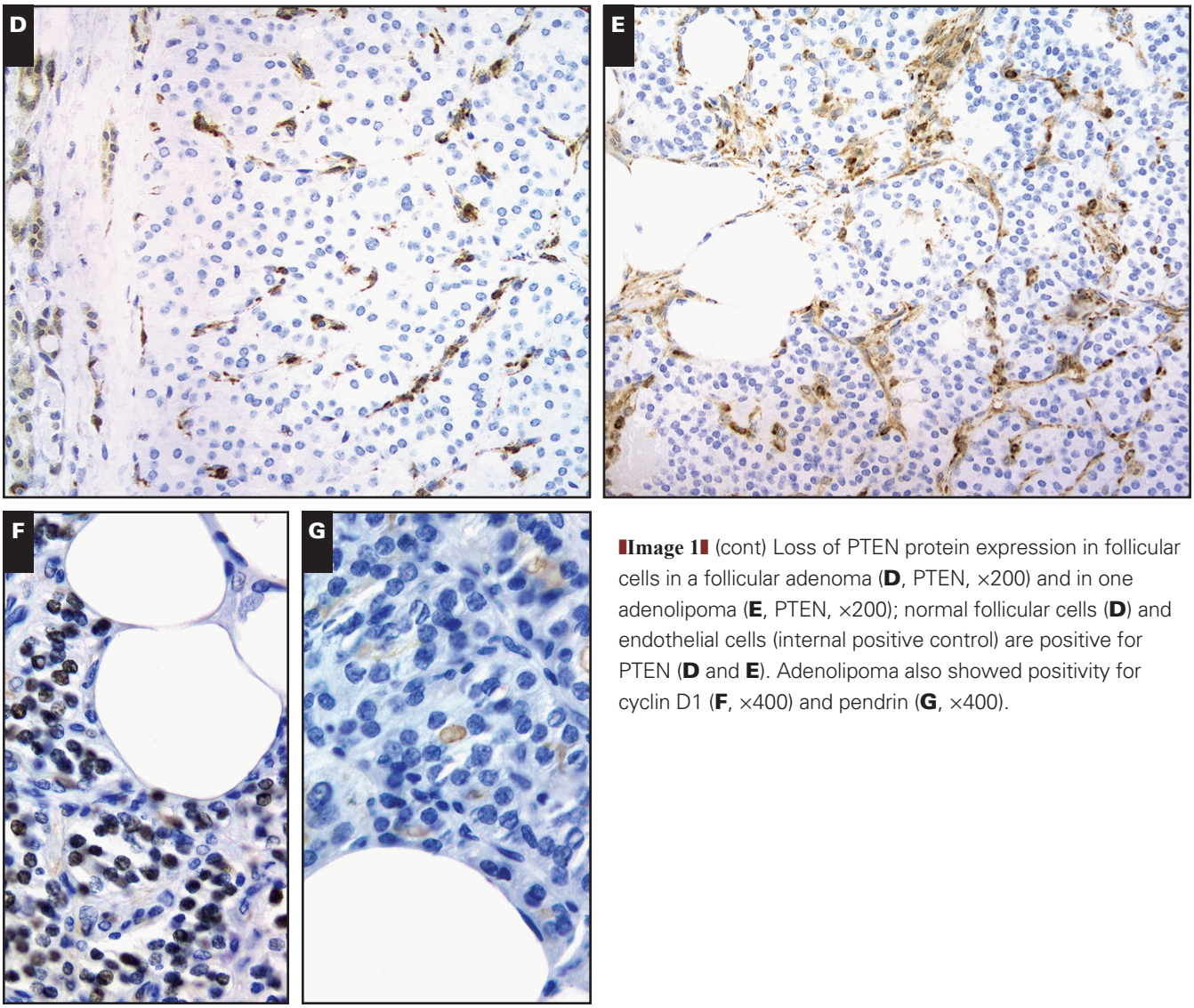

Image 1 (cont) Loss of PTEN protein expression in follicular cells in a follicular adenoma (**D**, PTEN, ×200) and in one adenolipoma (**E**, PTEN, ×200); normal follicular cells (**D**) and endothelial cells (internal positive control) are positive for PTEN (**D** and **E**). Adenolipoma also showed positivity for cyclin D1 (**F**, ×400) and pendrin (**G**, ×400).

Discussion

In the present case, the patient had a multinodular goiter, including benign and malignant lesions that were characteristic enough to lead to the diagnosis of PHTS. Harach et al[6] were the first to note that the histologic findings of a multiple adenomatous goiter and/or multiple follicular adenomas (including adenolipomas and the so-called microadenomas), particularly in children and young adults, should alert to the possibility of an inherited trait, such as CS. More recently, the same characteristic multicentric, bilateral, adenomatous nodules in a background of lymphocytic thyroiditis with benign and/or malignant follicular cell–derived neoplasms were confirmed in the thyroids of patients with CS[7] and BRRS.[7,12,13]

PHTS is a term that encompasses several clinical syndromes such as CS and BRRS, caused by germline inactivating mutations of the *PTEN* tumor suppressor gene. According to the criteria at our disposal, we herein described a case of PHTS clinically fitting with the diagnosis of CS.[1] Although germline mutations in *PIK3CA*, *AKT1*, *SDHB*, and *SDHD* genes as well as *KLLN* epimutation have been reported in rare cases of CS and Cowden-like syndrome,[12,14,15] a germline inactivating mutation of the *PTEN* gene was confirmed in our case.

A recent study confirmed an elevated age-adjusted standardized incidence ratio for carcinomas of the breast, thyroid, endometrium, colorectum, kidney, and melanoma in individuals with PHTS.[3] Follicular cell–derived carcinoma is a major criterion and an important feature in PHTS.[6,7,13,16,17] Thyroid cancer was reported in 14% of patients with *PTEN* mutation and CS,[18] while in patients with *PTEN* mutation (CS and BRRS) and thyroid pathology, it rose to 75%.[7] The main histologic subtypes of thyroid carcinoma were papillary carcinoma (60%-80%), follicular carcinoma (14%-45%), and anaplastic carcinoma (6%).[7,18] C-cell hyperplasia

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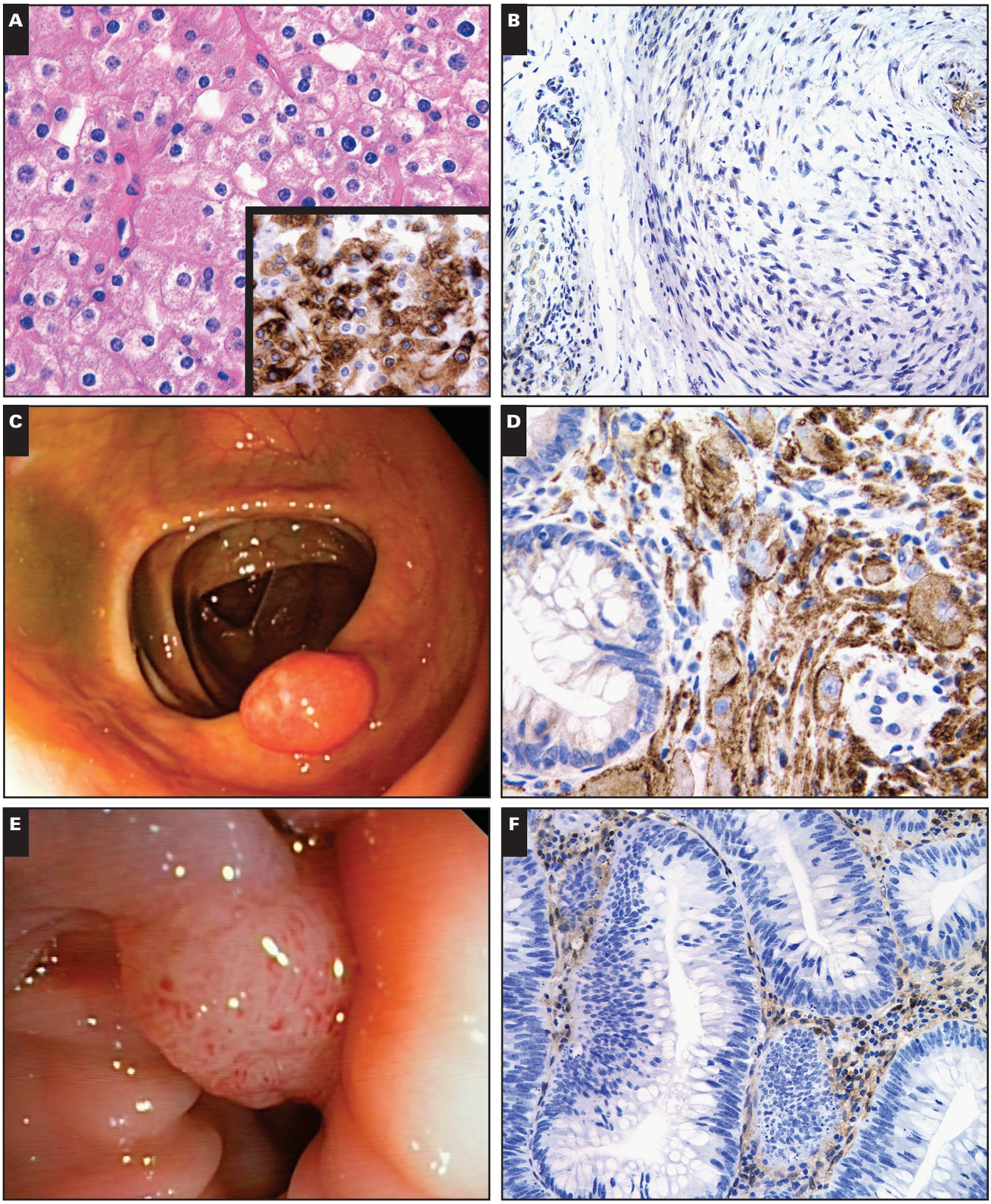

Image 2 Extrathyroid lesions in Cowden syndrome. Renal oncocytoma (**A**, H&E, ×400) showing strong positivity for PTEN protein (**A**, inset, ×400). Weak staining for PTEN in the vascular component of one hamartomatous lesion of the soft tissues (**B**, ×200). Endoscopic appearance of one ganglioneuromatous polyp (**C**), which was positive for PTEN in the immunohistochemical study (**D**, PTEN, ×400). Endoscopic image of one tubulovillous adenoma (**E**) that showed negativity for PTEN in the epithelial component (**F**, PTEN, ×400).

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(but not medullary carcinoma) was also reported in some patients.[6,7] This association with benign and malignant follicular tumors can be explained because PTEN downregulates the antiapoptotic/proproliferative AKT (protein kinase B) and mitogen-activated kinase (MAPK) pathways, the two main pathways involved in progression of thyroid tumorigenesis.[19] In fact, as occurred in the present case, the loss of PTEN expression in the adenomatous thyroid nodules, whether in all nodules or in a subset of nodules, appears to be both sensitive and specific for CS.[9]

The detection of *BRAF*^{V600E} mutation in the papillary carcinoma of a patient with CS[20] suggests the same model of tumor progression as in sporadic thyroid tumors. For this reason, in our patient, we investigated *BRAF*, *NRAS*, and *KRAS* genes (MAPK pathway) in the papillary carcinoma, follicular adenoma, adenolipomas, and adenomatous thyroid nodules. Despite our negative results, the participation of *RET/PTC*, *TRK* rearrangement or other less common alterations associated with mPTC could not be excluded.

Pathologists can play a crucial role in the recognition of pathologic findings associated with familial nonmedullary thyroid cancer.[21] The finding of multiple thyroid nodules is not unusual, particularly in older people, but these nodules are usually heterogeneous hyperplastic nodules combining different patterns of growth with colloid nodules, regressive changes (calcification, fibrosis, cystic degeneration, etc), and focal lymphocytic infiltration. In PHTS, however, it is precisely the combination of multiple, bilateral cellular follicular adenomas, adenomatous nodules, and multiple tiny foci of cellular proliferation composed of small follicles lacking abundant colloid (the so-called microadenomas), in a background of lymphocytic thyroiditis, especially when found in children and young adults, that should alert the pathologist to the possibility of PHTS. Interestingly, in addition to the peculiar pathologic thyroid findings associated with PHTS, there are also characteristic thyroid features in familial adenomatous polyposis[22]; in both cases, the morphologic features, along with the immunohistochemical findings for PTEN protein and β-catenin, respectively, are specific enough to indicate genetic testing for the patient.[21] The cribriform-morular variant of papillary thyroid carcinoma is associated with familial adenomatous polyposis.[21,22] Although the tumors in PHTS are usually benign and well demarcated, because of multicentricity and increased risk of recurrence or early progression to carcinoma, ultrasound surveillance and total thyroidectomy have been recommended.[6,13,18]

Patients with CS and *PTEN* mutation have a higher frequency of upper gastrointestinal polyps than previously believed,[23] and they are also prone to developing upper and lower polyps and cancer.[23-26] As this case exemplified, patients with PHTS have a high prevalence of colon polyposis with multiple histologic types of polyps such as hyperplastic, adenomatous, hamartomatous, lipomatous, ganglioneuromatous, and inflammatory.[24,26] To our knowledge, there are no studies concerning the immunohistochemical expression of PTEN protein in extrathyroidal lesions of patients with PHTS, and we believe the potential diagnostic usefulness of the loss of PTEN expression in the storiform collagenoma and tubulovillous adenoma in the present case deserves consideration.

To summarize, we present the case of a 28-year-old woman in whom a characteristic presence in the thyroid gland of multiple, bilateral, follicular adenomas (and adenolipomas), with several adenomatous nodules, including the so-called microadenomas, foci of adipose infiltration, mPTC, and lymphocytic thyroiditis, has led us to suspect CS. Immunohistochemical negativity for PTEN protein in the thyroid tumors supported the diagnosis, and this disorder was confirmed through genetic testing for the *PTEN* gene.

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