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Thyroid Carcinoma in the McCune-Albright Syndrome: Contributory Role of Activating $G_s\alpha$ Mutations

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McCune-Albright syndrome (MAS) is defined by the triad of café-au-lait skin pigmentation, polyostotic fibrous dysplasia, and hyperfunctioning endocrinopathies, such as precocious puberty, hyperthyroidism, GH excess, and Cushing's syndrome. This disorder is caused by sporadic, postzygotic activating mutations in the *GNAS1* gene, which codes for the $G_s\alpha$ protein in the cAMP signaling cascade. Nodular and diffuse goiters (with and without hyperthyroidism), as well as benign thyroid nodules, have been reported in association with MAS. Herein we report two cases of thyroid carcinoma in patients with MAS. The first is a case of papillary thyroid cancer detected incidentally during a hemithyroidectomy for hyperthyroidism in a 14-yr-old girl. The second is one of a 41-yr-old woman with long-standing MAS and an enlarging thyroid nodule, which was diagnosed as a clear cell thyroid carcinoma, a rare variant of thyroid cancer. Molecular analysis revealed that foci of malignancy and adjacent areas of hyperplasia and some areas of normal thyroid harbored activating mutations of Arg^{201} in the *GNAS1* gene. These findings suggest that the infrequent development of thyroid carcinoma in MAS patients involves additional mutational or epigenetic events. (*J Clin Endocrinol Metab* 88: 4413–4417, 2003)

MCCUNE-ALBRIGHT SYNDROME (MAS) is a rare, sporadic disease initially defined by the triad of precocious puberty, café-au-lait pigmentation of the skin, and polyostotic fibrous dysplasia (1). The syndrome may also be associated with autonomous hyperfunction of other endocrine tissues (1), involvement of nonendocrine tissues (2, 3), and renal phosphate wasting (4).

The underlying molecular mechanism of MAS is an activating mutation in the gene (*GNAS1*) coding for the α-subunit of the stimulatory G protein ($G_s\alpha$) involved in the cAMP cascade, referred to as a *gsp* mutation (5). This mutation results in inappropriately elevated levels of intracellular cAMP with ensuing endocrine cell hyperfunction and increased cell proliferation (6, 7). These mutational substitutions occur at the Arg (201) position (R201), most commonly with cysteine (R201C) or histidine (R201H) substitutions.

The mosaic pattern of phenotypic manifestations in MAS is attributable to a postzygotic somatic mutation of *GNAS1*, which occurs during embryonic development. Somatic activating mutations of Arg (201)or Gln (227) in $G_s\alpha$ have been implicated in the etiology of sporadic, isolated endocrine tumors, including pituitary adenomas (8–11), thyroid adenomas and carcinomas (9, 10, 12, 13) and Leydig cell tumors (14), as well as monostotic and polyostotic fibrous dysplasia (15) isolated ovarian cysts (16), and intramuscular myxomas (3).

Although hyperthyroidism, goiter, and benign thyroid adenomas are well known to be associated with MAS (17, 18) only a single case of thyroid cancer has been reported in MAS (19). Herein we describe a second MAS patient with thyroid carcinoma, review both cases, examine the role of activating $G_s\alpha$ mutations in thyroid oncogenesis, and discuss the implications for clinical management.

Subjects and Methods

Case 1

A 14-yr-old Filipino girl with café-au-lait skin pigmentation and a long-standing history of fibrous dysplasia was noted to have a 1.5-cm right-sided thyroid nodule on physical examination. The patient had no symptoms of thyrotoxicosis; however, laboratory evaluation revealed a suppressed serum TSH, less than 0.03 μU/ml (normal 0.50–5.70) as well as an elevated serum-free T_3 level of 520 pg/dl (normal 220–400), despite a normal serum-freeT_4 concentration, 1.12 ng/dl (normal 0.89–1.80). The patient underwent neck ultrasonography, which indicated increased vascularity within the nodule. A ^{99m}Tc-pertechnetate thyroid scan showed an area of hyperfunctioning tissue corresponding to the anatomic location of the palpable abnormality. In an effort to achieve euthyroidism in the most expedient manner before scheduled craniofacial surgery, a right hemithyroidectomy was performed.

There was no history of radiation exposure, other than routine radiographs for the evaluation of fibrous dysplasia. There was no family history of thyroid pathology, malignancies, or endocrinopathies. The patient's physical examination was remarkable only for the thyroid abnormality, stigmata of fibrous dysplasia, and typical café-au-lait skin pigmentation. Additional laboratory testing revealed the following co-

Abbreviations: LT4, Levothyroxine; MAS, McCune-Albright syndrome; PTC, papillary thyroid carcinoma; Tg, thyroglobulin; WBS, whole-body scan.

agulation factor abnormalities: factor-XI, 20% of control (normal 60–150%), factor-XII, 27% of control (norma: 60–150%), and factor-VII, 46% of control (normal 55–160%). These abnormalities resulted in elevations of prothrombin time (15.6 sec; nanoliter range: 11.8–14.7 sec) and activated partial thromboplastin time (54.5 sec; nanoliter range 23.4–34.5 sec). Because coagulation defects have not been previously described in MAS, this likely represents an incidental finding.

Pathology evaluation of the surgical specimen revealed a multifocal papillary thyroid cancer, with characteristic papillary configuration, follicular cells with clear nuclei, nuclear grooves, and intranuclear inclusions (Fig. 1, A and B). Areas of hyperplasia and normal thyroid were also present (Fig. 1, C and D, respectively). Serial sections of the areas of cancer, hyperplasia, and normal thyroid shown in Fig. 1 as well as normal lymph node were subjected to mutation analysis (Fig. 2A).

In view of the diagnosis of papillary thyroid cancer, the initially scheduled craniofacial surgery was delayed, and the remaining thyroid lobe was excised during a second neck operation 2½ months later. There was no evidence of carcinoma in the specimen from the completion thyroidectomy. Six weeks after completion thyroidectomy, while hypothyroid and on a low-iodine diet, the patient underwent a diagnostic ^{131}I (4 mCi) whole-body scan (WBS), which showed uptake in the thyroid bed only. A serum thyroglobulin (Tg) at that time was 3.7 ng/ml, and the TSH was 43.8 μU/ml. The patient subsequently received a therapeutic dose of 99 mCi of ^{131}I p.o. A posttherapy WBS showed the same focus of uptake noted in the diagnostic WBS. The patient was subsequently placed on suppressive levothyroxine (LT4) therapy. At the time of this report, *i.e.* approximately 40 months since the diagnosis of thyroid carcinoma, the patient remains without evidence of disease.

Case 2

A 42-yr-old Caucasian woman with known MAS (20) presented 11 yr earlier with a 1.2-cm round, firm, and mobile nodule in the right lobe of the thyroid. An ^{123}I radionuclide scan revealed slightly elevated uptake in a heterogeneous pattern and the patient was treated with LT4. The treatment was ineffective, and by the age of 40 yr, the patient's nodule was 2.1 cm in diameter. A fine-needle aspiration biopsy of the nodule showed findings suspicious for a carcinoma with clear cell features. The possibility that the lesion represented a metastasis from a primary nonthyroidal malignancy was excluded with computed tomography and magnetic resonance imaging of the neck, chest, abdomen, and pelvis. The patient's serum Tg at that time was 2.4 ng/ml (normal 3–40 in subjects with an intact, normally functioning thyroid gland). A right hemithyroidectomy was performed to establish a tissue diagnosis.

Other than routine radiographs, there was no history of radiation exposure to the neck. Although the patient had two siblings who had reportedly been diagnosed with melanoma, there was no family history of thyroid carcinoma. The patient was diagnosed at the age of 3 yr with MAS on the basis of the presence of menstrual bleeding, café-au-lait skin pigmentation, and radiographic signs of fibrous dysplasia. At the age of 30 yr, the patient had transient proteinuria. Renal biopsy demonstrated membranous nephropathy. This resolved and renal function has remained normal. At the age of 34 yr, the patient developed persistent vaginal bleeding and was found to have an enlarged leiomyomatous uterus, corresponding to a size of a 10-wk pregnant uterus, and a 10-cm left adnexal mass. A total hysterectomy and left salpingo-oophorectomy were performed. Three years before the development of thyroid carcinoma, the patient was diagnosed with type II diabetes, which has been well-controlled. Approximately 1 yr before the diagnosis of cancer, the patient was also noted to have multiple, biopsy-confirmed, intramuscular myxomas. The association of fibrous dysplasia and myxomas is known as Mazabraud syndrome (3, 21).

Surgical pathology of the excised tissue showed the presence of an epithelial neoplasm of the thyroid containing lipid-rich clear cells (Fig. 3A) with areas of vascular and capsular invasion (Fig. 3B). A lymph node in the surgical specimen was negative for tumor. The adjacent thyroid gland showed hyperplasia and normal thyroid (Fig. 3, C and D, respectively). Serial sections containing the areas shown in Fig. 2 were analyzed for *GNAS1* mutations (Fig. 2B). Neoplastic cells demonstrated strongly positive staining for both Tg and thyroid-specific transcription factor-1 (data not shown), confirming the thyroidal origin of the cancer. A description of the pathologic findings of this case has been reported previously (19).

A completion thyroidectomy was performed 4 months after the initial right hemithyroidectomy. The pathology examination of the remainder of the gland was negative for evidence of cancer but showed evidence of lymphocytic thyroiditis. This operation was followed 6 wk later by a 5-mCi ^{131}I diagnostic WBS under hypothyroid and low total-body iodine status. The only ^{131}I uptake was in the thyroid bed. At that time, the patient's serum Tg was 6.8 ng/ml, with a serum TSH level of 45.9 μU/ml. The patient received a treatment dose of 150 mCi ^{131}I. A posttherapy WBS again revealed uptake in the thyroid bed area only. Following ^{131}I administration, the patient was placed on LT4 suppressive therapy. At the time of this report, *i.e.* approximately 51 months since the diagnosis of thyroid carcinoma, the patient remains disease free.

Analysis of mutations in the GNAS1 gene

The study was approved by the Institutional Review Boards of the National Institutes of Health, Bethesda, Maryland, and the Children's Memorial Institute for Education and Research, Chicago, Illinois. Written informed consent was obtained. Paraffin-embedded sections of the

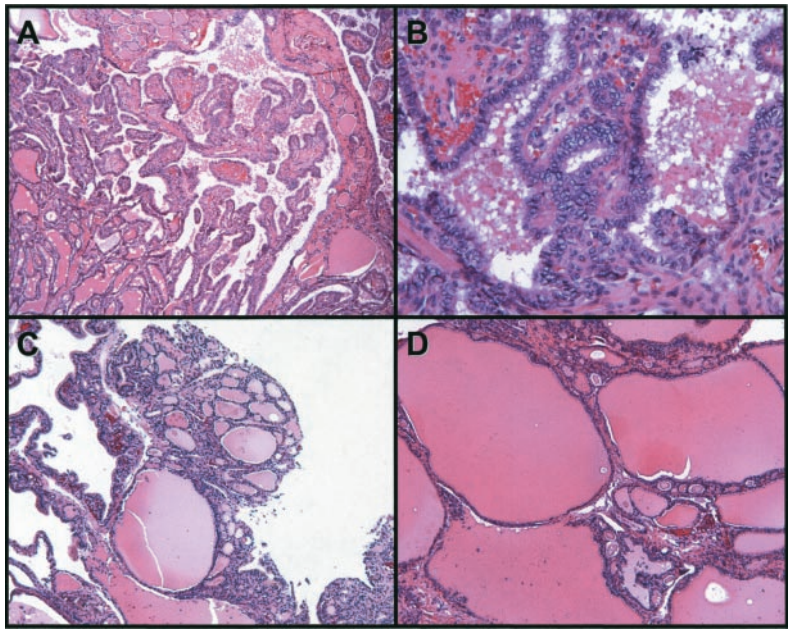

FIG. 1. Photomicrographs of tissue sections from case 1 (hematoxylin and eosin stain). A, Thyroid carcinoma with classic papillary architecture (magnification, ×60). B, Higher-power view of A reveals that the papillary fronds are lined by pseudostratified tumor cells with evidence of optically clear nuclei and nuclear grooves (×230). C, Hyperplasia with follicles of varying sizes lined by epithelial cells with increased nuclear to cytoplasmic ratios and multifocal pseudostratification (×75). D, A small portion of normal-appearing thyroid with colloid, located adjacent to the lesion (×75).

surgical thyroid specimens were stained with hematoxylin and eosin and used to identify discrete regions of interest. Small regions of tissue containing malignant, hyperplastic, and normal-appearing cells were then scraped into microfuge tubes with a sterile razor, and DNA was extracted as previously described (22). DNA samples from cells containing known heterozygous mutations of R201 in $G_s\alpha$ were used as positive controls and genomic DNA from a normal subject was used as a negative control.

A PCR and subsequent enzymatic digest-based technique for mutation detection was used (10). The sense primer, 5′-TTGTTTCAGGACCTGCTTCGCAGC-3′ was designed with a mismatch 3 bases from the 3′ end (underlined) that, when incorporated into the product, creates a restriction site for Pvu II only if a mutation encoding R201C is present. This primer also permits recognition of an Nla III site created by the mutation encoding R201H. The antisense primer was 5′-AGGTAACAGTTGGCTTACTGGAAG-3′ (GenBank Accession no. M21142.1, sense: bases 418–441, antisense: bases 518–495), and amplification resulted in a PCR product of 101 bp. Following amplification, the PCR product was incubated with Nla III (New England Biolabs, Inc., Beverly, MA) or Pvu II (Amersham Pharmacia Biotech, Piscataway, NJ) at 37 C overnight. The full digest volume was loaded on a 4% NuSieve 3:1/Tris-borate EDTA agarose gel prestained with Sybr Green I (Bio-Whittaker Molecular Applications, Rockland, ME) and bands were visualized by UV transillumination. Sequencing with the antisense primer was performed using an ABI Prism BigDye terminator cycle sequencing kit (Applied Biosystems Inc., Foster City, CA).

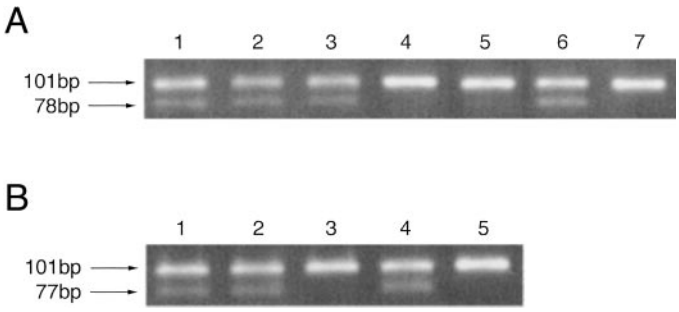

FIG. 2. Restriction enzyme analysis of Arg (201) mutations in PCR-amplified fragments of the *GNAS1* gene. A, PCR products from case 1 papillary thyroid carcinoma (lane 1), adjacent normal-appearing thyroid (lane 2), hyperplasia (lane 3), lymph node (lane 4), normal-appearing thyroid from completion hemithyroidectomy (lane 5), R201C-positive control (lane 6), and negative control (lane 7) were incubated with Pvu II. Normal PCR product (101 bp) is not cut by Pvu II, but products containing the mutant sequence for R201C are digested into fragments of 78 bp and 23 bp. B, PCR products from case 2 thyroid clear cell carcinoma (lane 1), hyperplasia (lane 2), normal-appearing thyroid (lane 3), R201H-positive control (lane 4), and negative control (lane 5) were incubated with Nla III. Normal PCR product (101 bp) is not cut by Nla III, but products containing the mutant sequence for R201C are digested into fragments of 77 bp and 24 bp.

Analysis of ras mutations and ret/papillary thyroid carcinoma (PTC) expression

DNA specimens isolated from regions containing carcinoma cells were amplified by PCR and tested for common oncogenic point mutations in the K-ras, H-ras, and N-ras genes by allele-specific oligonucleotide ligation (23) (Molecular Oncology Laboratory, Huntington Medical Research Institutes, Pasadena CA). Probes were specific for each possible single-base, nonsilent mutation in codons 12, 13, and 61 of the K-ras and N-ras genes and codons 12 and 61 of the H-ras gene. Results were confirmed by direct sequencing of exons 1 and 2 of the K-ras, H-ras, and N-ras genes. Immunostaining for gene products in the ret/PTC oncogene family was performed as previously described (24), using a rabbit polyclonal IgG antibody directed to the C terminus of ret (Santa Cruz Biotechnology, Santa Cruz, CA).

Results

Areas of tissue from case 1 that were microdissected for DNA extraction and PCR amplification included papillary thyroid carcinoma (Fig. 1, A and B), hyperplasia (Fig. 1C), adjacent normal-appearing thyroid (Fig. 1D), a normal lymph node, and normal-appearing thyroid from the opposite thyroid lobe (not shown). Restriction digests reveal that a heterozygous mutation encoding R201C in the *GNAS1* gene is present in the majority of the malignant cells (Fig. 2A, lane 1) as well as adjacent normal tissue (Fig. 2A, lane 2) and a region of hyperplasia (Fig. 2A, lane 3). There is no evidence of mutated *GNAS1* in lymph node tissue (Fig. 2A, lane 4). The left thyroid lobe, which did not harbor cancer, may contain a very small proportion of mutant cells, as evidenced by the faint band detected (Fig. 2A, lane 5). Identity of mutations was confirmed by direct DNA sequencing. The presence of

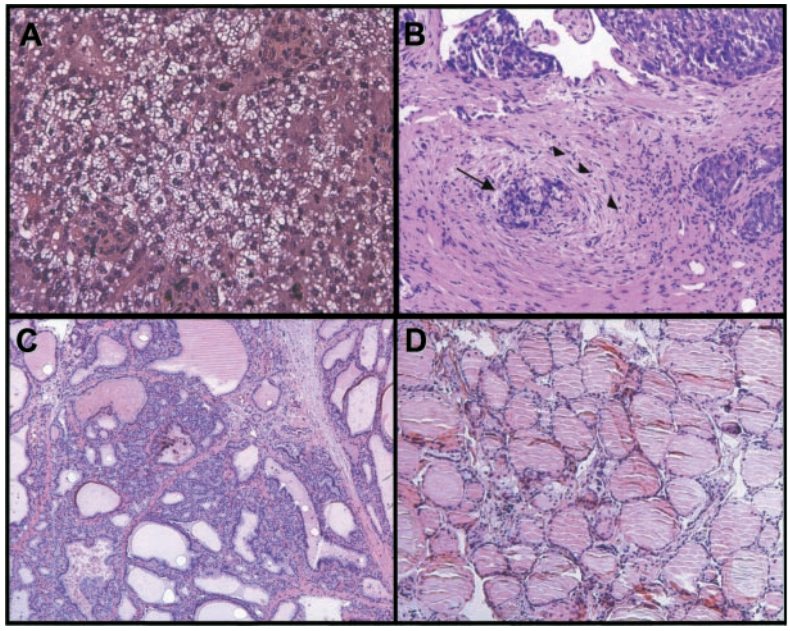

FIG. 3. Photomicrographs of tissue sections from case 2 (hematoxylin and eosin stain) (also see Ref. 19). A, Features of clear cell carcinoma of the thyroid with aggregates of clear-appearing tumor cells are shown (×230). B, A section of the tumor with the malignant feature of intravascular cancer cells (*arrow*) with perivascular desmoplastic changes (*arrowheads*). C, Hyperplasia (×75). D, A focus of normal-appearing thyroid with colloid formation (×100).

gsp mutations in histologically normal-appearing MAS tissue has been reported previously (2, 5).

Areas of thyroid tissue from case 2 that were microdissected included clear cell carcinoma (Fig. 3, A and B), hyperplasia (Fig. 3C), and normal-appearing thyroid (Fig. 3D). In this case, a heterozygous mutation encoding R201H in the *GNAS1*gene is present in the majority of the malignant cells (Fig. 2B, lane 1), and hyperplastic cells (Fig. 2B, lane 2) but not in normal-appearing thyroid (Fig. 2B, lane 3). Analysis of additional archival material from this patient revealed presence of the R201H mutation in uterine tissue but not in the kidney biopsy (data not shown). Identity of mutations was confirmed by sequencing.

Analysis of amplified carcinoma DNA from cases 1 and 2 by fluorescent oligonucleotide ligation and sequencing (23) failed to reveal mutations in codons 12, 13, and 61 of the K-ras, H-ras, and N-ras genes (data not shown). To determine whether the papillary carcinoma cells in case 1 expressed products of the ret/PTC oncogene family, immunohistochemical detection of the C terminus of the RET protein was performed. The tumor cells were negative for RET staining but infiltrating macrophages stained strongly as expected, thus providing an internal positive control (data not shown).

Discussion

Thyroid abnormalities represent one of the most common endocrinopathies in MAS. However, before the detection of thyroid cancer in case 2 (19), thyroid malignancies had never been reported in this syndrome (17, 18). Activating mutations of the $G_s\alpha$ or the TSH receptor genes are detected in a significant proportion of sporadic, hyperfunctioning follicular adenomas but are rarely associated with differentiated thyroid carcinomas (13, 25–28). In transgenic mouse models, sustained activation of the thyrocyte cAMP pathway generated by expression of mutant $G_s\alpha$ (6), cholera toxin (29), or a G_s-coupled A2 adenosine receptor (30) caused thyrocyte hyperplasia and hyperfunction, but not cancer, reinforcing the concept that this pathway alone is not sufficient to cause malignant transformation. Thyroid carcinoma in MAS is an uncommon occurrence, and it is doubtful that malignancies have been overlooked in other cases.

Prolonged periods of TSH hyperstimulation have been shown to lead to thyroid malignancy in rodents (31). This phenomenon has been much less commonly described in humans but has occurred in what are now rare cases of untreated congenital hypothyroidism (32, 33). In some sense, *gsp*+ MAS thyrocytes can be viewed as functionally similar to normal thyrocytes subjected to hyperstimulation by TSH in cases of untreated congenital hypothyroidism. With time, additional genetic errors may accumulate, eventually leading to neoplasia in a genetically or environmentally susceptible subgroup of patients. Interestingly, the histologic phenotype of clear cell thyroid carcinoma has been attributed to chronic TSH overstimulation because occasionally these rare tumors are seen in the context of long-standing untreated hypothyroidism (34).

The fact that the malignant cells in our patients share their embryonic *gsp*+ genotype with adjacent hyperplastic (but benign) cells and even some nearby normal-appearing cells indicates that malignancy is likely to have evolved as the result of additional genetic or epigenetic events. In fact, the faint band in lane 5 of Fig. 3A, which was derived from DNA from normal-appearing thyroid, suggests that a low number of cells bearing *gsp* mutations may exist in areas of normal functioning and appearing thyroid but at a level below that needed to create histopathological changes. It should be noted that lipid-rich, clear cell thyroid neoplasms are exceedingly rare, with only six other cases reported in the literature (reviewed in Ref. 19), and cases of papillary carcinoma arising within a hyperfunctional thyroid nodule are also exceedingly uncommon (28).

How can *gsp* cooperate with other genes to facilitate progression of the neoplastic process? Costimulation of the cAMP and inositol triphosphate/Ca^{2+} cascades can occur as the result of activating mutations of G protein-coupled receptors, and such mutations appear to be more tumorigenic in endocrine tissue than those that solely activate the cAMP cascade (35). This notwithstanding, G protein mutations that lead to constitutive activation of the inositol triphosphate/Ca^{2+} cascade have not been detected in human thyroid carcinomas (36). Although overexpression of the G_s-coupled A2 adenosine receptor in mouse thyroid is insufficient for the development of a malignant phenotype, thyroid carcinoma does develop after concomitant overexpression of the E7 protein of human papillomavirus type 16, which binds and inactivates the retinoblastoma protein, Rb1, involved in cell cycle control (30).

Further evidence of a potential early role of *gsp* in carcinogenesis comes from the fact that lesions of fibrous dysplasia can undergo malignant transformation, an alteration that is apparently promoted by ionizing radiation (37, 38). An overrepresentation of osteogenic sarcomas in patients with Mazabraud syndrome has been reported (21). Breast carcinoma has also been described in young women with MAS (39, 40). In addition, we have detected typical *GNAS1* mutations in malignant cells from MAS patients with breast cancer or embryonal cell carcinoma of the testes (unpublished data).

Data from human and rodent models discussed above suggest that the vast majority of *gsp*+ mutant thyroid cells do not become transformed. One possibility is that secondary cellular events such as increased phosphodiesterase activity or enhanced genomic stability blunt the functional effect of the *gsp* mutation *in vivo* (7, 41). It has also been suggested that interactions between *gsp* and other common thyroid oncogenes, such as *ras* and *ret/PTC*, are antagonistic, leading to cell death rather than transformation (41).

In conclusion, these two cases of thyroid carcinoma in patients with MAS extend the clinical spectrum of pathology encountered in this disorder. Although it appears that the development of thyroid carcinoma in MAS is an uncommon event, there is a need for heightened awareness and observation of the thyroid in patients with MAS. This is of particular importance when one considers that both functional and structural thyroid abnormalities are common in MAS. Clinicians can no longer assume that all thyroid nodules in MAS are benign and should have a lower threshold to aspirate them to exclude cancer. Our *GNAS1* mutational analysis data are consistent with previous work indicating that

activation of the G_s signaling cascade alone is insufficient for malignant transformation of thyroid or other endocrine cells. The nature of the additional mutational event(s) that participate in thyroid carcinogenesis remains to be determined.

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