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Familial Micronodular Adrenocortical Disease, Cushing Syndrome, and Mutations of the Gene Encoding Phosphodiesterase 11A4 (*PDE11A*)

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Abstract

We present the pathologic findings in the adrenal glands of 4 patients, aged 10 to 38 years, with Cushing syndrome and germline inactivating mutations of the gene *PDE11A4* that encodes phosphodiesterase11A4. The gene is expressed in the adrenal cortex and catalyses the hydrolysis of cyclic adenosine monophosphate and cyclic guanosine monophosphate. Two of the patients were mother and daughter; the third had no affected relative; the fourth patient inherited the mutation from her father. Three of the group, including the mother and daughter, had the same pathology, primary pigmented nodular adrenocortical disease, a disorder known to be caused by inactivating mutations of the *PRKAR1A* gene. In these cases, the adrenal glands were small and the pathologic change was deep in the cortex in which numerous pigmented micronodules developed. In the remaining patient, the glands were slightly enlarged primarily owing to a diffuse hyperplasia of the superficial cortex that extended into the epi-adrenal fat.

Keywords

Cushing syndrome; *PDE11A* mutation; primary pigmented nodular adrenocortical disease; micronodular adrenal hyperplasia; *PRKAR1A* mutation

In 1932, Harvey Cushing described the series of clinical findings that were later found to be caused by corticotrophin (ACTH)-secreting basophil adenomas of the pituitary gland (Cushing disease).[5] The main features of the disorder were young age at onset, central adiposity, kyphosis, and amenorrhea and impotence in females and males, respectively. It emerged subsequently that this constellation of symptoms and signs could also be caused by ACTH-independent conditions. In these, the primary cause of the manifestations almost always lay in the adrenal cortex, owing either to a unilateral tumor (adenoma or carcinoma)

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or a bilateral disorder (macro-nodular cortical hyperplasia or diffuse and nodular hyperplasia or primary pigmented nodular adrenocortical disease) (Cushing syndrome).[15,18] Rarely, they resulted from ectopic secretion of ACTH.[11]

Recently, genetic mutations responsible for several of the primary bilateral cortical disorders have been identified. The nodular hyperplasia of the McCune-Albright syndrome[12] is caused by postzygotic somatic mutations in the *GNAS* gene.[23] This gene encodes the α subunit of the stimulatory G protein and mutation results in activation of the Gsα subunit, uninterrupted stimulation of adenylyl cyclase, and persistently high levels of cyclic adenosine monophosphate (cAMP). Primary pigmented nodular adrenocortical disease (PPNAD), another primary bilateral adrenal cause of the Cushing syndrome,[21] is caused by inactivating germline mutations of the *PRKAR1A* gene.[4,6,7,13] *PRKAR1A* encodes the type 1A regulatory subunit of cAMP-dependent protein kinase[14]; its inactivation results in increased cAMP signaling.[13] PPNAD occurs as a sporadic isolated condition,[21] as a familial disorder,[6] and as a component of a multisystem tumorous familial syndrome, the Carney complex.[3,17,20,22]

There is a residual group of patients with bilateral primary adrenocortical pathology and Cushing syndrome with neither *GNAS* nor *PRKAR1A* gene mutations. A genome-wide scan of patients with primary adrenal disease and their tissues identified inactivating mutations disrupting the expression of a third gene, phosphodiesterase11A4 (*PDE11A4*), in 4 of them.[8] This gene is located at chromosome area 2q31.2, expressed in the adrenal cortex among other tissues and catalyses the hydrolysis of cAMP and cyclic guanosine monophosphate. In another patient from this group, a mutation in another gene, *PDE8B*, was found.[10] *PDE8B* is located on chromosome 5q13 and catalyses the hydrolysis of cAMP.[9] The mutations of the *GNAS*, *PRKAR1A*, *PDE11A*, and *PDE8B* genes all result in increased AMP signaling.[1]

Herein, we describe the adrenal findings in the 4 patients with the *PDE11A4* gene mutation, 1 PPNAD, the other indeterminate, possibly a variant of PPNAD.

PATIENTS AND METHODS

Patients

The 4 patients were females ranging in age from 10 to 38 years. Two (cases 1 and 2) were related as mother and daughter. The third patient had no affected relative. The fourth patient (case 4) inherited the mutation from her father; he had an enlarged adrenal gland but normal results of adrenocortical testing and no Cushing syndrome. Three underwent bilateral adrenalectomy. Unilateral adrenalectomy only was done in the case of patient 3 because technical difficulty prevented excision of the second gland. Follow-up period ranged from 3 weeks to 25 years. Patients 1 and 2 were cured of Cushing syndrome. Patient 3 became hypoadrenal postoperatively and initially required steroid support; presently, she is eucortisolemic, but still has a paradoxical response to dexamethasone stimulation (although less elevated than preoperatively) 5 years after the unilateral adrenalectomy. The fourth patient died 3 weeks after surgery. Certain findings in case 1 were reported earlier.[19]

Methods

Fresh specimen(s) of the adrenal cortex were obtained and frozen in each case for molecular genetic studies[8] after which the glands were immersed in 10% neutral buffered formalin. Blocks of the fixed tissue were embedded in paraffin wax. Five micron-thick sections were made for staining with the hematoxylin and eosin (H&E) method and immunostaining with antibodies directed against synaptophysin (ICN, SY38, dilution 1/40, Dual Envision), inhibin (Serotec, clone R1, dilution 1/60, Advance), melan A (Dako, clone 103, dilution 1/500 BRD, Advance), and vimentin (Dako, clone V9, dilution 1/500, Dual Envision). The number of sections of the glands available in the cases was 5, 17, 3 (unilateral adrenalectomy), and 6, respectively. Cortical thickness was measured at 100× magnification, using a 1 cm ocular reticule divided into 100 units. The areas of cortex inside and outside the adrenal capsule were determined by scanning sections with the Bacus Laboratories Slide Scanner (Bacus Laboratories Inc., Lombard, Illinois). The BLISS system captured images at 480 × 752 pixel resolution (magnification × 10). Each final image was composed of multiple tiles arranged to create a composite image. Computer-assisted analysis of the images was carried out using the Website Browser (Bacus Laboratories) that permitted outlined areas to be measured in square millimeters.

RESULTS

Clinical, Imaging, Laboratory, Genetic Findings

Selected results are presented in Table 1.

Pathology

Gross—The weights of the adrenal glands, gross findings, and the original pathologic diagnoses are shown in Table 2. The findings were similar in cases 1, 2, and 3. The cut surface of the gland resected in case 3 is shown in Figure 1A.

Microscopic—The microscopic findings were similar in cases 1, 2, and 3 and will be presented together to avoid repetition. The findings in case 4 were different and are described separately. Selected findings in the 4 cases are displayed in Table 3 and cytoplasmic immunocytochemical results in Table 4.

Cases 1, 2, and 3—H & E staining showed that the cortex was occupied by numerous, small, round, and oval nodules and irregularly shaped zones of enlarged cortical cells, many of which were situated in the deep cortex protruding into the medulla (Fig. 1). The nodules ranged from less than 1 to 4mm in diameter and were confined within the capsule of the gland. The cells had finely or coarsely granular eosinophilic cytoplasm; commonly, cells with clear vacuolated cytoplasm were admixed; some nodules featured fatty metaplasia. Many of the cells contained lipochrome. The nuclei were larger than those of the extra-nodular cortical cells and had open chromatin pattern. Scattered nuclei were considerably enlarged, hyperchromatic, and had prominent nucleoli. The low-power microscopic and corresponding diagrammatic appearance of a transverse section of the adrenal resected in case 3 are shown in Figures 1B and C.

The cortex between the nodules was composed of small cells with vacuolated cytoplasm; differentiation of the cortex into the zona fasciculata and zona reticularis was altered or lost. The amount of remaining full thickness cortex varied. In case 3, only 2 measurements were possible because of almost total replacement of the cortex by nodules (Table 3). The adrenal capsule was intact. There was a very occasional small poorly circumscribed cortical excrescence in the periadrenal fat (Figs. 1B–D) composed of enlarged eosinophilic cells, the largest measured 0.2 mm.

Case 4—The cortex was 0.6-mm thick (average of 23 random measurements) [normal thickness at age 10 y, 0.5mm (average of 50 random measurements, range=0.3 to 0.8 mm)]. The low-power microscopic and corresponding diagrammatic appearances of the patient's left adrenal gland are presented in Figure 2. The normal sharp distinction between the zona fasciculata (vacuolated clear cells in columns) and zona reticularis (granular eosinophilic cells in reticular pattern) was lost because of the presence of cells with mixed lightly eosinophilic and clear cytoplasm (hybrid cells) in both zones. There were scattered well-defined PPNAD-type nodules in the mid and deep cortex (Fig. 2B); some protruded into the medulla; some coalesced. The nodules (Fig. 3A) were composed of large cells with granular eosinophilic, rarely intensely eosinophilic cytoplasm. The nuclei were regular with moderate-sized nucleoli. A few nodules showed fatty metaplasia and occasional lymphocytes. There was a rare clear cell nodule (Fig. 3B). One nodule had cytoplasmic lipochrome. Occasional groups of cells showed differential cytoplasmic staining, featuring an eosinophilic periphery and an amphophilic or weakly basophilic center (Fig. 2C). Cortical cells protruded into the lumen of the central vein.

There were 2 capsular abnormalities: (1) absence of capsule (gaps) for distances up to 1.3mm with extension of the hyperplastic intraadrenal cortical parenchyma through the defect into the epi-adrenal fat (Figs. 2A, B, 3B). The interruptions in the capsule were not caused by expanding cortical nodules. Elsewhere, the continuity of the capsule was maintained but in places its collagen bundles were separated by cortical cells; here, the capsule seemed to be undergoing gradual dissolution (Fig. 3D). A few capsular "pockets" of cortical cells were present, some with cells in short linear array, findings that were regarded as normal.[2]

The cortical protrusions into the epi-adrenal fat measured up to 2mm in dimension; some were confluent (Figs. 2A, B). The cells had granular eosinophilic cytoplasm and were arranged in sheets with occasional well-defined PPNAD-type nodules, 1 of which had very large and huge cells containing lipochrome. The protrusions were usually solid and sharply circumscribed from the retroperitoneal fat, and occasionally partially limited by a pseudocapsule. Elsewhere, cortical cells percolated among retroperitoneal adipocytes without stromal response.

Immunostaining results are presented in Table 4 and Figure 4.

DISCUSSION

We have presented the pathology in the adrenal glands of 4 patients with Cushing syndrome caused by inactivating mutations of the *PDE11A4* gene. *PDE11A4* is one of an 11-member gene family, *PDE1—PDE11*. *PDE11A4* codes for a dual-specificity cyclical nucleotide phosphodiesterase, an enzyme that catalyses the hydrolysis of phosphodiester bonds.

The first genetic abnormalities identified as causative of primary bilateral nodular adrenal hyperplasia and the Cushing syndrome were activating mutations of the *GNAS* gene in the McCune-Albright syndrome.[23] More recently, a series of inactivating mutations of the *PRKAR1A* gene[13] were identified as causative of PPNAD, a different Cushing syndrome-associated pathologic phenotype. It seemed possible that the Cushing syndrome associated with the *PDE11A4* gene mutations might result in 1 of the foregoing pathologies.

In fact, 3 of the 4 patients (cases 1, 2, and 3) had PPNAD, a mother and her daughter with the same *PDE11A4* gene mutation, and a third unrelated patient with a different *PDE11A4* mutation. In these patients, the glands were small [largest total adrenal weight=6.9 gm (normal=8 to 9 gm)]. Nodules of large eosinophilic cells with lipochrome were present in the zona reticularis often straddling the corticomedullary junction. There was very minor involvement of the superficial cortex evidenced by a few transcapsular cortical extensions into the periadrenal fat.

The findings in the remaining patient (case 4) were very different. The adrenal glands were slightly enlarged owing to hyperplasia of the superficial cortex that protruded through gaps in the adrenal capsule into the periadrenal fat in which large extracapsular aggregates of cortical cells were present, some sharply circumscribed, others lacked circumscription and percolated between retroperitoneal fat cells. A few PPNAD-type nodules were present in the deep cortex. The patient inherited the *PDE11A4* mutation from her father who had an enlarged right adrenal gland but no Cushing syndrome and normal total glucocorticoid secretion.

The most parsimonious interpretation of these findings is that the 4 patients all had PPNAD because all exhibited small, pigmented adrenocortical nodules. However, the enlargement of the glands in case 4 and the extensive involvement of the epi-adrenal fat were very different from the findings in the other 3 cases, and from those in cases of sporadic PPNAD.[21] Also, some of the extranodular cortical immunocytochemical findings in case 4 differed for those in the other 3 cases. In addition, the nodules in case 4 showed stronger staining with melan A than in cases 1, 2, and 3. For these reasons, the findings in case 4 represent may not represent PPNAD although this possibility cannot be ruled out; they may constitute a different pathologic phenotype. As different mutations in the *PRKARIA* gene result in different clinical phenotypes,[7,16] it is possible that different *PDE11A4* mutations could cause different pathologic phenotypes. Further, PPNAD-type nodules are not diagnostic of primary pigmented nodular adrenal disease; isolated ones may be encountered in normal adrenal glands and multiple such nodules occur in idiopathic Addison disease[21] and adrenoleukodystrophy.[24]

In case 4, the protusion of the hyperplastic cortex through the capsule into the epi-adrenal fat and the intravascular protrusion of the cortical cells could be interpreted as evidence of an invasive and therefore, aggressive disorder. However, both of these findings are occasionally encountered in normal adrenal glands[2] and also in PPNAD.[21] The extraadrenal cortical extensions showed some evidence of pseudoencapsulation and did not evoke a host stromal response, as might be expected with an infiltrating malignant neoplasm. This extension of the cortical parenchyma beyond its capsular confines is an unusual, possibly unique, form of pathologic parenchymal hypertrophy that may be limited to the adrenal gland. The intravascular cortical protrusions in the case were likely related to the discontinuous anatomy of the adrenal vein musculature and not evidence of malignancy.[2]

Several of the terms used in connection with adrenal cortical pathology lack precision—micronodular and macronodular,[15] for example—are imprecise or are arbitrarily defined. The term “pigmented micronodular” is more specific but nodules that appear brown or black grossly may not have lipochrome microscopically, as in case 4. And nodules that have much lipochrome when examined microscopically likely had none at inception. Although the patients described in this article all had small “pigmented” or “dark brown” nodules grossly, the histopathology in the cases was not the same: the findings in case 4 were substantially different from those in the other 3, as we have described.

The results of our study indicate a need to establish a morphogenetic classification scheme for the Cushing syndrome-associated multiple types of primary bilateral adrenocortical pathology that apparently exist.

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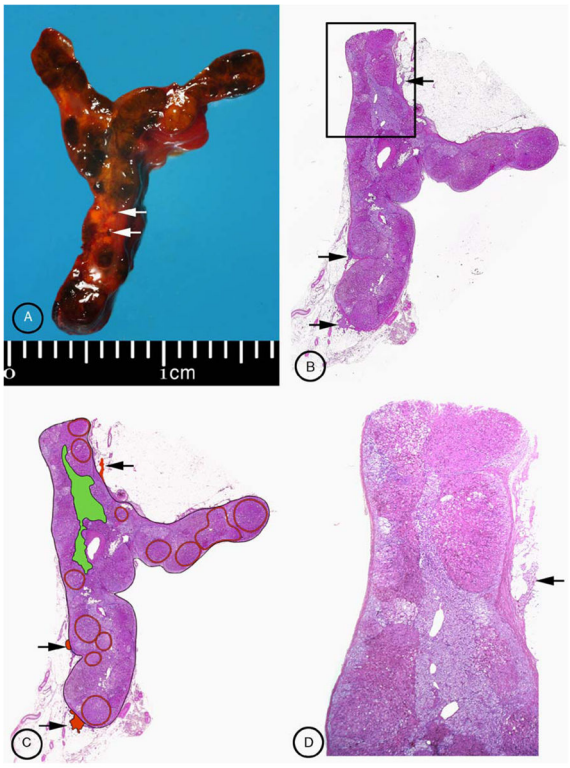

FIGURE 1.

(Case 3). Primary pigmented nodular adrenocortical disease. A, The cut surface of the fresh gland displayed pigmented nodules, ranging in size from less than 1mm (arrows) to 3mm in greatest dimension. B, Panoramic view of a microscopic transverse section of the adrenal gland showed a normal shape with sharply defined outline. Many cortical nodules, several confluent, and 3 small excrescences (arrows) were present. C, Diagrammatic representation of the findings in B. Cortical micronodules are enclosed in circles. Cortical excrescences in the periadrenal fat are shown in red. The medulla is depicted in green. D, The area in rectangle in B showed that the cortex was completely replaced by a series of juxtaposed nodules. A small cortical excrescence was present (arrow). This corresponds to the lesion indicated by the upper arrow in B and C.

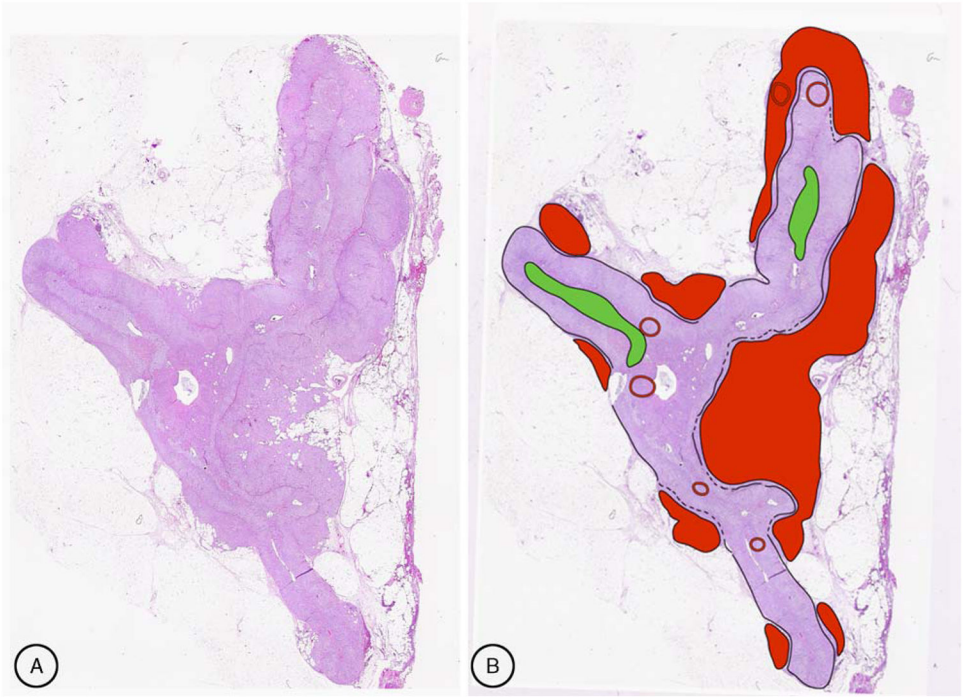

FIGURE 2.

(Case 4). Primary adrenocortical hyperplasia with extraadrenal cortical extension. A, Panoramic view of a microscopic transverse section of the left adrenal gland showed general preservation of the shape of the gland but loss of the normal sharp peripheral outline because of the presence of poorly circumscribed masses of cortical cells applied to the external aspect of the capsule. B, Diagrammatic representation of the findings in A. The adrenal capsule is indicated by a solid line, capsular attenuation by a broken line, and capsular breaks by an absent line. Cortical micronodules are enclosed in circles and permeating cortical excrescences indicated in red. The medulla is shown in green. The hyperplastic cortex and extracapsular cortical parenchyma occupied approximately equal areas.

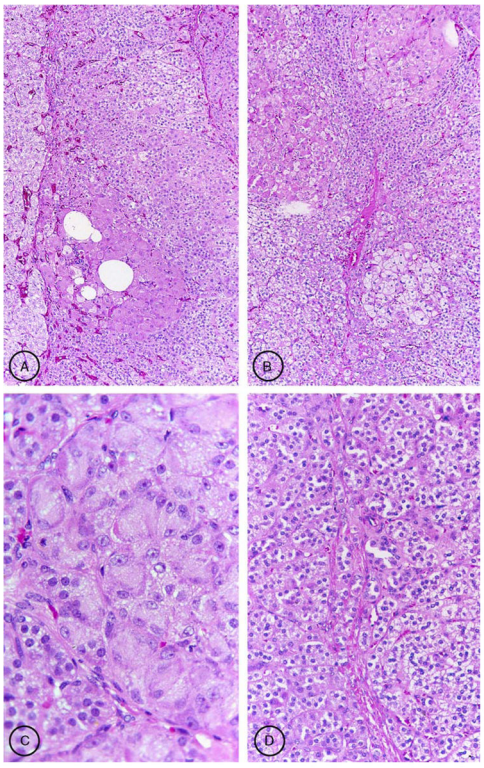

FIGURE 3.

(Case 4). A, An irregularly shaped nodule with fatty metaplasia composed of large eosinophilic and some vacuolated clear cells was present in the deep cortex abutting the medulla (left). A very attenuated capsule separated the cortex proper from extracapsular cortical tissue (upper right). B, A capsular break (upper portion of illustration) resulted in continuity of the hyperplastic cortex proper (left) and a solid excrescence (right) that contained 2 nodules, 1 with eosinophilic and the second with vacuolated clear cells (bottom left). C, Cortical nodule was composed of large cells with eosinophilic (top right) and amphophilic cytoplasm peripherally and centrally, respectively. D, The hyperplastic cortex (right) was separated from a solid cortical excrescence (left) by the adrenal capsule whose collagen bundles were separated by cortical cells.

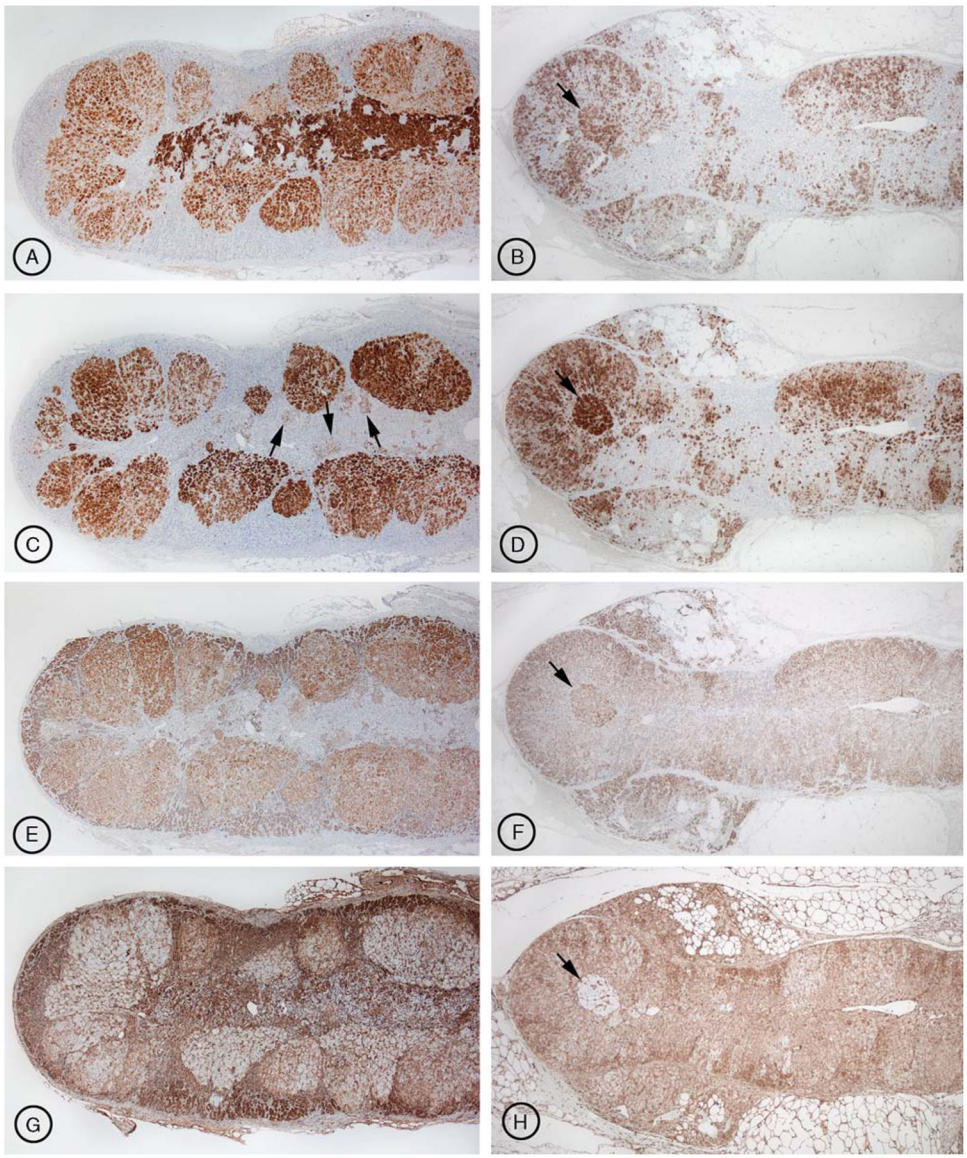

FIGURE 4.

Immunocytochemical results (case 3, A, C, E, and G; case 4, B, D, F, and H). Synaptophysin. A, Many cortical nodules were stained, often strongly; the extranodular cortex was unstained. The medulla was heavily stained. B, A vague cortical nodule (arrow) was less heavily stained than those in case 3; there was patchy staining throughout the extranodular cortex. Inhibin. C, Cortical nodules were strongly stained. Irregular clumps of enlarged cells between the nodules were lightly stained (arrows). D, A cortical nodule was strongly stained (arrow). There was patchy staining of the internodular cortex. Melan A. E, Juxtaposed nodules were moderately stained. A subcapsular rim of cortical cells was heavily stained. F, The outer cortex and extraadrenal cortical cells were stained. A nodule (arrow) was stained. Vimentin. G, The extranodular cortex, particularly the superficial aspect, was stained. Nodules were weakly stained or unstained. H, The intraadrenal and extraadrenal

cortex were stained, but not as strongly as in case 3. A cortical nodule was not stained (arrow).

TABLE 1

Clinical, Imaging, Selected Laboratory, and Genetic Findings in 4 Patients With Cushing Syndrome

Case	Sex	Age at Onset of Cushing Syndrome	Adrenal Gland Imaging	24 h Urinary Free Cortisol	Plasma ACTH	Urinary 17-hydroxycorticosteroids Following High-dose Dexamethasone Suppression Test	PDE11A4 Mutation (Horvath et al)[8]
1	F	22	Normal	↑	↓	Paradoxical increased excretion	(919C)→(R307X)
2	F	20	Normal	↑	↓	Not done	(919C)→(R307X)
3	F	38	Normal	↑	↓	Paradoxical increased excretion	1655_1657delTCT/ insCCfs15X
4	F	10	Minimal irregular enlargement	↑	↓	Not done	171delTfs41X

↑ indicates increased; ↓, decreased or undetectable.

TABLE 2

Weight and Gross Features of Adrenal Glands, and Original Pathologic Diagnoses in 4 Patients With Cushing Syndrome and PDE11A Mutations

	Weight (gm)				
Case	**Rt**	**Lt**	**Normal Total***	**Gross Features**	**Original Pathologic Diagnosis**
1	0.9	3.1	6–11 (mean, 8)	Multiple small black-brown nodules surrounded by a very thin rim of yellowish tissue	Multiple micronodular black adenomata
2	4.7	2.2	6–11 (mean, 8)	Multiple black-brown nodules measuring 0.5 to 4 mm	Diffuse micronodular hyperplasia (pigmented micronodular adrenal disease)
3	Not excised	5.4	6–11 (mean, 8)	Externally, brown multinodularity. Cut surface showed multiple brown nodules with very rare zones of orange cortex	Pigmented micronodular hyperplasia
4	Unknown†	Unknown†	1.5–2.5 (mean, 2)	Golden-yellow to brown with multiple darker lesions throughout	Pigmented nodular hyperplasia

*Based on data in ref Lack.

†The weight recorded included retroperitoneal fat. Computed axial tomography revealed minimal enlargement of both adrenals, compatible with hyperplasia. The surgeon described bilaterally enlarged, nodular, minimally pigmented adrenal glands.

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TABLE 3

Selected Adrenocortical Histopathologic Findings in 4 Patients With Cushing Syndrome and *PDE11A* Mutations

		Cortical Dimensions		Cortical Nodules		
Case	**Normal Zonation**	**Mean Thickness, mm (Range)**	**Ratio Intraadrenal to Extraadrenal cortex**	**Intraadrenal**	**Extraadrenal**	**Capsular Breaks**
1	Absent	0.5 (0.4–0.6)	28:1	Many	None	Rare
2	Absent	0.4	235:1	Many	None	None
3	Absent	0.4 (0.3–0.9)	129:1	Many	None	Rare
4	Absent	0.6 (0.4–1.1)	1.2:1	Occasional	Occasional	Many

+ indicates present; −, absent.

TABLE 4

Immunocytochemical Staining Results in 4 Cases of Cushing Syndrome and Micronodular Adrenocortical Disease*

	Synaptophysin		Inhibin		Melan A		Vimentin	
Case	**Nodules**	**Extranodular Cortex**	**Nodules**	**Extranodular Cortex**	**Nodules**	**Extranodular Cortex**	**Nodules**	**Extranodular Cortex**
1	+2 to +1†	Rare subcapsular cells positive	+3, occasionally +1	Not stained	Patchy +1	Patchy +1‡	Unstained to +1	+3
2	+3 to +2†	Scattered positive cells§	+3	Scattered positive cells	Patchy +1 to +2	+2‡	Unstained to +1	+2, interrupted subcapsular band unstained
3	+3 to +2†	Occasional positive cells	+3	Scattered positive cells	+1 to +2	+3‡	+1	+3
4	+2†	Patchy +2†	+3	Many positive cells and patches	+3 to +1	+3, outer >inner, focally unstained	+3 to +1	+3 to +1, outer >inner

*Staining scale = +1 (staining present), +2 (moderately strong staining), +3 (strong staining).

†Intensified staining of cell membrane and a globular paranuclear body.

‡Narrow subcapsular band.

§Some cells had a stained globular paranuclear body.