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Germline *PRKACA* Amplification Leads To Cushing Syndrome Caused By 3 Adrenocortical Pathologic Phenotypes

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Abstract

We describe the pathology of 5 patients with germline *PRKACA* copy number gain and Cushing syndrome: 4 males and 1 female, aged 2 through 43 years, including a mother and son. Imaging showed normal or slightly enlarged adrenal glands in 4 patients and a unilateral mass in the fifth. Biochemically, the patients had corticotropin-independent hypercortisolism. Four underwent bilateral adrenalectomy; unilateral adrenalectomy was performed in the patient with the adrenal mass. Pathologically, 3 patients, including the 1 with the tumor (adenoma), had primary pigmented nodular adrenocortical disease with extranodular cortical atrophy and mild intracapsular and extracapsular extension of cortical cells. The other 2 patients had cortical hyperplasia and prominent capsular and extracapsular micronodular cortical hyperplasia. Immunoperoxidase staining revealed differences for synaptophysin, inhibin-A, and Ki-67 (nuclei) in the atrophic cortices (patients 1, 2, and 3) and hyperplastic cortices (patients 4 and 5), and for Ki-67 (nuclei) and vimentin in the extracortical nodules in the 2 groups of patients. β-Catenin stained the cell membrane, cytoplasm, and nuclei of the adenoma. The patients were well at follow-up (1–23 years); 24-hour urinary cortisol excretion was elevated in the patient who had unilateral adrenalectomy.

Keywords

adrenal; cortical nodules; Cushing syndrome; genetics; *PRKACA*

Introduction

In 1932, Cushing immortalized his name by enunciating the syndrome of painful adiposity, kyphosis, and amenorrhea in females and impotence in males, and correlating the symptoms with basophil adenoma of the pituitary and bilateral adrenal cortical hyperplasia [1].

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Experience since has shown that there are 2 types of the syndrome. One, corticotropindependent disease (80% of cases), features bilateral adrenocortical hyperplasia (Cushing disease). The other, corticotropin-independent disease, is primary in an adrenal gland and is caused by a cortisol-secreting adenoma or carcinoma; exceptionally, the disorder is due to ectopic corticotropin secretion (Cushing syndrome).

Recent studies have shown that rare patients with the syndrome have a primary bilateral, genetically determined adrenocortical disorder. In these patients, the 2 adrenal glands are normal sized, smaller than normal, or slightly larger than normal; exceptionally, they feature adenomas. Different microscopic patterns have occurred in the group, including primary pigmented nodular adrenocortical disease (PPNAD) [2], caused by germline mutation of *PRKAR1A* [3]; micronodular adrenocortical disease, caused occasionally by germline mutation of *PDE11A* or *PDE8B* [4,9]; and bimorphic adrenocortical disease [5], caused by somatic *GNAS1* mutation [6].

Recently, we described [7] patients with Cushing syndrome who had 1) adrenal adenomas caused by *PRKACA* somatic amplification or 2) PPNAD or isolated micronodular adrenocortical disease or adrenal independent macronodular adrenocortical disease resulting from germline *PRKACA* copy number gain.

Herein, we describe the pathologic adrenal and genetic findings in 5 patients previously reported with Cushing syndrome and germline copynumber gain of *PRKACA* gene [7]. The variability of the histologic phenotypes was impressive.

Patients and Methods

Five patients with Cushing syndrome were studied. Two were evaluated at the National Institutes of Health by C.A.S.; the other 3 patients were referred to him for molecular genetic, clinical, and pathologic study. Four patients underwent bilateral adrenalectomy, and 1 had unilateral adrenalectomy. Patient records, histologic slides, paraffin blocks, and peripheral blood specimens for molecular genetic studies were obtained with appropriate patient and parental consent and with approval of the Mayo Clinic Institutional Review Board.

The resected adrenal glands were examined in the pathology laboratory, weighed, and immersed in 10% buffered formalin. Fixed tissue blocks were embedded in paraffin and sectioned to obtain 4-μm-thick sections for routine histologic and immunoperoxidase studies. The sections were stained with hematoxylin-eosin, Masson trichrome, and reticulin methods. Additional sections were exposed to antibodies to the following: vimentin (Dako; clone V9, dilution 1/500 BRD, Ventana Ultraview, CC1), synaptophysin (Leica [Novocastro]; clone 27G12, dilution 1/50 BRD, Ventana Optiview, CC1), inhibin-A (Ventana; clone R1, predilution, Ventana Optiview, CC1), melan A (Dako; clone A103, dilution 1/50 BRD, Ventana Optiview, CC1), CD56 (Dako; clone 123C3, dilution 1/100 BRD, Ventana Ultraview, CC1), calretinin (Leica [Novocastro]; clone 5A5, dilution 1/50 BRD, Ventana Ultraview, CC1), β-Catenin (Ventana; clone 14, predilution, Ventana

Ultraview, CC1), and Ki-67 (Dako; clone MIB-1, dilution 1/20 BRD, Ventana Ultraview, CC1).

With the use of standard methods [7], DNA was extracted from peripheral blood lymphocytes of the 5 patients and tested for genetic mutations, deletions, and genomic alterations (copy number gain) associated with Cushing syndrome, including those affecting *PRKACA*, *PRKAR1A* [8], *PDE11A* [4], and *PDE8B* [9]. The primary relatives of the 5 patients were not studied.

Supplementary Study

For classifying the large lesion in patient 3, the pathologic reports on 15 Mayo Clinic patients with Cushing syndrome and adrenocortical adenoma and the reports on 3 patients with macronodular hyperplasia were retrieved to record the gross characteristics of the lesions.

Results

Patients

Patient age, sex, presentation, treatment, follow-up, chromosomal microarray analysis, and exome sequencing are presented in Table 1. Results of adrenal imaging and hormonal measurements are shown in Table 2. Biochemically, the patients had corticotropin-independent Cushing syndrome. For all 5 patients, chromosomal and microarray analysis (reported in reference 7) revealed similar chromosome 19 copy number gain that included the *PRKACA* gene. Sequencing showed a normal *PRKACA* coding sequence in the 5 patients. There were no *PRKAR1A*, *PDE11A*, or *PDE8B* mutations.

Pathology

The cortical findings in patients 1, 2, and 3 were similar (patient 3 also had a cortical adenoma)—they are described as a group. The findings in patients 4 and 5 were comparable but different from those in patients 1, 2, and 3—they are described individually.

Gross and Imaging Findings

The gross findings are shown in Table 3, and imaging findings are shown in Figure 1.

Microscopic Findings

The microscopic findings for the 5 patients are summarized in Table 3.

Patients 1, 2, and 3—The glands featured multiple, circumscribed cortical micronodules (Figure 2)(micronodules were arbitrarily defined as spheroidal lesions ≤0.8 mm in diameter; 0.8 mm was the diameter of the largest cortical nodule in the group, with the exception of a 1.8-cm tumor in patient 3). The nodules were composed of moderately large, poorly outlined cells that had fine granular eosinophilic cytoplasm and were arranged in clusters and columns; there were a variable number of vacuolated clear cells. Some eosinophilic cells contained lipochrome, likely the result of prolonged cell exhaustion from overactivity. Adipocytes and lymphocytes were present in occasional nodules. Nuclei were vesicular,

usually with a single nucleolus. A few very large cells had correspondingly enlarged nuclei. Mitotic figures were not seen. In patients 1 and 2, cortical cells extended through openings in the gland capsule into the epiadrenal fat (Figure 2). In patient 3, capsular openings were not seen (only a small amount of capsule was available), but small groups of cortical cells separated strands of the capsular collagen and formed micronodules in the capsule. A few extracapsular clusters of cortical cells were also present. In patients 1 and 2, the extranodular cortex was atrophic (0.3 mm thick) (normal adult cortical thickness, about 1 mm; range, 0.7–1.3 mm [10]). It lacked normal zonation into outer zona fasciculata and inner zona reticularis layers and featured small, finely vacuolated cells. The findings were typical of PPNAD [3,4].

The 1.8-cm brown to dark-brown tumor in patient 3 was circumscribed and surrounded partly by a fibrous pseudocapsule and partly by compressed, vacuolated clear cells (Figure 3). The granular eosinophilic cells and the minor component of clear cells were arranged in lobules of various size. The cells were larger than those in the micronodules; some had lipochrome. Nuclei were vesicular; a few had grooves, others vacuoles. There was an occasional mitotic figure. Pathologically, the lesion was an adenoma. The compressed atrophic cortex at its periphery had micronodules, as described earlier.

Patient 4—The cortex was hyperplastic, measuring 0.4 to 1.1 mm thick (mean of 20 measurements, 0.8 mm; normal cortical thickness at 2 years, about 0.5 mm). Adrenal cortex from normal 2-year-old infants was not available for thickness measurements; at age 1 to 7 months, normal cortical thickness is 0.49 mm (mean of 455 measurements); at age 3 to 7 years, 0.5 mm (mean of 521 measurements). It had an overall eosinophilic appearance with patchy zonation into an outer layer of large eosinophilic cells and a deeper layer of smaller, finely vacuolated cells. There were a few foci of strongly eosinophilic cells deep in the cortex and a single focus of adipose tissue (0.7 × 1 mm) in which there were cortical cells. The capsular collagen fibers were separated by groups of cortical cells and were hidden among the cortical cells, masked by the eosinophilia of the permeating intracapsular cortical cells, and easily overlooked with hematoxylin-eosin staining but readily identified with Masson trichrome staining (Figure 2D). An occasional opening in the capsule allowed cortical cells to be extruded into the epiadrenal fat, where there were also a few separate isolated unencapsulated clusters of cortical cells. There were no micronodules in the cortex proper. There was no lipochrome.

Patient 5—The cortex was composed of clear, vacuolated cells with a few subcapsular foci of eosinophilic cells; it ranged from 0.6 to 0.8 mm in thickness (mean of 16 random measurements, 0.7 mm). There were exceptional poorly outlined foci of large, strongly eosinophilic cells deep in the cortex proper but no typical micronodules. Large, oval intracapsular and unencapsulated extracapsular aggregates of weakly and strongly eosinophilic cells arranged in clusters (≤1.5 mm in diameter) separated capsular collagen fibers and formed confluent micronodules, mantling the external surface of the capsule. There was a rare capsular opening with extension of cortical cells into the epiadrenal fat. There was no lipochrome.

Immunoperoxidase Staining

The cortical micronodules in patients 1, 2, and 3 stained with synaptophysin, inhibin-A, melan A, Ki-67 (moderate number of stained nuclei), and β-catenin antisera and did not stain for vimentin (2 of the 3 patients) (Figure 4). The atrophic cortex stained with vimentin, synaptophysin (scattered cells), inhibin-A (scattered cells), melan A, CD56, β-catenin, and Ki-67 (rare positive nuclei) antibodies. Some nodules were partly positive and partly negative for the same antiserum (Figure 4A). The detailed staining results are presented in Table 4.

The adenoma in patient 3 was positive for melan A, CD56 and Bcatenin (cytoplasm, membrane and nuclei with the latter (Figure 3B). Certain lobules, but not necessarily the same ones, stained for vimentin, synaptophysin and inhibin-A.

For patients 4 and 5, the intracapsular and extracapsular micronodules stained with all the antisera: vimentin, synaptophysin, inhibinA, melan A, CD56, β-catenin, and Ki-67 (scattered nuclei). The hyperplastic cortex stained for synaptophysin, inhibin-A, melan A, CD56, Ki-67 (nuclei) and β-catenin (also rare nuclear positivity); there was no staining for vimentin. The staining results for the intracapsular and extracapsular cortical cells were similar to those for the hyperplastic cortex, with the exception of the β-catenin nuclear staining (Figure 5).

Molecular Genetics

For the 5 patients, genetic analysis showed similar chromosome copy number gain that included the *PRKACA* gene (Table 1). There were no *PRKAR1A* [8], *PDE11A* [4], or *PDE8B* [9] mutations.

Supplementary Study

Table 5 compares the gross findings from 2 unselected groups of Mayo Clinic patients with Cushing syndrome and cortical adenoma and with macronodular hyperplasia.

Discussion

We report pathologic findings for the 5 patients with germline copy number gain of *PRKACA* and Cushing syndrome that we recently reported [7]. *PRKACA* encodes cyclic adenosine monophosphate (cAMP)-dependent kinase catalytic subunit alpha. cAMP is a signaling molecule important for multiple cellular functions. It activates protein kinase A by facilitating dissociation of the inactive enzyme into a dimer of regulatory subunits bound to 4 cAMP and 2 free monomeric catalytic subunits. Interestingly, copy number gain of the second subunit of the gene, *PRKACB*, was recently found in a patient with Carney complex but without Cushing syndrome [11].

Because the *PRKACA* copy number gain was in the germline in our patients, familial involvement was a likely possibility; in fact, patients 2 and 3 were mother and son. Patient 4 had as yet an unclassified form of tongue overgrowth [12,13]. It is likely that *PRKACA* copy number gain may provide an explanation for other patients with PPNAD with an as yet unidentified genetic aberration [14].

Pathologically, patients 1, 2, and 3 had an established disorder, PPNAD [2, 3]. Patients 4 and 5 did not have PPNAD, although they had multiple grossly visible gold and yellow nodules simulating that condition. Microscopic examination showed that the nodules were not in the cortex proper but in the adrenal capsule and immediately outside it. Zones and clusters of the cells separated strands of capsular collagen in patient 4, thereby expanding it. This was not immediately evident in the hematoxylineosin–stained sections; the dissociated eosinophilic capsular collagen fibers were hidden among the similarly staining intracapsular cortical cells (Figure 2D). Gaps in the adrenal capsule permitted cortical cells to extend into the epiadrenal fat. In patient 5, extracapsular micronodules were confluent and formed a continuous band on portions of the capsule.

Thus, 3 different histologic phenotypes occurred in the 5 patients. In patients 1, 2, and 3, cortical micronodules occurred primarily in the cortex proper that was atrophic (in addition, patient 3 had a cortical neoplasm, an adenoma). In patients 4 and 5, micronodules occurred within and outside the capsule and the cortex proper was hyperplastic, without nodules. The lumpy appearance of glands evident grossly in the 5 patients was the result of cortical cell proliferation in different anatomical locations.

The multiple micronodules in the cortex proper in patients 1, 2, and 3 suggest that the genetic copy number gain affected scattered cells or small groups of cortical cells in the cortex and that their hypertrophy and proliferation resulted in micronodules. In patients 4 and 5, the entire cortex proper was affected, as evidenced by diffuse cortical hyperplasia. In both groups, there were intracapsular and extracapsular cortical nodules, which were much more prominent in patients 4 and 5. The findings for the latter patients were similar to those described for some patients with *PRKAR1A* [3], *PDE11A* [4], and *PDE8B* [9] mutations. The observations for the 2 groups of patients are compared in Table 6. The third pathologic finding, the adenoma, a neoplasm, was likely not happenstance. A cortical adenoma was described in 1 of the 4 patients in the original report on PPNAD (they had a germline *PRKAR1A* mutation) [2, 4], and the tumor has been found bilaterally in McCune-Albright syndrome (somatic *GNAS* mutation) [5,6]. Thus, germline and somatic adrenal mutation may cause adrenal cortical hyperplastic or neoplastic lesions (benign), or both.

Intracapsular cortical cells and openings in the capsule through which cortical cells extend into the periadrenal fat are occasionally seen in normal adrenal glands [10]. They have been described in exaggerated form in several genetically mediated bilateral adrenocortical diseases causing Cushing syndrome [4,5] and were a prominent finding in this study in patients 4 and 5 (and to a lesser degree in patients 1 and 2). This particular behavior and site of hyperplasia appears to be unique to the adrenal glands among capsulated organs; its presence likely points to some type of genetic aberration.

The histogenesis of the cells inside and outside the adrenal capsule is unknown, but they manifestly originate in the superficial cortex and in the capsule. Embryonic cells have been described in both these locations in the normal human adult cortex [15] and may be a possible source. In normal development of the cortex and in replacement of effete cortical cells, cell proliferation is centripetal [16], not centrifugal, as was the case in the patients we studied.

The main immunocytochemical distinguishing findings between the 2 groups were with the vimentin, synaptophysin, CD56, and β-catenin antibodies. There was strong staining for vimentin in the atrophic cortex in patients 1, 2, and 3 (similar to that in familial micronodular adrenocortical disease associated with *PDE11A* mutation [4] and in bimorphic adrenocortical disease due to activation of the stimulatory G protein (*GNAS*), and lack of staining in the hyperplastic cortex in patients 4 and 5. Occasional β-catenin–stained nuclei occurred in the extracapsular cortical nodules in patients 1, 2, and 3; there were many such stained nuclei in the nodules in patients 4 and 5. The strong β-catenin cell membrane, cytoplasmic, and nuclear staining of the cells in the adenoma in patient 3 suggested that it was a malignant neoplasm [17], but the lesion was benign pathologically and clinically. The weak Ki-67 cytoplasmic staining of the micronodules in patient 2 [18] is unexplained.

From past experience with genetic bilateral adrenal disorders [4, 5], it was not surprising to find several types of adrenocortical pathology associated with copy number gain of *PRKACA*. The current findings—and those reported in other recently recognized Cushing syndrome–associated primary, bilateral, genetically mediated adrenal disorders [4,5]—have revealed a surprising spectrum and flexibility of the adrenal cortical morphologic response to genetic mutation and copy number gain. The same pathologic phenotype has resulted from different genetic mutations, and the same mutation has caused different histologic phenotypes. There is not an exact pathologic-geneotypical correlation, unfortunately. However, recognition of similar bilateral cortical pathology in an infant or a young patient, particularly if it is unusual pathologically, should raise suspicion of a genetic disorder.

Cushing in his wildest dreams could never have imagined where the findings in his patients, Minnie G. [1,19] and E.G.F [1], would lead—and the story is not yet complete.

Acknowledgment

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Abbreviations

cAMP	cyclic adenosine monophosphate
PPNAD	primary pigmented nodular adrenocortical disease

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Highlights:

- 5 patients with germline PRKACA copy number gain and Cushing syndrome.
- Bilateral adrenal cortical pathology.
- Findings included primary pigmented nodular adrenocortical disease, cortical hyperplasia, and cortical adenoma.
- The disorder affected mother and son.
- One patient had an enlarged tongue.

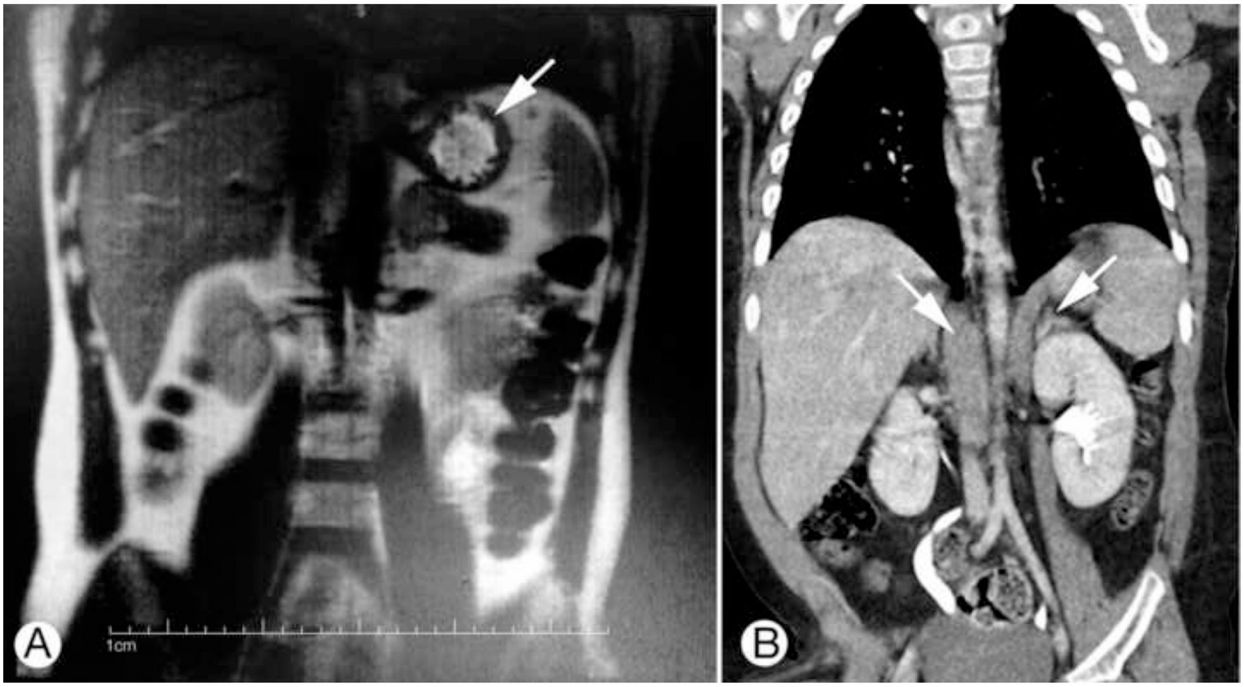

Figure 1.

Radiologic Imaging of Adrenals (Patients 3 and 4). A, Patient 3. Coronal magnetic resonance imaging with contrast agent showed a left adrenal mass with an irregular periphery (arrow). B, Patient 4. Coronal computed tomography with contrast agent identified normal-sized adrenals (arrows) with suggestive nodularity (left gland).

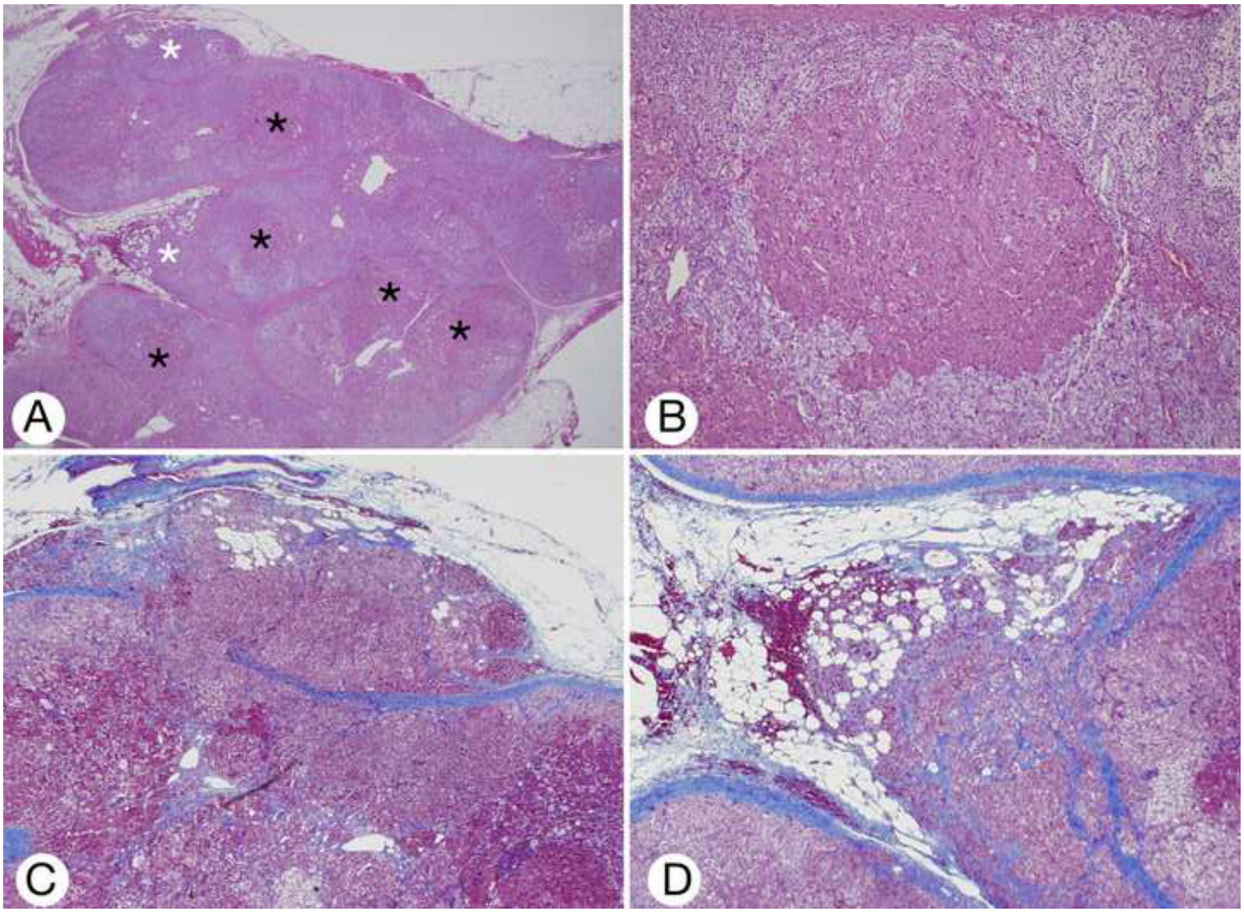

Figure 2.

Left Adrenal (Patient 3). A, An intermediate-power microscopic view showed multiple, intra-adrenal cortical nodules (black asterisks) and circumscribed and uncircumscribed zones of extracapsular cortical cells (white asterisks). B, A circumscribed cortical nodule composed of eosinophilic cells with uniform nuclei was located between the atrophic cortex (above) and the medulla (below). C, A break in the capsule permitted extracapsular extrusion (circumscribed) of cortical cells (Masson trichrome) (indicated by the upper white asterisk in panel A). D, Higher-power magnification of the area indicated by the lower white asterisk in panel A showed capsular collagen fibers (stained blue) separated by cortical cells. The cells extended among the epiadrenal adipocytes, forming a circumscribed, bulbous minimass (Masson trichrome).

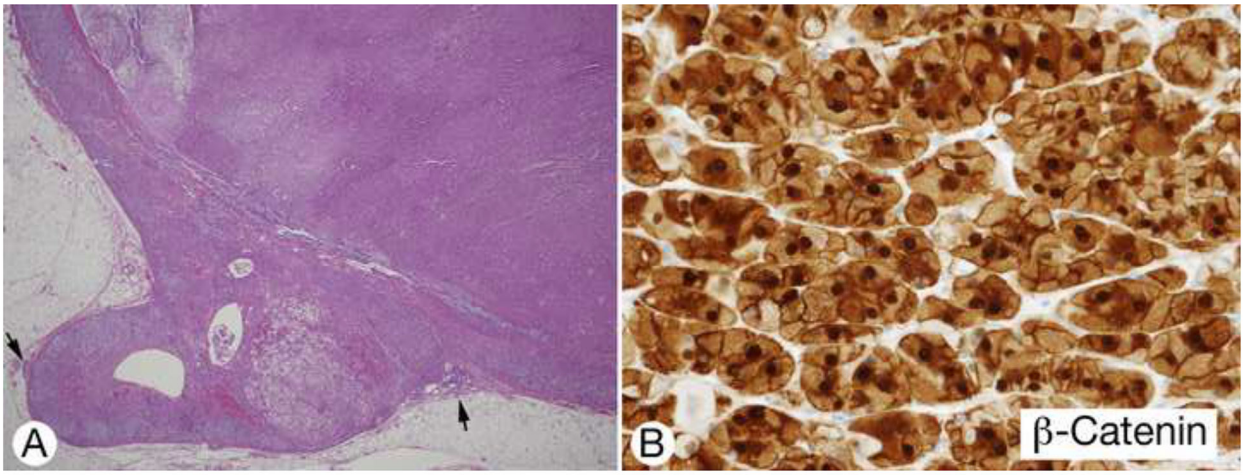

Figure 3.

Left Adrenal (Patient 3). A, The cortical adenoma featured a sheet of eosinophilic and clear, vacuolated cortical cells surrounded by a fibrous pseudocapsule that separated it from the attached gland, which had cortical micronodules. Small foci of extracapsular cortical cells were present (arrows). B, β-Catenin stained the cell membrane, cytoplasm, and nuclei of the tumor in panel A.

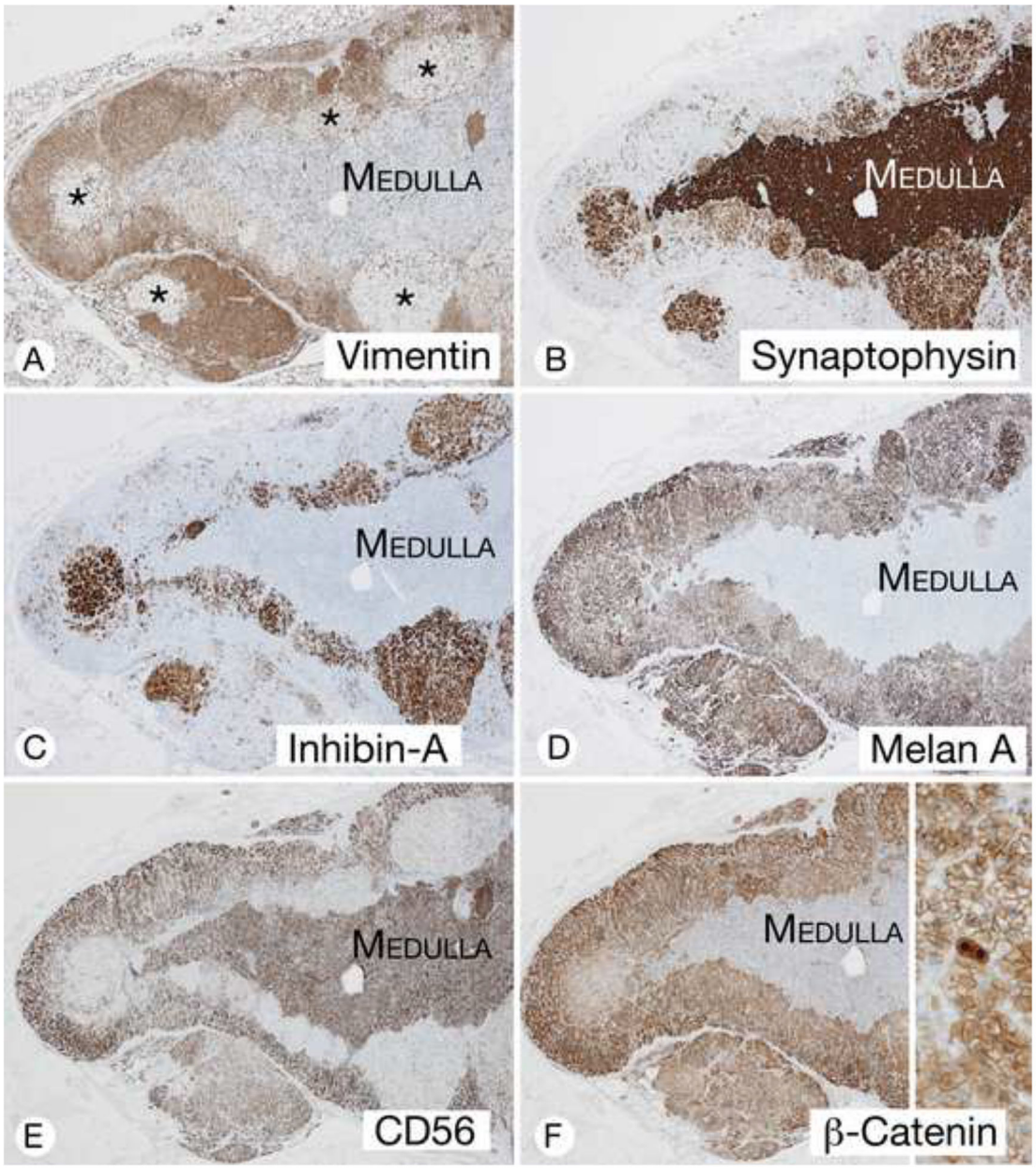

Figure 4.

Multiple Cortical Micronodules in Left Adrenal (Patient 2). The micronodules were positive for synaptophysin (B), inhibin-A (C), and melan A (D); variably positive for CD56 (E); and variably negative for vimentin (asterisks) (A). The extracortical nodule (bottom, A-F) showed zonal positive and negative staining. The atrophic cortex stained with vimentin (A), melan A (D), CD56 (E), and β-catenin (F) antibodies.

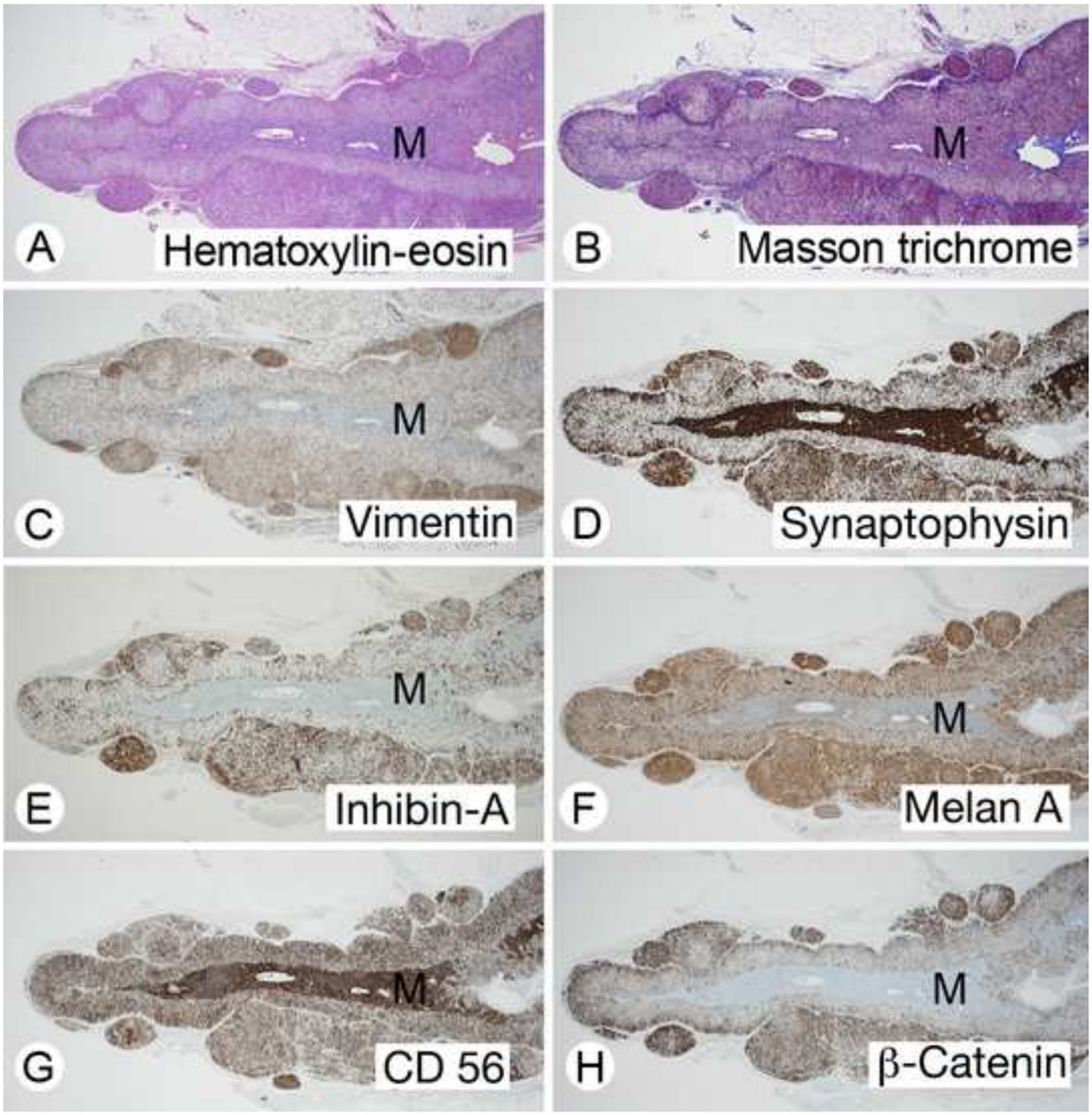

Figure 5.

Micronodules in Left Adrenal (Patient 5). The micronodules (A) were shown to be extracapsular with Masson trichrome staining (B). The micronodules were variably stained with vimentin, synaptophysin, inhibinA, melan A, CD56, and β-catenin (C, D, E, F, G, and H). The hyperplastic cortex stained strongly but variably with melan A, CD56, and β-catenin (F, G, and H), and scattered cells were stained by synaptophysin and inhibin-A antisera (D and E). M indicates medulla.

Table 1.

Clinical Findings, Treatment, Follow-up, and Molecular Genetic Findings for 5 Patients With Cushing Syndrome

Patient	Age, y	Sex	Presentation	Adrenalectomy	Follow-up; Years After Adrenalectomy	Chromosomal Microarray Analysis	Sequencing Of *PRKACA*
1	7	M	Cushing syndrome	Bilateral	Well; 1	CGH array: chromosome 19 duplication, including the *PRKACA* gene	Normal
2[a]	35 and 43[b]	F	Cushing syndrome	Bilateral (asynchronous)	Well, tubular mammary carcinoma; 23	616 kb gain on chromosome 19 at p19 p13.2, including the *PRKACA* gene	Normal
3	23	M	Avascular necrosis of left femoral head	Unilateral	Well, elevated morning urinary cortisol; 8	616 kb gain on chromosome 19 at p19 p13.2, including the *PRKACA* gene	Normal
4	2	M	Large at birth, transient hypoglycemia, macroglossia	Bilateral	Well; 1	Duplication at p13.13-p13.12 on chromosome 19, including the *PRKACA* gene	Normal
5	2	M	Cushing syndrome	Bilateral	Well; 4	Duplication at p13.13-p13.12 on chromosome 19, including the *PRKACA* gene	Normal

Abbreviation: CGH, comparative genomic hybridization.

[a] Patient 2 is the mother of patient 3.

[b] Cushing syndrome recurred following unilateral adrenalectomy, necessitating contralateral adrenalectomy 8 years following unilateral surgery.

Table 2.

Adrenal Computed Tomographic (CT) Findings and Clinical Diagnosis for 5 Patients with Germline *PRKACA* Copy Number Gain

	CT Findings		
Patient	**Left Adrenal**	**Right Adrenal**	**Clinical Diagnosis**
1	Normal	Normal	Corticotropin-independent Cushing syndrome
2	Enlarged; maximum diameter, 1.2 cm	Normal	Corticotropin-independent Cushing syndrome
3	Ovoid mass; 2.6×2.4 cm	Normal	Corticotropin-independent Cushing syndrome
4	Consistent with nodular hyperplasia	Consistent with nodular hyperplasia	Corticotropin-independent Cushing syndrome
5	Normal	Normal	Corticotropin-independent Cushing syndrome

Table 3.

Gross and Light Microscopic Adrenal Findings for 5 Patients With Cushing Syndrome and Germline *PRKACA* Amplification

	Gross Adrenal Findings				Light Microscopic Adrenal Findings		
	Weight, g		Cut Surface				
Patient	Left	Right	Left	Right	Cortical Micronodules	Nonnodular Cortex	Capsular and Extracapsular Cortical Cells and Nodules
1	Not weighed	Not weighed	Small black and brown micronodules	Golden brown	Present Acidophilic cells, adipocytes, lymphocytes, and lipochrome	Atrophic Small vacuolated cells Normal zonation absent	Present Extracapsular mainly
2	15[a]	12[a]	Black nodules, 0.1–0.8 cm	Black nodules, 0.6 and 0.8 cm; punctate black areas	Present Inconspicious with H-E; well seen with Masson trichrome Acidophilic cells, occasional large clear cells, and lipochrome	None visible Cortex replaced by micronodules	Present Extracapsular mainly
3	15.3	Not resected	Light to dark brown mass, 3.5×1.9×1.8 cm	Not resected	Present Acidophilic cells and lipochrome	Atrophic Small vacuolated cells Normal zonation absent	Present Extracapsular mainly
4	7	4	Orangeyellow; slightly nodular	Orange-yellow; slightly nodular	Absent	Hyperplastic Acidophilic and vacuolated clear cells Normal zonation absent	Present Extracapsular mainly
5	3.5	3	Diffuse nodules ≤0.2 cm	Multiple golden yellow nodules ≤0.2 cm	Absent	Hyperplastic Clear cells Normal zonation absent	Present Intracapsular mainly

Abbreviation: H-E, hematoxylin-eosin.

[a]Weight included periadrenal fat.

Table 4.

Immunoperoxidase Staining Results for 5 Patients With Cushing Syndrome and Germline *PRKACA* Amplification[a]

	Patients 1, 2, and 3			Patients 4 and 5	
Antibody	**Cortical Micronodules**	**Atrophic Cortex**	**Capsular and Extracapsular Cortical Nodules**	**Hyperplastic Cortex**	**Capsular and Extracapsular Cortical Nodules**
Vimentin	Weak positivity for patient 1 Negative for patients 2 and 3	Finely granular Outer more than inner	Finely granular Cell membrane	Negative	Negative
Synaptophysin	Coarsely granular Paranuclear body	Few, scattered, finely granular cells Paranuclear body (patients 2 and 3)	Coarsely granular Paranuclear stained body	Coarsely granular Outer more than inner Paranuclear body	Coarsely granular Paranuclear body
Inhibin-A	Finely granular	Few, scattered, finely granular cells	Finely granular	Finely granular Outer more than inner	Finely granular
Melan A	Coarsely granular	Finely granular Outer more than inner	Coarsely and finely granular	Coarsely granular Outer more than inner	Coarsely granular
CD56	Patchy, weak to moderate Cell membrane	Finely granular Cell membrane	Patchy, weak to moderate Cell membrane	Finely granular Cell membrane	Finely granular Cell membrane
β-Catenin	Strong to moderate Cell membrane, occasionally nuclear	Strong Cell membrane, occasionally nuclear	Moderately strong Cell membrane, occasionally nuclear	Moderately strong Cell membrane, rare nuclei	Moderately strong Cell membrane, outer more than inner Many nuclei
Ki-67	Moderate number of stained nuclei Weak cytoplasmic positivity (patients 1 and 2)	Rare stained nuclei	Scattered stained nuclei	Moderate number of stained nuclei	Moderate number of stained nuclei

[a]Staining was diffuse, cytoplasmic, and strong unless otherwise stated.

Table 5.

Comparison of Gross Features of Cushing Adenoma and Cushing Macronodular Hyperplasia

Feature	Adenoma (15 Lesions)	Macronodule (3 Lesions)
Laterality	Unilateral, single	Bilateral, multiple
Periphery	Distinct Usually encapsulated	Indistinct Agglomerated
Total weight, mean (range), g	13.2 (4.5–29)	150 (74–218)
Color	Brown, black, red, variegated	Usually yellow; sometimes mottled yellow and brown
Extranodular cortex	Atrophic	Atrophic
Extracapsular extension	No	Yes

Table 6.

Summary of Adrenal Pathologic Findings for 5 Patients With Cushing Syndrome and Germline *PRKACA* Amplification

Patient	Cortex	Intra-adrenal Cortical Nodules[a]	Capsular and Extracapsular Cortical Nodules[a]
1, 2, and 3	Atrophic	3+	1+ to 2+
4 and 5	Hyperplastic	None	3+

[a]Frequency is indicated as 1+, infrequent; 2+, occasional; or 3+, frequent.