Cushing Syndrome Caused by Adrenocortical Tumors and Hyperplasias (Corticotropin-Independent Cushing Syndrome)

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Abstract

Endogenous Cushing syndrome (CS) is caused by excess adrenal glucocorticoid secretion that is adrenocorticotropic hormone (ACTH)-dependent or independent; ACTH-independent adrenocortical causes of CS account for up to 20% of CS in adults, and 15% in children over age 7 years. In younger children, ACTH-independent CS may account for as many as half of the CS cases. In both adults and children, adrenocortical lesions causing CS include the common, isolated and sporadic, solitary cortisol-producing adenoma, the rare adrenocortical cancer, and a spectrum of recently recognized, bilateral hyperplasias (bilateral adrenocortical hyperplasias, BAHs): micronodular adrenal disease and its pigmented variant, primary pigmented nodular adrenocortical disease are mostly genetic processes. Macronodular BAHs, ACTH-independent macronodular hyperplasia or massive macronodular adrenocortical disease are less frequently genetic and almost never present in children (except in McCune-Albright syndrome); they present often with atypical CS in middle-aged or elderly adults. The majority of benign adrenocortical tumors associated with CS are associated with defects of the cAMP signaling pathway, whereas adrenal cancer is linked to aberrant expression of growth factors and germline or somatic mutations of tumor suppressor genes such as TP53. Adrenalectomy is the preferred mode of treatment for all adrenocortical causes of CS.

Endogenous Cushing syndrome (CS) is a rare disorder [1]. There are significant differences in the pathophysiology and epidemiology of hypercortisolism among age groups [2]; different criteria are being used for the confirmation and differential diagnosis of this disorder in children [3, 4]. CS may be caused by corticotropin (ACTH)-producing pituitary tumors, a disorder also known as ‘Cushing disease’, or by ACTH-independent, cortisol-producing adrenocortical tumors (ADTs). In children, ectopic production of ACTH is extraordinarily rare [2, 5]; it has been reported only in a handful of cases confined at the extremes of pediatric age, infants with neuroblastomas or other neuroendocrine tumors [5] and adolescents with carcinoids, sporadic...
or in the context of multiple endocrine neoplasia type 1. Bilateral adrenocortical hyperplasias (BAHs) are far more common as causes of CS in children than in older patients [1, 2].

Epidemiology

Adrenocortical neoplasms account for less than 0.5% of all clinically significant tumors; however, autopsy studies indicate that as many as 10% of adults over the age of 40 years may have an ADT, usually a simple nodule that is not larger than 1 cm; up to 36% may have micronodular hyperplasia [6]. CS is a manifestation of approximately one third of all ADTs. In children, a significant number of ADTs presenting with CS are malignant, but the opposite is true in adults. There is a female-to-male predominance for ADTs in all ages (although this is probably not true for infants and toddlers).

Clinical Presentation

In most patients the onset of CS is rather insidious [1–4]. The most common presenting symptom of the syndrome is weight gain, but it is not universally present. Pathognomonic for CS in childhood is weight gain associated with growth retardation [7]. Other common problems reported include facial plethora, headaches, hypertension, hirsutism, amenorrhea, and hypogonadism (or delayed sexual maturation in children) [8]. Virilization is rare in ADTs unless the tumor produces adrenal androgens in addition to glucocorticoids; skin manifestations, including acne, violaceous striae and bruising and acanthosis nigricans, are also common. Sleep disruption, muscular weakness and mental changes are frequent.

Diagnostic Evaluation of ACTH-Independent CS

Diagnostic testing for CS is complicated by developmental differences in the regulation of hypothalamic-pituitary-adrenal axis between young and older individuals, as well as other factors, such as exposure to steroid hormones, medications and other exogenous substances, stress, and chronic illness [3, 8]; these factors may influence normal values for several of the tests employed in the workup for CS [2–4, 8]. In children, the diagnosis of CS is facilitated by the inhibitory effects of hypercortisolemia on height gain [7]. Indeed, the deceleration of growth velocity with a concurrent and unabated weight gain are the hallmark of CS in childhood [7–9]. In contrast, in lieu of these apparent signs, extensive biochemical investigation is needed for the confirmation of the diagnosis of CS in adult patients [1–4], especially if their situation is
complicated by moderate weight gain and other conditions, which collectively have been called 'pseudo-Cushing' states [10]. Adult patients may also have ectopic sources of ACTH as causes of CS in up to 10–15% of the total number of cases [11]. In general, the diagnostic evaluation proposed in figure 1 is what we recommend for both adults and children with CS [1–4, 8, 10–12] (fig. 1).

The appropriate therapeutic interventions in CS depend on accurate diagnosis and classification of the disease. The history and clinical evaluation (including growth charts in children) are important to make the initial diagnosis. Upon suspicion of the syndrome, laboratory and imaging confirmations are necessary. An algorithm of the diagnostic process is presented in figure 1. The first step in the diagnosis of CS is to document hypercortisolism. This step is usually done in the outpatient setting. Because of the circadian nature of cortisol and ACTH, isolated cortisol and ACTH measurements are not of great value in diagnosis. One excellent screening test for hypercortisolism is a 24-hour urinary free cortisol (UFC) excretion corrected for body surface area. A normal 24-hour UFC value is <70 μg/m^2/day (with the radioimmunoassay values). Falsely
high UFC may be obtained because of physical and emotional stress, chronic and severe obesity, pregnancy, chronic exercise, depression, alcoholism, anorexia, narcotic withdrawal, anxiety, malnutrition and excessive water intake (more than 5 liters/day). These conditions may lead to sufficiently high UFCs to cause what is known as pseudo-CS. On the other hand, falsely low UFC may be obtained mostly with inadequate collection. Another baseline test for the establishment of the diagnosis of CS is a low-dose dexamethasone suppression test; the cortisol cutoff level should be <1.8 μg/dl (50 nmol/l); if it is greater than 1.8 μg/dl, further evaluation is necessary. If the response to both the 1 mg dexamethasone overnight suppression test and the 24-hour UFC are both normal, a diagnosis of CS may be excluded with the following caveat: 5–10% of patients may have intermittent or periodic cortisol hypersecretion and may not manifest abnormal results to either test. If periodic or intermittent CS is suspected, continuous follow-up of the patients is recommended. Diurnal plasma cortisol variation, including midnight cortisol values, is a very good test for the establishment of the diagnosis of CS: in our institution, it has become the test of choice for the confirmation of endogenous hypercortisolemia and is routinely done in patients with confirmed elevated urinary cortisol levels on the outside. There are several caveats for the interpretation of the test of which the most important ones are: (1) the venous catheter has to be placed at least 2 h before the test and (2) if the patient comes from another time zone, a 1-h/day adjustment should be taken into account prior to obtaining the test. In general, serum cortisol levels are drawn at 11:30 p.m. and 12:00 midnight and at 7:30 a.m. and 8:00 a.m., while the patient is lying in bed and asleep; midnight cortisol levels above 5 μg/dl are abnormal and confirm the diagnosis of CS, whereas an inverted diurnal rhythm is seen in BAHs and some other adrenal tumors. If one of the tests suggests CS or if there is any question about the diagnosis, tests that distinguish between pseudo-Cushing states and CS may be obtained. One such test is the combined dexamethasone-CRH test. Once the diagnosis of CS is confirmed there are several tests to distinguish ACTH-dependent disease from the ACTH-independent syndrome. A spot plasma ACTH may be measured; if this measurement is <5 pmol/l it is indicative of ACTH-independent CS, although the sensitivity and specificity of a single ACTH measurement are not high because of the great variability in plasma ACTH levels and the instability of the molecule after the sample's collection. Even if one assumes that the sample was collected and processed properly (collected on ice and spun down immediately in a refrigerated centrifuge for plasma separation; the sample should then be immediately processed or frozen at −20°C), ACTH levels that are between 5 and 20 pmol/l are not informative in this era of high sensitivity assays; levels above 20 pmol/l are more suggestive of an ACTH-dependent condition, but again that is not a certainty until single ACTH levels are repeatedly over 70 pmol/l.

The standard 6-day low- and high-dose dexamethasone suppression test (Liddle's test) is used to differentiate Cushing disease from ectopic ACTH secretion and adrenal causes of CS. In the classic form of this test, after 2 days of baseline urine collection, 0.5 mg of dexamethasone (adjusted per weight for children <70 kg by dividing the
dose by 70 and multiplying by the weight of the child) every 6 h are given per os starting at 6.00 a.m. on day 3 (‘low-dose’ phase of the test) for a total of 8 doses (2 days); this is continued with a 2-mg dose of dexamethasone per os (adjusted per weight for children <70 kg by dividing the dose by 70 and multiplying by the weight of the child) on day 5 (‘high-dose’ phase of the test) given every 6 h for another 8 doses (final 2 days). UFCs and 17-hydroxysteroid (17-OHS) excretion are measured at baseline, during, and 1 day after the end of the dexamethasone administration. Approximately 90% of patients with Cushing disease will have suppression of cortisol and 17-OHS values, whereas less than 10% of patients with ectopic ACTH secretion will have suppression. UFC values should suppress to 90% of baseline value and 17-OHS excretion should suppress to less than 69% of baseline value. The criteria are similar if one uses serum cortisol values obtained at 8 a.m. of the morning after the last dose of dexamethasone, e.g. serum cortisol on day 7 should be 90% of baseline serum cortisol values (obtained at 8 a.m. the day before dexamethasone administration).

The Liddle test has been modified to (1) giving 2 mg every 6 h (without the preceding low-dose phase); (2) administering dexamethasone intravenously over 5 h at a rate of 1 mg/h, or (3) giving a single high dose of dexamethasone (8 mg, in children adjusted for weight <70 kg) at 11 p.m. and measuring the plasma cortisol level the following morning. This overnight, high-dose dexamethasone test has sensitivity and specificity values similar to those of the classic Liddle test: a 68% suppression of serum cortisol levels from baseline is what differentiates Cushing disease (more than 68% suppression) from other causes of CS (adrenal or ectopic ACTH production; less than 50% suppression) [12]. An ovine CRH stimulation test may also be obtained for the differentiation of Cushing disease from ectopic ACTH secretion [11], but it is less useful in the diagnosis of ADTs [8].

In addition to the biochemical testing, the most useful tests in the diagnosis of cortisol-producing ADTs are imaging [computed tomography (CT) or magnetic resonance imaging (MRI)] and Liddle’s test (especially in the diagnosis of BAHs). CT is preferred over MRI for cortical (vs. medullary) tumors because it allows for better delineation of the adrenal contour (fig. 2). With the use of contrast material, adrenal CT is an excellent diagnostic tool in the investigation of ADTs although more testing is required for the delineation of a cortisol-producing benign ADT. Most adrenocortical carcinomas are unilateral and quite large by the time they are detected. Ultrasound may not be used to image the adrenal glands for the diagnostic workup of CS, because its sensitivity and accuracy are much less than CT or MRI. Catheterization studies may not be used to confirm the source of cortisol secretion in ADTs.

**Histological Types of Benign ADTs Causing CS**

Benign ADTs causing CS include the common adrenocortical adenoma (ADA) and BAHs [13] such as primary pigmented nodular adrenocortical disease (PPNAD) and
ACTH-independent macronodular adrenocortical hyperplasia (AIMAH) – also known as massive macronodular adrenocortical disease (MMAD). The various types of adrenocortical lesions, their histology and other information are given in table 1. The common cortisol-producing ADA of the zona fasciculata needs little introduction, although histological variants of this common lesion do exist.

In all ages, the most common ADT causing CS is a unilateral adenoma (fig. 2); however, up to 10% of patients may have bilateral tumors [13]. Table 1 lists no less than 6 types of BAHs. They are divided into two groups of disorders, macro- and micronodular hyperplasias on the basis of the size of the associated nodules (fig. 2). In macronodular disorders, the greatest diameter of each nodule exceeds 1 cm; in the

Fig. 2. a–c CT of the adrenal glands from 3 patients with micronodular BAHs. d The right adrenal gland with PPNAD from the patient whose CT is shown in c: multiple small, pigmented nodules are seen that are characteristic of PPNAD. CT from a patient with MMAD/AIMAH and CS with massive enlargement of the adrenal glands on both sides (arrows) (e) and her left adrenal with visible multiple macronodules (f). g CT from an adolescent with a common, solitary, cortisol-producing adrenal adenoma (arrow). h The tumor from the patient in g: this is a classic cortisol-producing adenoma with yellow fat and brown discoloration, the result of pigment (lipofuscin and rarely neuromelanin). i CT from a patient with a left-sided cortisol-producing tumor that was inhomogeneous with hemorrhage and calcifications. j The tumor from the patient in i: calcification and blood are visible; the tumor was premalignant.
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<tr>
<th>Adrenocortical lesions</th>
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<th>Histopathology</th>
<th>Genetics</th>
<th>Gene/locus</th>
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<tr>
<td><strong>Benign</strong></td>
<td></td>
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<tr>
<td>Common adenoma</td>
<td>all ages</td>
<td>adenoma of the zona fasciculata</td>
<td>MEN 1, FAP, MAS, HLRCS, CNC, Carney triad, other</td>
<td>menin, APC, GNAS, FH, PRKAR1A; 2p16, 9q34, other</td>
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<tr>
<td>Macronodular hyperplasias (multiple nodules more than 1 cm each)</td>
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<td>Bilateral macroadenomatous hyperplasia (BMAH)</td>
<td>middle age</td>
<td>distinct adenomas (usually 2 or 3) with internodular atrophy</td>
<td>MEN 1, FAP, MAS, HLRCS, other; isolated (AD); other</td>
<td>menin, APC, GNAS, FH, ectopic GPCRs</td>
</tr>
<tr>
<td>BMAH of childhood (c-BMAH)</td>
<td>infants, very young children</td>
<td>as above; occasional microadenomas</td>
<td>MAS</td>
<td>GNAS</td>
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<td>AIMAH, also known as MMAD (AIMAH/MMAD)</td>
<td>middle age</td>
<td>adenomatous hyperplasia (multiple) with internodular hyperplasia of the zona fasciculata</td>
<td>isolated, AD</td>
<td>ectopic GPCRs; WISP-2 and Wnt signaling; 17q22-24, other</td>
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<tr>
<td>Micronodular hyperplasias (multiple nodules less than 1 cm each)</td>
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<tr>
<td>Isolated PPNAD (i-PPNAD)</td>
<td>children; young adults</td>
<td>microadenomatous hyperplasia with (mostly) internodular atrophy and nodular pigment (lipofuscin)</td>
<td>isolated; AD</td>
<td>PRKAR1A, PDE11A; 2p16; other</td>
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<tr>
<td>CNC-associated primary pigmented nodular adrenocortical disease (c-PPNAD)</td>
<td>children; young and middle ages</td>
<td>microadenomatous hyperplasia with (mostly) internodular atrophy and (mainly nodular) pigment (lipofuscin)</td>
<td>CNC (AD)</td>
<td>PRKAR1A, 2p16; other</td>
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<tr>
<td>Isolated micronodular adrenocortical disease (i-MAD)</td>
<td>mostly children; young adults</td>
<td>microadenomatous with hyperplasia of the surrounding zona fasciculata and limited or absent pigment</td>
<td>isolated, AD; other</td>
<td>PDE11A, PDE8B; other; 2p12-p16, 5q, other</td>
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micronodular group nodules are less than 1 cm. Although nodules less than 1 cm can occur in macronodular disease (especially the form associated with McCune-Albright syndrome), and single large tumors may be encountered in PPNAD (especially in older patients), the size criterion has biologic relevance, as we rarely see a continuum in the same subject: most patients are either macro- or micronodular. There are two additional basic characteristics that we use in this classification of BAHs [13]: that of the presence of pigment and that of status (hyperplasia or atrophy) of the surrounding cortex. Pigment in adrenocortical lesions is rarely melanin; most of the pigmentation in both ADAs and BAH that produce cortisol is lipofuscin (fig. 2). The latter appears macroscopically as light brown to, sometimes, dark brown or even black discoloration of the tumorous or hyperplastic tissue; microscopically, lipofuscin can be seen but it is better detected by electron microscopy.

PPNAD is a genetic disorder with the majority of cases associated with Carney complex, a syndrome of multiple endocrine gland abnormalities in addition to lentigines and myxomas; the adrenal glands in PPNAD are most commonly normal or even small in size with multiple pigmented nodules surrounded by an atrophic cortex (fig. 3). The nodules are autonomously functioning resulting in the surrounding atrophy of

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**Table 1.** (continued)

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<td>mitotic figures, atypia of cortical cells; capsular invasion; metastases</td>
<td>isolated</td>
<td>TP53, β-catenin, INHA; 2p, 2q, 9q, 11q, other</td>
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<td>children; young adults</td>
<td>as above</td>
<td>LFS (AD); BWS, RTS, other</td>
<td>TP53, CHK22, IGF2, other</td>
</tr>
<tr>
<td>Brazil variant</td>
<td>children; young adults</td>
<td>as above; milder clinical course</td>
<td>AD; other</td>
<td>TP53, INHA, SF1; 9q34 amplification; other</td>
</tr>
</tbody>
</table>

MEN 1 = Multiple endocrine neoplasia type 1; FAP = familial adenomatous polyposis (polyposis coli); MAS = McCune-Albright syndrome; HLRCS = hereditary leiomyomatosis and renal cancer syndrome; FH = fumarate hydratase; AD = autosomal dominant; CNC = Carney complex; GPCR = G-protein-coupled receptors; LFS = Li-Fraumeni syndrome; BWS = Beckwith-Wiedemann syndrome; RTS = Rubinstein-Taybi syndrome.
ADRENAL CUSHING SYNDROME

Children and adolescents with PPNAD frequently have periodic or atypical CS [14, 15]. AIMAH/MMAD is another rare disease, which leads to CS [16, 17]. The adrenal glands are massively enlarged with multiple, huge nodules that are typical, yellow-to-brown cortisol-producing adenomas (fig. 2). Most cases of MMAD are sporadic, although few familial cases have been described; in those, the disease appears in children. In some patients with MMAD, cortisol levels appear to increase with food ingestion (food-dependent CS) and in response to posture and other activities [16]. In these patients, aberrant expression of the neuroendocrine G-protein-coupled receptors (GPCRs) has been demonstrated in their adrenocortical tissue. Food-dependent CS has not been described in younger patients or in children, although bilateral macronodular

Fig. 3. Paradoxical stimulation of 17-OHS (a) and UFC (c) in patients with PPNAD (I) versus those with MMAD (d) and solitary cortisol-producing adenomas (H) during the course of Liddle's test. *p > 0.05; **p > 0.001. b 50% increase of UFCs on day 6 of the test detects more than 70% of the patients with PPNAD versus other cortisol-producing tumors of the adrenal cortex. d PPNADs express synaptophysin within the cortical nodules, an unexpected feature for a cortical lesion.
adrenal hyperplasia can also be seen in McCune-Albright syndrome [17]. In this syndrome there is a somatic mutation of the GNAS gene leading to constitutive activation of the Gsα protein and continuous, non-ACTH-dependent stimulation of the adrenal cortex. CS in MAS is rare and usually presents in the infantile period (before 6 months of age); interestingly, a few children have had spontaneous resolution of their CS [13, 14].

Because both PPNAD and MMAD and other BAHs can present with bilateral adrenal masses (fig. 2), a useful biochemical test is the 6-day-long Liddle test, modified to identify stimulation of UFC secretion, rather than suppression [18]. In this test, patients with micronodular forms of BAH respond with a gradual increase of UFC and 17-OHS secretion in response to the administration of dexamethasone (by 2 days of 0.5 mg dexamethasone every 6 h and 2 days of 2 mg dexamethasone every 6 h) (fig. 3). Although the cause of this ‘paradoxical’ rise in glucocorticoid synthesis by the adrenal cortex in response to dexamethasone is not known, it is a glucocorticoid receptor-mediated phenomenon [19] that may be due to the abnormal expression of various substances by the PPNAD cortex (such as synaptophysin and others, fig. 4) [20].
Adrenal Cancer and CS

Malignant neoplasias of the adrenal cortex account for 0.05–0.2% of all cancers, with an approximate prevalence of two new cases per million of population per year; adrenal cancer occurs at all ages, from early infancy to the 8th decade of life [21, 22]. A bimodal age of distribution has been reported, with the first peak occurring before the age of 5 years, and the second in the 4th to 5th decade. In all published series, females predominate, accounting for 65–90% of the reported cases. Several studies have shown a left-sided prevalence in adrenal cancer; however, others have reported a right-sided preponderance. In approximately 2–10% of the patients, adrenal cancer is found bilaterally. Overall, there appears to be a higher prevalence of adrenocortical carcinoma among patients with incidentally discovered adrenal masses than in the general population, although numerical estimates vary widely in the literature. Among the radiologically detectable masses, independent of size, one in 1,500 lesions may be an adrenal carcinoma; using the 5-cm cutoff as the most commonly accepted criterion for clinical investigation of an ADT, carcinoma may be found in as many as 7% of the patients with adrenal tumors over 5–7 cm in size [23].

In some areas of the world, higher incidence of adrenal cancer, especially in children, has been documented. This is particularly true for Southern Brazil, where environmental mutagens and a frequent TP53 mutation have been postulated as the relevant pathogenic event [24]. In these areas, evaluation of incidentally discovered adrenal masses may be necessary for lesions smaller than 5 cm. Although the incidence of adrenal incidentalomas appears to be higher in some familial neoplasia syndromes like multiple endocrine neoplasia type 1 and familial adenomatous polyposis, it is unclear whether this finding is accompanied by a higher predisposition to adrenal cancer.

CS is most common among pediatric patients with adrenal carcinoma present with a hormonal syndrome which makes their detection easier and leads to their early surgical resection and medical treatment. CS is less frequent among adults with the disease.

Clinical and Molecular Genetics of ADTs Associated with CS

As we already mentioned, aberrant cAMP signaling has been linked to genetic forms of cortisol excess that lead to CS [25], mostly BAHs. Macronodular adrenocortical hyperplasia may be due to GNAS mutations associated with either McCune-Albright syndrome or sporadic ADTs. Micronodular BAH, and its better-known variant, PPNAD, may be caused by germline-inactivating mutations of the PRKARIA gene [26]. Most patients with PPNAD also have Carney complex, as mentioned above [27].

Over the last several years, it has become apparent that there are several forms of micronodular BAH that are not caused by germline-inactivating mutations of the
PRKAR1A gene (table 1). We described one such case associated with an atypical, episodic, form of CS in a young child [14]. Her adrenal histology showed moderate diffuse cortical hyperplasia, multiple capsular deficits, and massive circumscribed and infiltrating extra-adrenal cortical excrescences that in many cases formed micronodules that were nonpigmented. Synaptophysin, a marker for PPNAD, also stained the nodules, in addition to the surrounding cortex [14, 20].

Recently, we reported that inactivating mutations of the PDE11A and PDE8B genes could be found in a subgroup of patients with PPNAD and other forms of BAH [28–30]. PDE11A is a dual-specificity phosphodiesterase catalyzing the hydrolysis of both cAMP and cGMP; it is expressed in several endocrine tissues, including the adrenal cortex [28, 29]. The PDE11A gene was mapped to the 2q31-35 chromosomal region and tumors from patients with PDE11A-inactivating mutations demonstrated 2q allelic losses (51). The PDE11A locus, like that of other PDEs, has a complex genomic organization; of the four possible splice variants, only A4 appears to be expressed in the adrenal cortex, whereas A1 is ubiquitous, and A2 and A3 have a more limited expression pattern. More recent data show that PDE11A is widely expressed in adrenocortical tissue and its expression appears to be modified in a variety of tumors beyond PPNAD and other forms of BAH.

PDE11A mutations and polymorphisms were found as low-penetration predisposing factors to ADTs [29]. The PDE11A data support the notion that this gene is not necessarily causative of BAH but that it is associated with a low-penetrance predisposition to the development of BAH and possibly other ADTs leading to CS and, perhaps, other conditions.

More recently, a single PDE8B mutation was identified in a young child with BAH and CS [30]; PDE8B is another cAMP-specific PDE with wide expression in endocrine tissues, including the adrenal cortex. Its involvement in ADT formation beyond this rare case of isolated micronodular adrenocortical disease remains to be seen.

**BAHs and cAMP Signaling**

The cause of all forms of BAH studied to date appears to be linked to increased cAMP signaling. However, the histopathological changes in the adrenal glands of patients with the various mutations or functional abnormalities of this pathway differ significantly (fig. 2). PRKAR1A mutations are associated with the pigmented micronodular variant of BAH that is known as PPNAD, whereas PDE11A (and possibly PDE8B) mutations appear to be predisposing to a variety of lesions from isolated PPNAD to nonpigmented micronodular hyperplasia; GNAS mutations are associated with the macronodular and clearly nonpigmented forms of BAH. It is also interesting that sporadic ADTs (without any family history) can be associated with somatic mutations in all three of these genes.
It is noteworthy that in all forms of BAH associated with increased cAMP signaling there are patients with mutations in one of the causative genes that do not present with overt CS. The frequency with which carriers of mutations in one of these genes present with ‘classic’ CS appears to be higher in PRKAR1A mutation carriers than in PDE11A, PDE8B, or GNAS-associated disease with significant, however, interindividual variability and without a clear genotype-phenotype correlation. Interestingly, the age at which CS presents in these disorders is exactly the reverse, with McCune-Albright syndrome patients (GNAS mutation carriers) presenting almost always in infancy, whereas at least some of the patients with PDE11A, PDE8B mutations appear to present mostly in early childhood years and PRKAR1A mutation carriers in late adolescence and young adulthood. Thus, a number of factors are likely to affect the expression of these mutations, developmental, hormonal, and perhaps, gender-related ones. The presence of allelic losses of the corresponding normal allele in adrenal tissues seems to also be a determining factor in the development of disease associated with PRKAR1A and PDE11A, PDE8B mutations, since all these genes were identified using LOH studies [26, 28, 30].

PDE11A and PDE8B are the first PDEs to be linked to an inherited condition associated with tumor formation but may not be the only enzyme of this large family of proteins that predisposes to tumors. Our genome-wide dataset [28] suggests that other PDEs are likely to be involved in adrenal tumorigenesis in a similar manner: not by causing tumors per se, but by being a predisposing factor. Very little is known about PDE11A, PDE8B or other PDEs in adrenocortical tissue which, however, appears to exhibit significant PDE activity in vitro. Our preliminary data suggest that several PDEs are expressed in the cortex; PDE11A is expressed at levels that are higher than those of most other such enzymes with the exception of PDE8B [28, 30].

The high frequency of PDE11A-inactivating mutations in the population [29], the possibility that other members of this large family of proteins are involved in ADT formation, and the identification of clinically silent carriers [28–30] raise an interesting question: is it possible that PDE11A and PDE8B mutations (or mutations in a similar gene) underlie the high frequency of ‘incidentalomas’ [6] in the general population? At the moment, this question cannot be answered; larger and prospective studies need to be performed.

**Surgical Treatment of ADTs Causing CS**

Patients with benign ADTs are operated today mostly via a laparoscopic procedure (LP) that is preferred for both bilateral and unilateral lesions. LP has minimized morbidity and improvement is immediate after resection in these patients who are hypertensive preoperatively or have other complications of CS [31].Replacement with glucocorticoids is necessary for up to 1 year after surgery for patients with unilateral adrenalectomies, whereas for patients after a bilateral procedure replacement with
both gluco- and mineralocorticoids is necessary for life. For patients with cancer, the treatment of all primary tumors is also surgical, although open laparotomy for staging is preferable over LP. If complete resection of an adrenocortical carcinoma cannot be achieved, as much as possible of the tumor should be removed. Solitary recurrences or metastases should also be removed surgically, if possible. Long-term disease-free status has been produced by complete resection of adrenocortical carcinoma, whereas long-term remissions have followed surgical resection of hepatic, pulmonary, or cerebral metastases. Therapy with o,p'-DDD (mitotane) is initiated either as an adjuvant to surgical treatment or for patients with inoperable cancer [32, 33]. o,p'-DDD is an adrenocytolytic agent which is given at maximally tolerated oral doses (up 10 g/m²/day). It ameliorates the endocrine syndrome in approximately two thirds, whereas tumor regression or arrest of growth has been observed in as many as one third of the patients. Occasionally, for the correction of hypercortisolism, steroid synthesis inhibitors (aminogluthethimide, metyrapone, trilostane, ketoconazole) or glucocorticoid antagonists (RU 486) are required. Patients taking mitotane (o,p'-DDD) may develop hypoaldosteronism or hypocortisolism, and fludrocortisone or hydrocortisone should be added as needed. Radiation therapy is occasionally helpful for palliation of metastases.

Concluding Remarks

CS caused by ADTs is most commonly caused by a solitary adenoma; BAHs are a more frequent cause of CS than previously thought. Defects of the cAMP signaling pathway are frequent in ADTs associated with CS. Cancer associated with CS is extremely rare. Surgical advances have made ADTs causing CS a disease that is cured in most cases with the exception of cancer.

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References

Adrenal Cushing Syndrome


