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Review

Clinical, Pathophysiologic, Genetic, and Therapeutic Progress in Primary Bilateral Macronodular Adrenal Hyperplasia

Jérôme Bertherat,[1,*] Isabelle Bourdeau,[2,*] Lucas Bouys,[1,*] Fanny Chasseloup,[3,*] Peter Kamenický,[3,*] and André Lacroix[2,*]

[1]Department of Endocrinology and National Reference Center for Rare Adrenal Disorders, Cochin Hospital, Assistance Publique Hôpitaux de Paris, 24 rue du Fg St Jacques, 75014 Paris, France
[2]Division of Endocrinology, Department of Medicine and Research Center, Centre hospitalier de l'Université de Montréal (CHUM), Montréal, Québec H2X 0A9, Canada
[3]Service d'Endocrinologie et des Maladies de la Reproduction Université, Paris-Saclay, INSERM, Physiologie et Physiopathologie Endocriniennes, 94276 Le Kremlin-Bicêtre, France

Correspondence: André Lacroix, Division of Endocrinology, Department of Medicine, Research Center, CHUM 1000 Saint-Denis Street, Montréal, QC H2X 0A9, Canada. E-mail: andre.lacroix@umontreal.ca.
*All authors contributed equally.

Abstract

Patients with primary bilateral macronodular adrenal hyperplasia (PBMAH) usually present bilateral benign adrenocortical macronodules at imaging and variable levels of cortisol excess. PBMAH is a rare cause of primary overt Cushing's syndrome but may represent up to one-third of bilateral adrenal incidentalomas with evidence of cortisol excess. The increased steroidogenesis in PBMAH is often regulated by various G protein–coupled receptors (GPCRs) aberrantly expressed in PBMAH tissues; some receptor ligands are ectopically produced in PBMAH tissues, creating aberrant autocrine/paracrine regulation of steroidogenesis.

The bilateral nature of PBMAH and familial aggregation led to the identification of germline heterozygous inactivating mutations of the *ARMC5* gene, in 20% to 25% of the apparent sporadic cases and more frequently in familial cases; *ARMC5* mutations/pathogenic variants can be associated with meningiomas. More recently, combined germline mutations/pathogenic variants and somatic events inactivating the *KDM1A* gene were specifically identified in patients affected by glucose-dependent insulinotropic peptide (GIP)-dependent PBMAH. Functional studies demonstrated that inactivation of KDM1A leads to GIP-receptor (GIPR) overexpression and over- or downregulation of other GPCRs. Genetic analysis is now available for early detection of family members of index cases with PBMAH carrying identified germline pathogenic variants. Detailed biochemical, imaging, and comorbidity assessment of the nature and severity of PBMAH is essential for its management. Treatment is reserved for patients with overt or mild cortisol/aldosterone or other steroid excesses, taking in account comorbidities. It previously relied on bilateral adrenalectomy; however, recent studies tend to favor unilateral adrenalectomy or, less frequently, medical treatment with cortisol synthesis inhibitors or specific blockers of aberrant GPCR.

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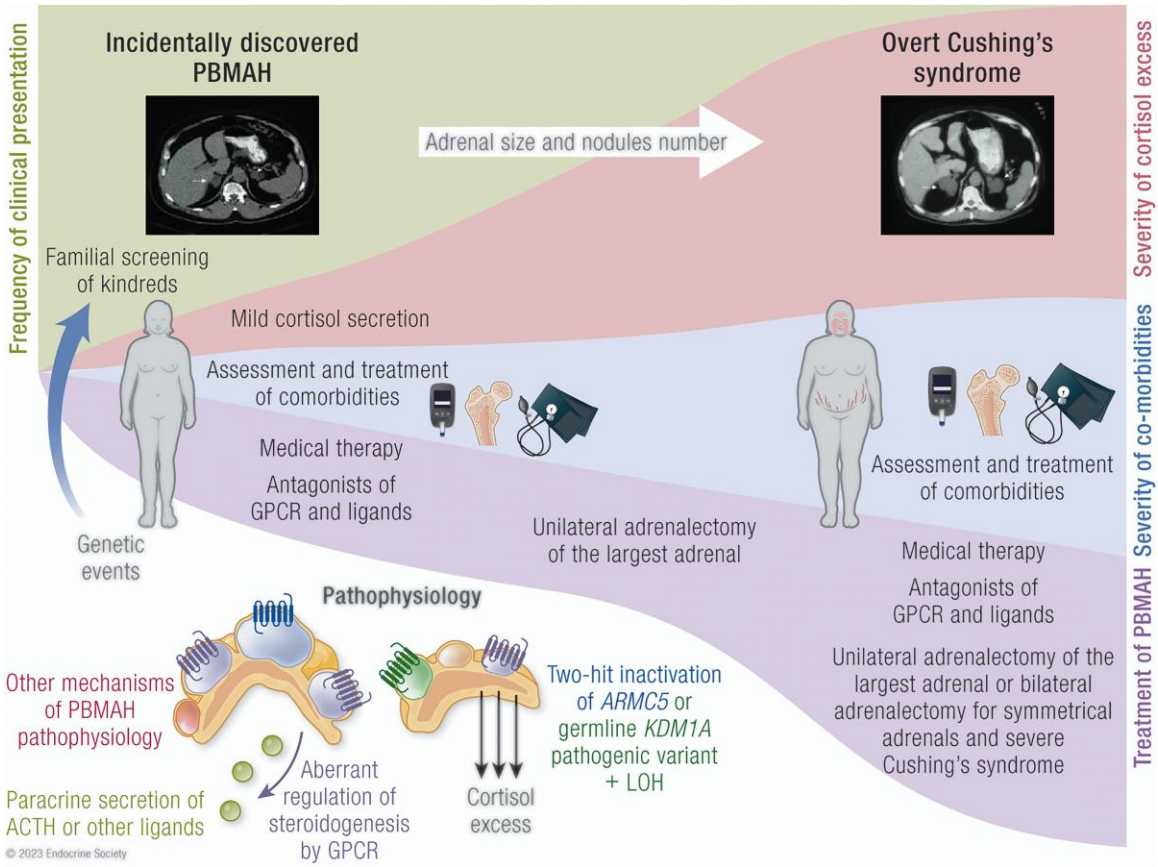
Graphical Abstract



Key Words: Cushing's syndrome, adrenal hyperplasia, heredity, *ARMC5*, *KDM1A*, adrenalectomy

Abbreviations: 5-HT, serotonin (5-hydroxytryptmine); ACTH, adrenocorticotropic hormone; ADRA2A, α-2A adrenergic receptor; ADRB, β-adrenergic receptor; AVP, arginine-vasopressin; AVPR1, arginine-vasopressin receptor type I; AVS, adrenal vein sampling; cAMP, cyclic adenosine monophosphate; CBG, cortisol binding globulin; CS, Cushing's syndrome; CT, computed tomography; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DM, diabetes mellitus; DST, dexamethasone suppression test; E3, E3 ubiquitin ligase; FAP, familial adenomatous polyposis; 18-FDG, fluorine-18 fluorodeoxyglucose; FDG-PET, fluorodeoxyglucose–positron emission tomography; GIP, glucose-dependent insulinotropic peptide; GIPR, GIP receptor; GnRH, gonadotropin-releasing hormone; GPCR, G protein–coupled receptor; hCG, human chorionic gonadotropin; HLRCC, hereditary leiomyomatosis and renal cancer; HTR4, 5-HT receptor type 4; HU, Hounsfield Units; IHC, immunohistochemistry; IV, intravenous; LC-MS, liquid chromatography-mass spectrometry; LH, luteinizing hormone; LHCGR, luteinizing hormone/chorionic gonadotropin receptor; LOH, loss of heterozygosity; MAS, McCune-Albright syndrome; MC2R, melanocortin type 2 receptor; MEN1, multiple endocrine neoplasia type 1; MGUS, monoclonal gammopathy of unknown significance; MRI, magnetic resonance imaging; 17-OHP, 17-hydroxyprogesterone; PBMAH, primary bilateral macronodular adrenal hyperplasia; PCR, polymerase chain reaction; PDE, phosphodiesterase; PET/CT, positron emission tomography/computed tomography; PKA, protein kinase A; POMC, proopiomelanocortin; PPNAD, primary pigmented nodular adrenocortical disease; TRH, thyrotropin-releasing hormone; UFC, urinary free cortisol; WHO, World Health Organization.

Described for the first time by Kirschner et al in 1964 (1), primary bilateral macronodular adrenal hyperplasia (PBMAH) is characterized by the presence of bilateral adrenocortical benign macronodules larger than 1 cm that present with variable levels of excess cortisol secretion. PBMAH has been referred to by different names throughout the years, such as adrenocorticotropic hormone (ACTH)-independent massive bilateral adrenal disease (AIMBAD) (2), massive macronodular adrenal hyperplasia or disease (MMAD) (3, 4), giant macronodular adrenal hyperplasia (5), huge bilateral adrenocortical multinodular hyperplasia (6), ACTH-independent macronodular adrenal hyperplasia (AIMAH) (7), macronodular adrenal hyperplasia (MAH) (8), bilateral macronodular adrenal hyperplasia (BMAH) (9), primary macronodular adrenal hyperplasia (PMAH) (10, 11), and more recently, with the 2022 World Health Organization (WHO) pathological classification of adrenal tumors, bilateral macronodular adrenal disease (BMAD) (12). The ACTH-independent nature of the disease was reconsidered after the description of paracrine intra-adrenal ACTH secretion controlling local steroidogenesis in PBMAH (13), and the name PBMAH was then proposed and has been since mostly used in the literature (14); the term PMAH was alternatively used based on the finding that some patients initially present with unilateral lesions (10, 11), but we prefer to use PBMAH, as the pathophysiology involves both adrenal glands even if the progressive phenotype may be transiently macroscopically unilateral. The 2022 WHO pathological classification based its suggestion to eliminate the term hyperplasia and to replace it by disease on the finding that

ESSENTIAL POINTS

- PBMAH patients most often present with bilateral macronodular adrenal incidentalomas with mild dysregulated cortisol secretion
- In PBMAH, cortisol secretion is most often dysregulated by the fluctuations of ligands which activate a diversity of G protein–coupled receptors aberrantly expressed in the nodular tissues
- Previously believed to be sporadic, PBMAH can rarely be associated with hereditary syndromic diseases (multiple endocrine neoplasia type 1 [MEN1], familial polyposis coli/familial adenomatous polyposis [FAP], and hereditary leiomyomatosis and renal cancer [HLRCC] syndrome)
- *ARMC5* mutations/pathogenic variants are responsible for 20% to 25% of the apparent sporadic cases and a larger number of familial cases and can also be associated with meningiomas; *KDMIA* mutations are specifically responsible for ectopic expression of GIPR in sporadic or familial cases of GIP-dependent PBMAH with Cushing's syndrome and can be associated with adrenal myelolipoma, monoclonal gammopathy of undetermined significance (MGUS), or multiple myeloma
- Progressive increase in hyperplasia and macronodules development can lead to sufficient cortisol and other steroid excess resulting in the multiple comorbidities of Cushing's syndrome that require therapeutic interventions
- Genetic screening of adult family members of PBMAH patients carrying *ARMC5* or *KDM1A* mutations should be offered, in order to detect affected members and provide early detection of PBMAH to prevent the insidious effects of progressive cortisol excess
- Bilateral adrenalectomy has been the treatment of choice in the rare cases of PBMAH with severe overt Cushing's syndrome, but for most patients with modest cortisol excess causing comorbidities, unilateral adrenalectomy has been effective in reverting cortisol excess although it requires long-term surveillance for relapse and comprehensive therapy of residual comorbidities

in PBMAH, germline and somatic mutations underlie macronodules formation (see "Genetic Aspects"); they suggested to use the term hyperplasia in bilateral diseases secondary to chronic ACTH stimulation (12). As long-term elevation of ACTH in Cushing's disease, ectopic ACTH syndrome, or congenital adrenal hyperplasia often results not only in diffuse hyperplasia but also in macronodular forms (presumably also caused by secondary somatic events), we prefer to use secondary BMAH for those entities and we will use PBMAH in this review to refer to the primary etiology.

The study of the pathophysiology and genetics of this disease has been very illustrative in the field of Cushing's syndrome (CS). In particular, PBMAH has been a model to identify the role of aberrant (or illegitimate) membrane hormone receptors in adrenal CS (7, 15).

PBMAH was for a long time considered to be a rare cause of CS. This is certainly true for the classic presentation with massive bilateral adrenal enlargement and overt CS (graphical abstract, right section). Because the diagnosis of PBMAH nowadays is often incidentally found by radiological imaging and mostly in patients with mild dysregulated (pituitary ACTH-independent) cortisol secretion (graphical abstract left panel), it is now clearly perceived as a heterogeneous disease with various clinical, hormonal, and imaging presentations. The mild forms of the disease are also much more frequent, and nowadays clinicians are often confronted with the management of PBMAH patients.

With the increasing frequency of PBMAH diagnosis, its various and heterogeneous clinical aspects, pathogenic mechanisms, and recently recognized hereditary nature are now much better described than even a decade ago. This review will update the clinical aspects of PBMAH from the clinical presentation to management and will summarize the main mechanisms implicated in its pathophysiology (Graphical abstract).

Epidemiology

There is very scant large-scale epidemiological data on PBMAH. The limitations of the coding system used by most health care organizations preclude the generation of valid specific data on PBMAH from large epidemiologic studies (16). For patients that are diagnosed after the investigation of clinical signs of overt CS, which probably represent only a minor subset of PBMAH patients diagnosed today, the epidemiological studies of CS can be used. The incidence of CS ranges between 1.8 and 3.2/million/year and its prevalence ranges between 57 and 79 cases/million (17-22). Adrenal CS accounts for 20% to 30% of all causes, but PBMAH itself is less frequent than unilateral tumors causing CS. One epidemiological study centered on adrenal causes of CS reported a higher-than-expected incidence of 1.57/million/year in Korea (23). However, within specific series of CS patients, PBMAH is considered to represent less than 2% of the cases (24), but a rate as high as 6.2% was reported from a large institution (25).

Another way to approach the epidemiology of PBMAH is provided by the study of the adrenal incidentalomas incidence, since nowadays it represents the most frequent circumstance of its finding. In such patients, overt hypercortisolism is rare and therefore is often not identified in epidemiological studies dedicated to CS. Another limitation is that many of these patients are not treated by surgery and therefore lack pathological proof of the PBMAH diagnosis. It is considered that 15% to 20% of adrenal incidentalomas are bilateral lesions. Most of them are benign adrenocortical lesions (26). In some large series of benign adrenal incidentalomas, a rate of up to 22% of bilateral lesions was reported (27); in patients with modestly dysregulated cortisol secretion (defined by morning serum cortisol between 50 and 138 nmol/L following overnight 1 mg oral dexamethasone), bilateral lesions were present in 26% to 30% of patients (27, 28). Among the benign bilateral adrenocortical lesions, up to one-third may be in fact cases of PBMAH with mildly dysregulated cortisol secretion but without a diagnosis proven by pathology (29). This has been demonstrated in some patients by pathological analysis or the identification of a genetic cause of PBMAH (30). The epidemiology of adrenal incidentalomas suggests an increased

prevalence with age, ranging from 3% in the age group around 50 years to 10% in the group older than 70 years. (26). Most patients with PBMAH are diagnosed in this age range. If a subset of bilateral incidentalomas is bona fide PBMAH, this would imply that PBMAH is far more frequent than determined by the epidemiology of classic forms of CS.

Clinical Presentations

PBMAH can be now diagnosed in 3 different clinical circumstances: a) in patients with bilateral adrenal incidentalomas; b) in patients with clinical signs of cortisol (and aldosterone or other steroid) excess; and c) during familial screening of PBMAH or investigation of a multiple neoplasia syndrome related to PBMAH. At first consultation, a majority of patients have a sporadic presentation. Although clinical presentations can have some similarities between these 3 different circumstances, differences might be described and are mainly related to the degree of cortisol excess. Because familial screening is by definition a way to diagnose the very early form of the disease, it will be described in the second part of this section. The natural history of the disease is now partially understood, but clinical observation suggests a longer duration of cortisol dysregulation and probably a gradual enlargement of the adrenals before the diagnosis of PBMAH (Graphical abstract). The clinical presentation described here corresponds to the current practice and will probably change over time, notably in the group of patients presenting an identifiable genetic cause because of the progress of familial screening.

The age at diagnosis of patients with clinical signs of cortisol excess is most often between 45 to 65 years in adults (7, 8, 25, 30-33). However, rare cases of overt CS due to macronodular adrenal hyperplasia can be observed in the first months of life in infants with McCune-Albright syndrome (MAS) (34-36); but, considering the genetic mosaicism, the age of onset of adrenal hyperplasia in MAS and its unique clinical presentation, the adrenal hyperplasia observed in MAS and PBMAH may be considered as 2 distinct diseases.

There is a moderately increased prevalence in female individuals, who account for about 60% of the cases (25, 32). The clinical presentation is related to the variable levels of cortisol excess, ranging from asymptomatic PBMAH in patients with bilateral adrenal incidentalomas and no specific signs of cortisol excess to overt CS. When incidentally discovered, surgery is not always performed to confirm the pathological diagnosis; therefore, it might be difficult to differentiate PBMAH from bilateral adenomas. Careful imaging analysis might be helpful in those cases, especially when multiple nodules with internodular hyperplasia are visible on one, or even better, both sides (see "Imaging" section). Imaging analysis is also key to identify the bilateral nature of the lesions when the presentation is asymmetric. It should be noted in the natural history of the disease that, in some patients, the bilateral nature is more demonstrative after a few years of follow-up.

In patients with overt CS, classic clinical signs, such as weight gain with truncal obesity, supraclavicular and dorsal fat pads, proximal myopathy, purple striae, skin fragility, and easy bruising can be observed. These signs are probably present in < 25% of the PBMAH patients diagnosed today (32). Clinical signs of hyperandrogenism (hirsutism, acne) can also be observed (37, 38). Depression, and in some cases, psychosis, usually resulting from marked cortisol excess can be observed in PBMAH patients (39). Diabetes is diagnosed in 30% to 40% of the patients, and hypertension in 65% to 85% (25, 32). Aldosterone cosecretion can worsen arterial hypertension. As a consequence of increased cardiovascular risk, ischemic heart and cerebrovascular diseases can be observed (25). In patients with incidentally discovered PBMAH, diabetes and hypertension are frequently the only putative clinical consequence of CS and likely the cause of the increased mortality observed in this mild form of cortisol excess (28, 40); a recent study shows that comorbidities are more frequent in bilateral than in unilateral adrenal incidentalomas (27). Osteoporosis is reported in at least 20% of PBMAH patients, some of them with fractures (25). Venous thromboembolism can also be a complication of cortisol excess in PBMAH. Hypokalemia is present in about 10% of PBMAH patients, and can be a result of severe cortisol excess, but should prompt the investigation of cosecretion of aldosterone (32, 41).

PBMAH can also be a manifestation of inherited multiple neoplasia syndromes such as multiple endocrine neoplasia type 1 (MEN1) (42), familial adenomatous polyposis (FAP) (43) and hereditary leiomyomatosis and renal-cell cancer (HLRCC) (44), but it is rarely inaugural in these conditions. This will be discussed further in the "Genetic Aspects" section. For example, in a large series of 715 MEN1 patients, adrenal lesions (including a few cases of PBMAH) were the first neoplasia reported only in 6% of patients, while they were diagnosed concomitantly with the MEN1 diagnosis in 33% of cases, and afterward in 61% (45). Familial cases of PBMAH, without any features of multiple neoplasia syndromes, were first reported in the early 1990s (46-52); up to 45 families including 170 affected patients have been summarized recently (11, 53). The index cases were diagnosed with CS due to PBMAH between the ages of 38 and 69, while some affected relatives showed abnormal hormonal results and/or adrenal nodules or thickening on imaging as early as 17 years of age (52). These cases suggested a putative autosomal dominant inheritance which was later confirmed by the identification of 2 tumor suppressor genes predisposing to PBMAH: *ARMC5* in 2013 (54), responsible for around 80% of clear familial presentation of PBMAH, and *KDM1A* in 2021 (55, 56), causing PBMAH associated with GIP-dependent CS. These will be discussed in further detail in "Genetic Aspects." Thus, a third situation of diagnosis of PBMAH may be in the course of family screening of probands of patients with known mutations of *ARMC5, KDM1A* or other responsible genes (Graphical abstract and see "Genetic Aspects" section).

Imaging

Normal adrenals appear as retroperitoneal bilateral glands at the superomedial pole of each kidney (57), with an inverted V or Y shape (58), and a mean volume of 3.6 to 3.8 cm^3 for the right adrenal and 4.5 to 4.8 cm^3 for the left adrenal (59, 60). Adrenal volume is significantly higher in men than in women and is positively correlated with body weight (61).

Commonly, the radiological presentation of PBMAH is a bilateral enlargement of the 2 adrenal glands, each harboring one or several macronodules, more than 1 cm in diameter, with internodular hyperplasia (Fig. 1); less frequently, the macronodules are associated with a hypoplastic internodular tissue. But considering the high heterogeneity of PBMAH, no single imaging parameter can formally confirm the diagnosis, which can be only established after the pathological analysis

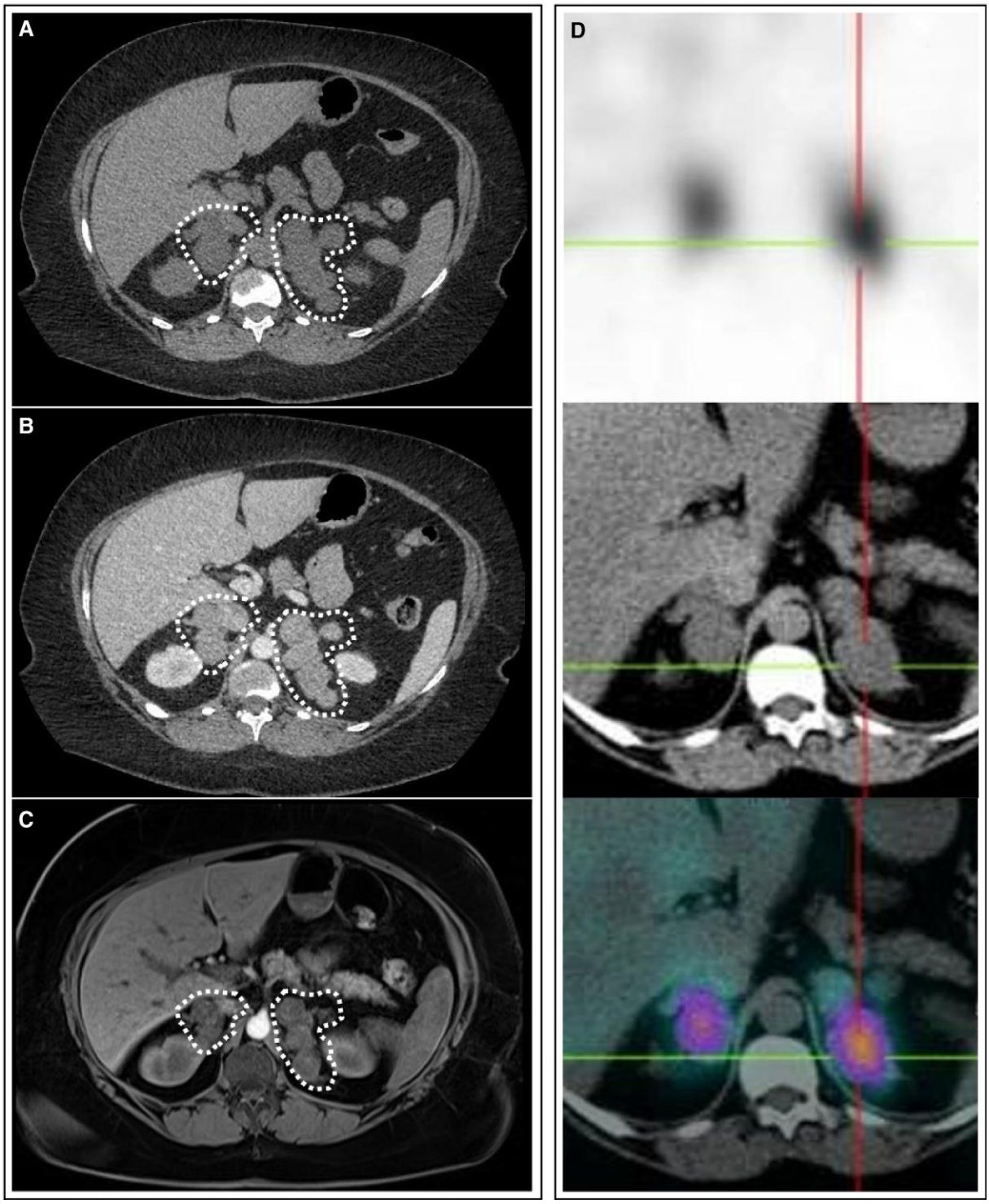


Figure 1. Adrenal morphological and functional imaging of a PBMAH patient with a germline pathogenic variant of *ARMC5*. (A) unenhanced adrenal CT showing several bilateral adrenal nodules, with an asymmetrical adrenal enlargement (maximal diameters: right, 56 mm; left, 80 mm), and attenuation values between 10 and 18 HU (adrenals are surrounded in white dotted lines) (B) enhanced adrenal CT at venous portal phase showing a homogeneous bilateral uptake of iodine contrast reagent by the macronodules (RPW >40% and APW >60%); (C) T1-weighted adrenal MRI; (D) NP-59 scintigraphy showing a bilateral uptake of the radiotracer with a left predominance, in keeping with CT and MRI aspect.

Practical tip/synthesis for Fig. 1:

- Bilateral adrenal incidentaloma is the most frequent presentation of PBMAH.
- In patients with presumed unilateral incidentaloma, careful revision of images by experienced adrenal radiologist is required to detect modest contralateral hyperplasia.
- Bilateral adrenal hyperplasia with 1 or several macronodules (>1 cm) is usual in PBMAH.
- Diffuse hyperplasia only or very asymmetrical macronodular adrenals can also occur.
- Nodule size and Hounsfield attenuation values used for unilateral lesions in CT scans do not apply to PBMAH for risks of malignancy.
- Functional imaging such as iodocholesterol uptake usually corresponds to adrenal size on CT scan.

of resected adrenal lesions. Thus, its diagnosis in non-operated patients is based on a combination of clinical, biological and radiological arguments and on the exclusion of differential diagnoses (Table 1). Since the vast majority of PBMAH are incidentally discovered on abdominal imaging performed for another condition (62), accurate radiological parameters are necessary to easily discriminate PBMAH from other causes of bilateral adrenal masses in addition to those summarized in Table 1, such as bilateral metastasis from extra-adrenal cancer, or bilateral adrenal hematoma (63, 64).

When unilateral masses are incidentally found, a careful examination of the contralateral adrenal should be performed in order to discriminate purely unilateral lesion from asymmetrical presentation of PBMAH. The radiological discrimination between PBMAH and other causes of benign adrenal hyperplasia, such as primary aldosteronism due to bilateral lesions, is less reliable and requires the concomitant analysis of clinical and biological profiles. In bilateral adrenal masses, considering that 2 different types of lesion or collision tumors may be detected concomitantly (65-73), each adrenal mass should be assessed individually in order to not misdiagnose potentially severe tumors such as adrenocortical carcinoma or pheochromocytoma (26).

Morphological Imaging

The 2 commonly used morphological imaging techniques to characterize PBMAH, and more generally adrenal masses, are computed tomography (CT) scans and magnetic resonance imaging (MRI). Both can provide information about the size, shape, and fat content of adrenal lesions. Because of its easier accessibility, shorter duration, and more affordable cost, CT scanning is more widely utilized than MRI, and its interpretation criteria are better known and more reproducible. In unilateral adrenal masses, the threshold of 4 cm is commonly used for the risk of malignant tumors such as adrenocortical carcinoma, with a poor specificity (20% to 42%), but a higher sensitivity (93% to 98%) (74-76). However, this does not apply to PBMAH, since in the largest series, patients can harbor adrenal masses of various sizes ranging from 10 to 130 mm without any cases evolving into adrenocortical carcinoma (ACC) (32, 62). However, the measurement of adrenal size is helpful in the assessment of PBMAH with an asymmetric presentation for which unilateral adrenalectomy is considered in order to control cortisol excess (see "Therapy of PBMAH"). The assessment of malignancy risk on adrenal imaging rests mostly on the evaluation of the fat content of the adrenal lesions, and adrenal adenomas are usually identified as benign adrenocortical masses based on their homogeneous high fat content. Because PBMAH are benign lesions, one might expect that imaging criteria based on attenuation value on CT scan or chemical shift on MRI used to diagnose a benign adrenocortical adenoma would apply to PBMAH. If the criteria used to classify benign adrenocortical incidentalomas can be used in bilateral lesions, they may be not valid in many cases of PBMAH. Indeed, in PBMAH, a size larger than 4 cm or an unenhanced density above 10 or 20 Hounsfield Units (HU) are frequently observed and are not associated with malignancy (77). Further studies with large number of PBMAH patients will be useful to characterize more precisely the radiological features of PBMAH.

Computed tomography scan

Normal adrenal glands have an attenuation close to the liver and show homogeneous enhancement after contrast reagent injection (58). Just one single study evaluating specifically PBMAH patients described the adrenals morphology, but did not assess the attenuation values (78) and most studies assessing attenuation values are focused on adrenal adenoma. On unenhanced CT scan, adrenal adenomas are classically characterized by an attenuation lower than 10 Hounsfield Units (HU) related to a rich fat content, with a 98% specificity and a 71% sensitivity (79, 80). Around 30% of adenomas are lipid-poor and thus present an unenhanced attenuation greater than 10 HU. However, these criteria accepted now for the characterization of unilateral adrenal mass have not been investigated enough in bilateral adrenal mass in general and more specifically in PBMAH.

In PBMAH, the adrenal glands are classically enlarged bilaterally, and contain at least 2 nodules of more than 1 cm in diameter (Fig. 1). The morphological aspect of the adrenal glands can be highly variable, ranging from diffuse hyperplasia without a clearly visible nodule, unique mass on each adrenal gland, to massively enlarged adrenals, up to 10 cm or more, with numerous nodules (7, 81). The largest published cohorts of PBMAH patients did not report the CT attenuation values or the MRI chemical shift. However, a few case reports and a small series described the attenuation values of PBMAH, which can be either less than 10 HU (82-84), or more than 10 HU and up to 25 HU on unenhanced CT (85-88), but none of them mentioned the washout parameters. Hence, the imaging parameters usually reported to discriminate benign from malignant adrenal lesions must be considered differently when the diagnosis of PBMAH is supported by the slow evolution pattern of adrenal imaging over time and the hormonal investigation. Large prospective studies including PBMAH cases should use different standardized protocols to clarify this unresolved question.

Magnetic resonance imaging

MRI is a second-line imaging modality in the assessment of adrenal lesions (58) and can be helpful in the situations where CT is contraindicated, such as pregnancy or allergy to iodine contrast reagent. Similar to CT, MRI is reliable to evaluate the fat content of an adrenal mass: the so-called chemical shift observed in lipid-rich lesions is defined by a drop of signal on the T1-weighted out-of-phase images compared with the T1-weighted in-phase images, which can be evaluated using either a qualitative visual method or a quantitative method (signal drop >16.5% (89)). Both qualitative and quantitative methods are equally accurate to discriminate lipid-rich from lipid-poor adenomas (90, 91). MRI is valuable to determine the fat content of adrenal masses with equivocal CT images between 10 and 20 HU attenuation, but it has been demonstrated that the sensitivity of MRI for the detection of adenoma decreases and is inferior to CT washout evaluation for the lesions with unhanced attenuation above 20 HU (92, 93). A recent prospective study demonstrated that a postcontrast washout measurement on MRI, in addition to the chemical shift evaluation, increases the sensitivity and specificity for the detection of a lipid-rich adenoma compared with the chemical shift alone. There was no significant difference between lipid-poor adenomas and non-adenomas regarding the washout parameters (94), which limits its value in routine

Table 1. Differential diagnosis of PBMAH with other etiologies of bilateral adrenal lesions and increased cortisol secretion

	PBMAH	Bilateral adrenal adenomas	ACTH-dependent nodular hyperplasia	Primary pigmented nodular adrenocortical disease (PPNAD)	Partial glucocorticoid resistance syndrome
Clinical presentation	• Mild or overt clinical signs or comorbidities of CS (or aldosterone/other steroids) • Bilateral incidentalomas	• Bilateral incidentalomas • Mild or overt clinical signs or comorbidities of CS	• Overt clinical symptoms and comorbidities of CS are usual in Cushing's disease or ectopic ACTH syndrome leading to nodular adrenal hyperplasia	Overt CS in children or young adults	• Possible arterial hypertension • Possible hirsutism • Possible fatigue • No signs of overt CS
Imaging	• Bilateral enlargement of the 2 adrenal glands and macronodules with internodular hyperplasia. • Nodules are isointense relative to muscle in T1-weighted sequences and hyperintense relative to liver with T2-weighted sequences	• Bilateral nodules without internodular hyperplasia • Use of washout or MRI signal shift to differentiate from primary malignant tumors	• Bilateral enlargement of the 2 adrenal glands • Nodules have the signal intensity of the liver on T2-weighted MRI images	Bilateral microdules smaller than 1 cm, not always visible on CT scan, but possibility of unilateral and more rarely bilateral macronodules	• Bilateral nodular hyperplastic adrenals • Rarely very large
Biochemical Investigation	• Possible hypokalemia	• Possible hypokalemia	• Hypokalemic metabolic alkalosis in severe ectopic ACTH syndrome	• Possible hypokalemia	• Possible hypokalemia
Hormonal characteristics	• Normal or high UFC • Low/suppressed ACTH level • Normal or elevated 17-OH-P • Normal or low DHEAS • Partial or lack of cortisol suppression after DST • Possible elevated ARR	• Normal or high UFC • Low/suppressed ACTH level • Normal 17-OHP • Partial or lack of cortisol suppression after DST	• High UFC • Normal/high ACTH • Higher DHEAS • Partial suppression after DST • Response to desmopressin/CRH in Cushing's disease, less frequent in benign NET tumors with ectopic ACTH	• High UFC, suppressed ACTH Lack of suppression or paradoxical stimulation cortisol after 6-day DST (Liddle's test)	• High UFC • Normal/high ACTH • Possible elevated adrenal androgens • Possible altered ARR • Reduced cortisol suppression after DST
Pathology	• Cortical nodules (2 cell types): small compact cells and larger cells with clear cytoplasm and higher lipid content • Internodular: diffuse nodular hyperplasia or, less frequently, atrophic internodular tissue	• Homogeneous lipid-rich nodules with corded/nested cell proliferation with vacuolated clear cytoplasm • Internodular: atrophic	• Diffuse adrenal hyperplasia, and nodular adrenocortical hyperplasia in late stages • Normal zonation of the cortex conserved in Cushing's disease/ectopic ACTH syndrome	• Multiple small pigmented nodules in both adrenals, possibility of some associated pigmented larger nodule(s)	• Not well studied
Genetics	• Germline pathogenic variant of *ARMC5* with LOH • Germline pathogenic variant of *KDM1A* with LOH • Other genetic events detailed in the genetic section	• Somatic gain-of-function variant of *PRKACA* • Somatic pathogenic variant of *CTNNB1* • Somatic gain-of-function variant of *GNAS*	• Somatic gain-of-function variant of *USP8* in Cushing's disease • Rare syndromic cases of Cushing's disease: *MEN1, RET, CDKN1B, AIP, PRKAR1A,* TSC1, TSC2, DICER1, *CABLES1*	• Frequent association with Carney Complex. Germline pathogenic variant of PRKAR1A, PDE11A, PDE8B. • Germline duplication of PRKACA	• *NR3C1* pathogenic variant

(*continued*)

Table 1. Continued

	PBMAH	Bilateral adrenal adenomas	ACTH-dependent nodular hyperplasia	Primary pigmented nodular adrenocortical disease (PPNAD)	Partial glucocorticoid resistance syndrome
Therapy	• Medical therapy • Specific therapy to block aberrant receptor or ligands in some cases • Surgical therapy	• Unilateral or bilateral adrenalectomy	• Pituitary surgery and/or specific medical therapies for Cushing's Disease • Steroidogenesis inhibitors and surgery of ACTH-secreting NET in ectopic ACTH secretion • Bilateral adrenalectomy in some cases	• Bilateral (rarely unilateral) adrenalectomy	• Therapy of comorbidities • Avoid unnecessary surgery • Glucocorticoid replacement for more severe resistance forms

Abbreviations: ACTH, adrenocorticotropic hormone; ARR, aldosterone/renin ratio; CS, Cushing's syndrome; CT, computed tomography; DHEAS, dehydroepiandrosterone sulfate; DST, dexamethasone suppression test; LOH, loss of heterozygosity; MEN1, multiple endocrine neoplasia type 1; MRI, magnetic resonance imaging; 17-OHP, 17-hydroxyprogesterone; PBMAH, primary bilateral macronodular adrenal hyperplasia; PDE, phosphodiesterase; PPNAD, primary pigmented nodular adrenocortical disease; UFC, urinary free cortisol.
Practical tip/synthesis:

- Table 1 summarizes the differential diagnosis between PBMAH and other etiologies of increased cortisol secretion in patients with bilateral adrenal lesions.
- Differential diagnosis is complicated by the high variability of clinical and hormonal findings depending on the stage of development of the various pathologies and the level of cortisol overproduction.
- Careful revision of images with radiologist expert in adrenal pathologies is recommended.
- Repeated hormonal determinations may be necessary as levels of secretion may be fluctuating.
- Unsuppressed ACTH levels when sustained clear cortisol excess is present (high UFC) supports ACTH-dependent causes (Cushing's disease, ectopic ACTH syndrome, or glucocorticoid resistance syndrome).
- Correlation or lack of correlation between degree of clinical manifestations and cortisol excess is important, for example, to diagnose patients with glucocorticoid resistance syndrome.
- In patients with clinical CS, elevated UFC, suppressed ACTH, no clearly enlarged adrenals, use of exogenous glucocorticoids undisclosed by the patient or of rare ovarian ectopic source of cortisol must be considered.

practice. Unfortunately, as for CT scans, there are very few specific MRI studies on series of bilateral adrenal mass to confirm the value of these criteria in PBMAH.

In PBMAH studies using MRI imaging, the large nodules were isointense relative to muscle with T1-weighted sequences and hyperintense relative to liver with T2-weighted sequences (Fig. 1); in contrast, the nodules in pituitary-dependent macronodular hyperplasia have a signal intensity similar to that of liver on T2-weighted MR images (4) (Table 1).

Volumetric studies

Manual contouring on CT images allows a three-dimensional volumetric modeling of adrenal glands, with a high inter-observer reliability (59, 60), and has been investigated in some adrenal conditions such as primary aldosteronism (95, 96) and primary pigmented nodular adrenocortical disease (PPNAD) (97). In PBMAH, this could be helpful in the decision of which adrenal to remove (see "Therapy of PBMAH"). A single cohort study of PBMAH published to date (98) reports a positive correlation between total adrenal volume and urinary 17-OH-corticosteroids values, but no significant correlation with 24-hour urinary free cortisol (UFC), except for the left adrenal measurement alone. The authors did not assess the performance of volumetric modeling for the surgical decision (see Therapy section).

Metabolic Imaging

NP-59 scintigraphy

131-iodine-norcholesterol scintigraphy, so-called NP-59 scintigraphy, has been developed in the mid-1970s (99, 100) and enables visualization of the radionuclide uptake of adrenocortical masses. NP-59 is useful to evaluate cortisol-secreting disorders (101-103): it can be valuable in the assessment of modest cortisol secretion in adrenal incidentalomas (104-106). It can be used to ascertain atypical cases of bilateral lesions to clarify whether lesions on both sides are of adrenocortical origin. In PBMAH, when an adrenal surgery is considered to control cortisol excess, NP-59 scintigraphy was used to determine which adrenal to remove (see "Surgical Therapy" section) (Fig. 1). NP-59 scintigraphy is no longer available in North America and is performed only in specialized centers elsewhere. NP-59 is costly and time-consuming, as delayed images are performed 3 to 7 days after tracer injection. These limitations obviate general recommendations about this imaging technique.

Fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography

Fluorine-18 deoxyglucose (18-FDG) is the most widely used tracer in positron emission tomography/computed tomography (PET/CT) imaging and has been demonstrated to be reliable in the discrimination of benign from malignant unilateral adrenal masses (107, 108). Due to inter-individual tracer uptake variations, an adrenal to liver maximum standardized uptake value (maxSUV) ratio of 1.45 has been proposed by a prospective study involving 77 patients treated by adrenalectomy (109) and a ratio of 1.29 was further retained by a retrospective study of 66 patients (110). To date, 18-FDG PET/CT accuracy has not been assessed in PBMAH specifically, but some investigators reported high maxSUV values in PBMAH (111, 112), suggesting that cortisol-

secreting adrenal masses have higher FDG uptake than nonsecreting lesions and aldosterone-secreting masses (113). However, a Chinese group recently reported a case of a 51-year-old female patient with PBMAH causing severe CS, with only mild adrenal FDG uptake (114). The knowledge about 18-FDG uptake in PBMAH is still quite limited and does not allow any recommendation on its usefulness; further studies are needed to assess the accuracy of 18-FDG PET/CT to discriminate PBMAH from malignant adrenal masses such as adrenocortical carcinoma (ACC) or bilateral adrenal metastases from extra-adrenal cancer, or in the evaluation of the lateralization of cortisol secretion as suggested in one study (113).

Future Perspectives in Adrenal Imaging

The recent exponential development of artificial intelligence in imaging allows to consider its use in adrenal masses characterization. It first necessitates the segmentation of the adrenal lesions, whether manually or with automation. To date, no automated algorithm for the adrenal segmentation has been released (58). Once the lesions are segmented, radiomics allow the analysis of texture, based on first-order features (quantitative analysis of each voxel) and second-order features (taking into account the spatial location and the relationships between voxels) (115). Recent applications in adrenal imaging promise a future added value of radiomics in the characterization of adrenal lesions, especially in the discrimination between benign and malignant masses (116-122); however, as PBMAH has almost never been associated with malignant progress, the usefulness of radiomics in the evaluation of PBMAH imaging remains to be determined.

Summary of the Recommendations for PBMAH Imaging

A reliable imaging is a key feature in the management in PBMAH, for differential diagnosis and for patient management to decide which adrenal to remove if a unilateral adrenalectomy is considered. Based on the general recommendations about adrenal tumors and incidentaloma, an unenhanced CT should be performed for every patient suspected to present PBMAH. If the unenhanced attenuation of adrenal masses does not exceed 10 HU, no further exploration is formally indicated. For questionable CT images, CT with washout or MRI with chemical shift evaluation may be helpful. 18-FDG injection could be discussed with the limitations that we currently lack large studies of bilateral adrenal mass to confirm the criteria used for benign unilateral adenomas. At this point, there is no recommendation to perform routinely 18-FDG PET/CT in the assessment of PBMAH patients.

The frequency of repeat imaging in the follow-up patients with clearly benign PBMAH with mild cortisol excess not requiring surgical intervention has not been carefully determined. Frequent imaging is not required unless sufficient progression of biochemical cortisol excess and comorbidities indicate intervention, which is in accordance with current recommendations for adrenal incidentalomas (26). Use of MRI, particularly in patients younger than 40 years, should be considered to avoid excessive radiation exposure.

Hormonal Evaluation

Evaluation of Cortisol Secretion

The sensitivity of the various hormonal investigations that can be performed in PBMAH patients will depend on the circumstance of diagnosis. In patients with overt CS who are usually diagnosed after the investigation of specific signs of cortisol excess, 24-hour urinary free cortisol (UFC) or midnight cortisol will be increased in most cases (Fig. 2). On the other hand, patients diagnosed after the investigation of bilateral incidentalomas will more often present with abnormal 1 mg dexamethasone suppression test (DST) than increased UFC or midnight cortisol (Graphical abstract and Fig. 2).

24-hour urinary free cortisol

24-hour urinary free cortisol (UFC) is one of the first-line measurements recommended for the diagnosis of CS (24, 123), with high performance for values above the upper limit of normal (124, 125) (Table 1). The measurement of free cortisol is not modified by the variation of transport proteins, affected by many conditions and treatments such as exogenous estrogens, thus avoiding the pitfalls of interpretation of serum cortisol, measuring both free cortisol and cortisol bound to cortisol binding globulin (CBG). However, UFC is affected by renal function, with a false decrease of its value when creatinine clearance is lower than 60 mL/minute (126). A reliable sample collection is crucial, with appropriate volume and urinary creatinine level, and the instructions must be clearly explained to the patients: the collection begins with the second void of day 1 (the first morning void is discarded), finishes with the first void of day 2, and must include all voids during the day and night between these 2 points (123). Despite the great diagnostic value of UFC in the investigation of a clinical suspicion of overt CS, it is elevated in only 25% to 46% of PBMAH patients (32, 62, 127) and can be normal in up to 63% of patients with mild CS (128), justifying repeated UFC measurements (at least twice) and its use to complement with other methods of assessment of cortisol secretion, such as dexamethasone suppression or midnight salivary cortisol (123).

Salivary and serum cortisol

A high plasma or salivary cortisol value at a time where it should be low, typically at midnight for patients with a conventional day-night cycle (129), is a valuable clue for the diagnosis of CS (123, 130). Midnight salivary cortisol reflects free cortisol and is well correlated with serum cortisol level (131-133); it is reproducible (134), easy to perform by chewing a cotton swab for a few minutes, and shows high accuracy in the detection of CS (125, 130, 135-139), including in mild forms (128), when elevated above the upper limit of normal, usually 4 nmol/L (123). It cannot be used for shift workers and depressive patients since the typical nychthemeral cycle of cortisol is disrupted in these patients (130). To date, midnight salivary cortisol has not been assessed specifically in large series of PBMAH patients. The detection of a flattened serum cortisol daily cycle (ie, measurement of cortisol every 4 hours during 24 hours, beginning at 08:00 AM), without the typical midnight nadir, also provides a good indication for CS with a high sensitivity when midnight serum cortisol is higher than 200 nmol/L (140, 141) but requires the

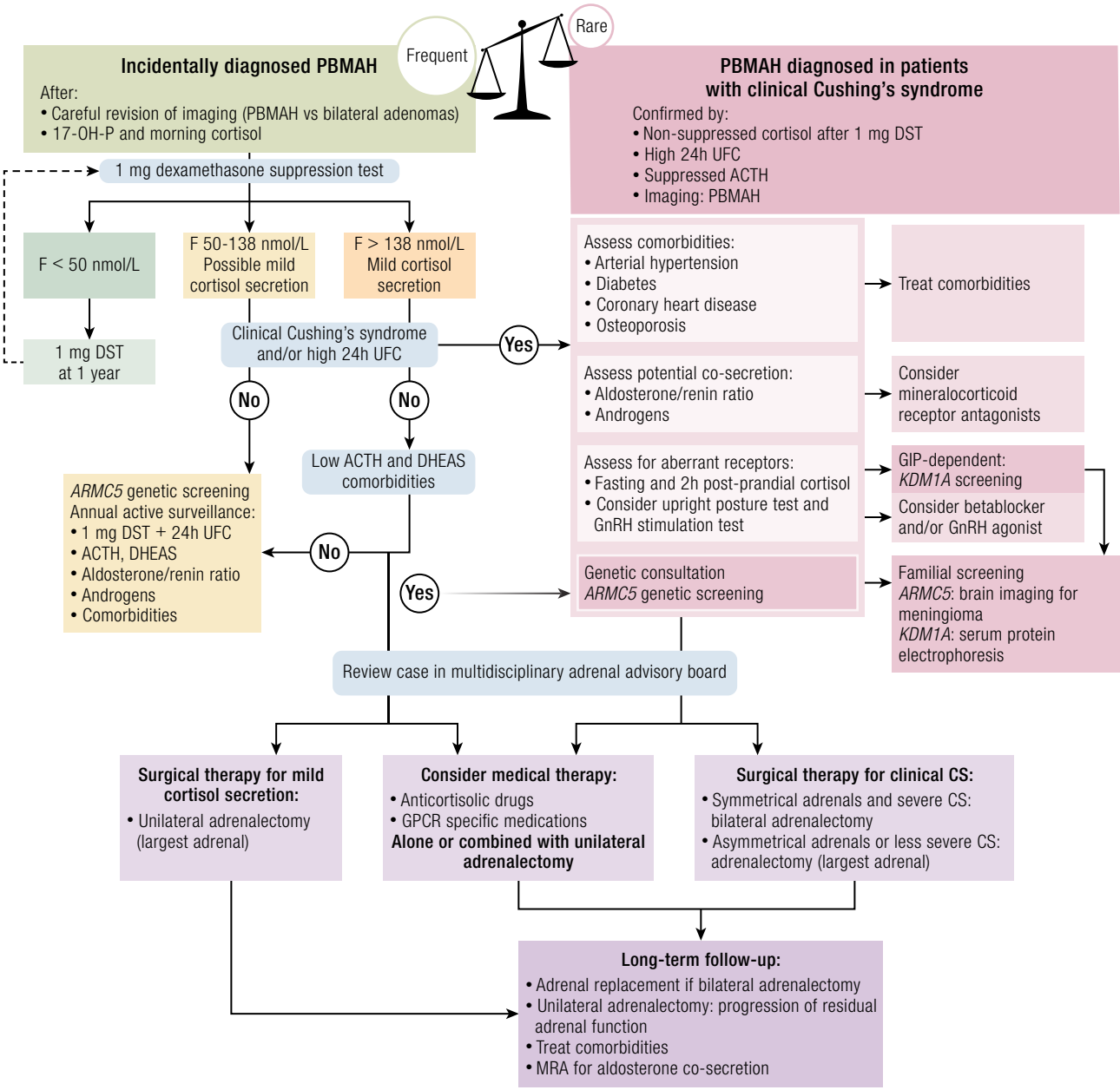


Figure 2. Flowchart of investigation and therapy of PBMAH. Patients with PBMAH can present in a large majority following incidental finding at imaging of bilateral incidentalomas varying in size and degree of mild (majority, left part of flowchart) to clinically apparent cortisol excess (less frequent, right part of flowchart). In rare patients with overt Cushing's syndrome and low plasma ACTH concentrations, adrenal imaging can reveal the presence of PBMAH with large bilateral macronodular adrenals. In incidentally found cases (left part of flowchart), other etiologies of bilateral incidentalomas should have been excluded (64); the 1-mg DST will categorize dysregulated cortisol secretion as either apparently not secreting (<50 nmol/L), possible mild secretion (50–138 nmol/L) or mild secretion (>138 nmol/L). It is essential in patients with PBMAH and abnormal cortisol secretion to evaluate in detail for the presence of cardiometabolic and bone health comorbidities. Elevated urinary free cortisol (UFC) levels, decreased plasma ACTH and DHEAS will indicate medical or surgical cortisol-lowering therapies. In patients without elevated UFC, mild cortisol excess, or comorbidities, active surveillance is recommended with annual clinical and hormonal evaluations. In all cases, genetic screening for germline *ARMC5* pathogenic variants is recommended to patients. Potential GIP-dependent regulation of cortisol secretion should be assessed in all PBMAH patients by determining serum cortisol before and 2 hours after mixed meal; a more than 50% increase would require further detailed hormonal investigation and genetic screening for *KDM1A* pathogenic variants. In all cases with clearly identified cortisol excess and comorbidities, discussion with patients and multidisciplinary adrenal advisory board should be conducted to offer best possible therapeutic recommendations. In rare patients requiring therapy for cortisol excess, the identification of aberrant hormone receptors can lead to specific medical therapy to block the aberrant receptors or their ligands. Otherwise, surgical removal of the largest adrenal gland in patients with mild cortisol excess can restore cortisol secretion within normal limits, particularly when adrenals are asymmetric in size. Bilateral adrenalectomy may be required in patients with very severe cortisol excess and comorbidities. Long-term follow-up of contralateral adrenal and adequate therapy of comorbidities is required.

(continued)

hospitalization of the patients, unlike late-night salivary cortisol, which can be performed in outpatient care. In a recent series involving 352 PBMAH patients, midnight plasma cortisol was superior to 200 nmol/L in only 32% of patients, while among them 75% had a cortisol after 1 mg dexamethasone suppression above 50 nmol/L, suggesting dysregulated cortisol secretion (62), thus questioning the diagnostic performance of midnight plasma cortisol or late-night salivary cortisol in the early phases of this disease.

To date, just a single study has assessed the plasma steroid profiling specifically in PBMAH patients compared with adrenal CS from other causes and healthy controls (142), showing distinctive steroid profiles for PBMAH patients. Similarly, Hsiao et al showed in 2009 a higher urinary excretion of 17-OH-corticosteroids in PBMAH patients than in other adrenal CS causes, while UFC values were comparable (143). The measurements of urinary 17-OH-corticosteroids are no longer widely available and current guidelines do not recommend its use (123) and steroid profiling in blood or urine by liquid chromatography-mass spectrometry (LC-MS) is not clinically available yet.

Adrenocorticotropic hormone

A suppressed plasma ACTH concentration—typically lower than 2 pmol/L—is usually observed in patients with primary adrenal overt CS, including those with PBMAH and clinical CS (Table 1); however, a plasma ACTH above 2 nmol/L is not rare when a mild cortisol excess is not sufficient to completely suppress ACTH. Assay methods for ACTH are highly variable, and in particular can fail to correctly estimate the lowest values; thus, a nonsuppressed ACTH value can be due to a pitfall of assay method and should be interpreted with caution (144).

The demonstration of local paracrine production of ACTH by steroidogenic cells in PBMAH tissues has led to reconsideration of the "ACTH-independent nature" of the condition (13, 14). In large PBMAH series, ACTH is not entirely suppressed in nearly 40% of patients (32, 62); it is unclear whether this is explained by the modest levels of deregulated cortisol secretion in early phases of PBMAH with insufficient suppression of ACTH of pituitary origin or if it reflects the paracrine secretion of ACTH from PBMAH tissues with modest levels detected in circulation. Thus, plasma ACTH levels should be considered carefully in this condition, and always in relation with other biochemical parameters.

Dexamethasone suppression tests

Assessment of the endogenous responses to glucocorticoid negative feedback is informative for CS diagnosis. The 1 mg dexamethasone suppression test (DST) is based on morning cortisol measurement after 1 mg oral dexamethasone at midnight, and it is now considered essential for evaluating patients with suspected endogenous CS. It is considered the most sensitive tool to detect mild cortisol excess in patients with adrenal incidentaloma (26).

In patients with mild cortisol secretion, daily cortisol secretion as measured by 24-hour UFC frequently remains in the normal range, and thus the diagnosis relies mostly on insufficient cortisol suppression. Post 1 mg dexamethasone cortisol concentrations inferior to 50 nmol/L (1.8 µg/dL) is considered normal and sufficient to exclude a mild cortisol secretion (26). This cutoff constitutes an appropriate screening test, with sensitivity over 95% and specificity of 80%, therefore, giving a low false-negative rate (123, 124, 145). Values above this threshold should be interpreted with caution. Plasma cortisol concentrations after 1 mg dexamethasone between 51 and 138 nmol/L (1.9–5.0 µg/dL) are suggestive of mild cortisol excess and should lead clinicians to assess comorbidities to establish an appropriate treatment strategy (Fig. 2 and see "Therapy of PBMAH" section). It is possible to observe false positives of cortisol after 1 mg DST because of variable absorption and variable metabolism of dexamethasone or because of different levels of cortisol binding globulin (CBG); for example, women taking oral contraceptives have increased CBG levels (146)). False negatives are also possible when the elimination of dexamethasone is reduced, as seen in renal or liver failure. It is recommended to measure serum dexamethasone at 8 AM following the 1 mg dose taken at 11 PM the night before, to ensure that adequate concentrations of 140 to 295 ng/dL (3.6 to 7.5 nmol/L) validate the test results (147).

The 2 mg/day–48-hour low-dose DST (also referred as the Liddle's test) has a lower diagnostic accuracy in this disease (123) but can be used in the setting of screening for aberrant receptors in patients with PBMAH. Screening for aberrant receptors can be conducted after ACTH suppression with regular administration of dexamethasone starting 48 hours before and during the period of testing to avoid any effect of ACTH on steroidogenesis (Fig. 2) (148-150).

Because *ARMC5*-mutated index cases display a more severe disease, plasma cortisol levels are less suppressed in patients carrying *ARMC5* pathogenic variants after 1 mg DST. In family members carrying *ARMC5* inherited pathogenic variants,

Figure 2. (Continued)

Practical tip/synthesis:

- Figure 2 presents the recommended flowchart of investigation and therapy of PBMAH.
- Careful review of imaging of patients with adrenal incidentaloma should include the careful search of bilateral lesions, as between 20% and 30% of patients with mild cortisol secretion have bilateral lesions and possible PBMAH.
- PBMAH identified as bilateral incidentaloma is much more frequent than for patients with clinical Cushing's syndrome and PBMAH.
- As illustrated, careful evaluation of degree of cortisol excess and its resulting comorbidities are essential to identify patients requiring medical or surgical therapy.
- All patients with PBMAH should be screened for low fasting and postprandial increase of serum cortisol and GIP-dependent PBMAH leading to screening for germline *KDM1A* pathogenic variants and family and potential associated MGUS/multiple myeloma screening.
- Other PBMAH patients should be screened for potentially associated genetic syndromes and *ARMC5* pathogenic variants, leading to familial screening.
- Surgical therapy is decided based on severity of cortisol excess/comorbidities relative size of each PBMAH adrenal.
- Long-term follow-up is required to monitor and treat residual cortisol deficit, sufficiency or excess, and comorbidities.

screening with 1 mg DST can be proposed as detailed in the "Genetic Aspects" section (32, 123).

ACTH stimulation test

The ACTH stimulation test evaluates adrenal steroidogenic function and is most often used for the diagnosis of adrenal insufficiency (151). In bilateral adrenal incidentalomas, it is also recommended for the diagnosis of CYP21 deficiency (64). In patients with PBMAH, this test is also used after unilateral adrenalectomy to evaluate the remaining adrenal function and to offer glucocorticoid replacement and education in case of insufficient response; however, in some cases of corticotroph deficiency, the test results could still be normal shortly after surgery (152). ACTH stimulation test has been used in PBMAH patients to evaluate the partially defective enzymatic steroidogenesis. Libe et al studied plasma cortisol and 17-hydroxyprogesterone (17-OHP) response after an intravenous (IV) injection of 250 mcg of ACTH 1-24 in 32 patients with PBMAH (153) (Table 1). Administration of ACTH provoked a greater percent rise in serum cortisol and 17-OHP in the subclinical group than in the overt CS group. The findings of elevation of basal and post ACTH 1–24 response of 17-OHP and of relatively reduced response of cortisol to ACTH 1-24 in PBMAH is explained by reduced steroidogenic enzymes (including 21-hydroxylase) and melanocortin type 2 receptor (MC2R) expression in the patients' hyperplastic adrenal lesions (154-156) (Fig. 3 upper panel); however, patients were genotyped and did not exhibit evidence of germline *CYP21A2* mutations (153). More recently, this was confirmed with steroid profiles measured by LC-MS in 73 patients with unilateral or bilateral incidentalomas (157); globally, the steroid increase was higher in patients with bilateral incidentalomas vs unilateral and tended to be higher in patients with incidentalomas than controls. However, the 1 mg DST is more accurate for the assessment of mild hypercortisolism.

Evaluation of Mineralocorticoid Secretion

Since cosecretion of cortisol and aldosterone has been described in PBMAH (158-160) as well as in unilateral adenomas (161-166) and considering that primary aldosteronism due to bilateral adrenal hyperplasia may be a differential diagnosis for PBMAH, the assessment of aldosterone secretion should be performed according to the current guidelines of the Endocrine Society (167) (Fig. 2). This includes the measurement of plasma aldosterone, renin activity or mass and the calculation of aldosterone/renin ratio (ARR), using a diagnostic threshold dependent on the units in which aldosterone and renin are expressed (167, 168). Ideally, the sampling should be performed in standardized conditions: in the morning, at least 2 hours after patient's ambulation, after 5 to 15 minutes of seating position, with normal serum potassium concentration and under a normally salted diet. The interfering antihypertensive therapies should have been discontinued 2 to 6 weeks before assessment, as recommended (167). However, PBMAH patients represent a widely hypertensive population (32, 62, 127, 169) and thus, in some patients, these standardized conditions may not be achieved. A recent study in patients taking antihypertensive drugs suggested that if plasma renin is suppressed, there is no need for medication washout, and the measurement of aldosterone remains interpretable; the medication washout should be considered only in patients with nonsuppressed renin values (170).

Evaluation of Adrenal Androgen and Estrogen Secretion

Only few cases of PBMAH with clinically apparent cortisol and androgens cosecretion (37, 171, 172), or with androgen hypersecretion alone (38) have been reported. In women, androgen cosecretion can lead to virilization (37). Exceptionally, estrogen cosecretion can occur and may be clinically detected in men presenting with gynecomastia, as reported in a PBMAH patient with elevated estrone blood levels (173). To identify these rare forms of PBMAH, the initial biochemical assessment should include gonadotropins and adrenal androgens measurement. Dehydroepiandrosterone sulfate (DHEAS) is often low, but not completely suppressed in PBMAH patients (54, 142), similarly to other benign causes of adrenal adenoma and CS (174, 175), a consequence of ACTH suppression (Fig. 2). It can also be lower in *ARMC5*-mutated PBMAH patients secreting higher amounts of cortisol, compared with patients with wild-type *ARMC5*, who have less severe cortisol overproduction (142).

Steroid Metabolomics

Urine steroid metabolomics refers to the study of urine steroid metabolite by mass spectrometry. Often associated with machine-learning data analysis, this measurement of 24-hour steroid metabolite excretion is made using gas or LC-MS and can detect many selected steroid metabolites (glucocorticoid, mineralocorticoid, and androgen metabolites).

The application of urine steroid metabolomics in adrenal CS could be a promising tool, as the use of steroid metabolomes may be able to identify different degrees in severity of adrenal CS and eventually different genetic etiology of adrenal cortisol excess (176).

For example, using a steroid panel in a group of 222 patients tested for CS identified 11-deoxycortisol, 21-deoxycortisol, 11-deoxycorticosterone, corticosterone, and cortisol as the main steroids increased in patients with CS compared with controls (177). This study was able to show that a combination of 7 steroids had a similar receiver operating characteristic curve (ROC) as salivary or urinary free cortisol with 87% sensitivity and 89% specificity. However, it is important to highlight that DST still yielded the highest sensitivity and specificity out of all the tests (100% and 97%, respectively), including the metabolome profile. Overall, various steroid metabolites seem to display distinctive patterns depending on the different subtypes of CS: a combination of 10 plasma steroids (11-deoxycortisol, cortisol, cortisone, corticosterone, 11-deoxycorticosterone, androstenedione, 18-oxocortisol, DHEA, DHEAS, and aldosterone) was able to discriminate 3 subtypes of CS (adrenal (n = 21), pituitary (n = 51), and ectopic (n = 12), but the rate of misclassified patients was still lower when using a combination of routine tests (5.8% vs 9.5% when using the 10-steroid panel) (177).

There remains some overlap between studies on other metabolites; therefore, it is unlikely that, in this setting, metabolome studies could offer a single-test replacement (176-178). Given the different excretion patterns of steroids depending on etiology, the metabolome can be helpful to classify adrenal incidentalomas either as benign or malignant (179). A pilot study using 32 metabolites offered an area under the receiver operating characteristic curve of 0.965 (+/− 0.054); however, for reproducibility, profiling of 10

steroids by liquid chromatography/tandem mass spectrometry (LC-MS/MS) was then selected for a larger study that included 2017 patients (75): alone, urine steroid metabolomics had a positive predictive value (PPV) of 34.6% (95% CI, 28.6-41.0). This was higher than imaging characteristics (density and washout, MRI signal, PET uptake) and size (PPV of 19.7% [95% CI, 16.2–23.5]). Interestingly, the combination of imaging characteristics, tumor diameter, and urine steroid metabolomics displayed the high PPV of 76.4% (95% CI, 67.2-84.1). Using multisteroid profiling in the EURINE-ACT study, which included 22% of patients with bilateral lesions, possibly mostly with early PBMAH, increased urinary glucocorticoid excretion progressively suppressed plasma ACTH, serum DHEAS, and urinary androgens (27); it remains to be confirmed whether PBMAH cases can be distinguished specifically by this approach from other etiologies of primary adrenal lesions with mild or overt cortisol excess.

A study of a combination of 9 steroids assayed in plasma by mass spectrometry suggest indeed qualitative differences in PBMAH patients by comparison with other adrenal causes of CS (142).

Plasma DHEA/DHEAS levels are usually more elevated in patients with Cushing's disease compared with patients with adrenal CS, and DHEAS levels have shown a linear correlation with plasma ACTH levels (23, 177, 180). The measurement of DHEA/DHEAS reflects the chronic ACTH stimulation and can assist, although imperfectly, subtyping CS etiologies (Table 1). This difference in androgen levels is also found in urine steroid metabolomics (178). Interestingly, DHEAS has also been proposed as a diagnostic tool for mild hypercortisolism in adrenal incidentaloma, but there are discrepancies between studies that currently limit its use for the diagnosis of mildly dysregulated cortisol secretion (23, 181, 182). However, the specific accuracy of DHEAS in PBMAH has not been demonstrated and should be addressed by further studies.

Differential Diagnosis of PBMAH With Other Etiologies of Cortisol-Secreting Bilateral Adrenal Lesions

PBMAH can be confused with 3 main differential diagnoses, as summarized in Table 1. Chronic ACTH stimulation in ACTH-dependent CS from Cushing's disease or from a benign neuroendocrine tumor secreting ACTH ectopically can result in adrenal nodular hyperplasia and thus can be misdiagnosed for PBMAH. Nonsuppressed values of ACTH and DHEAS, potential ACTH and cortisol inhibition by dexamethasone, or their stimulation by desmopressin and/or CRH allow their distinction; in addition, congruent pituitary MRI in the case of Cushing's disease or the visualization of endocrine tumor consistent with ectopic CS are valuable tools to differentiate these conditions. Another challenging diagnosis is partial glucocorticoid resistance consecutive to inactivating mutations of *NR3C1* (183), encoding the glucocorticoid receptor. The resulting lack of negative feedback leads to chronic increased ACTH levels stimulating the development of bilateral nodular adrenal hyperplasia. It is characterized by high ACTH and UFC levels, without any clinical sign of overt Cushing's syndrome, but can be possibly accompanied by hypertension, hypokalemia, and hirsutism resulting from increased production of mineralocorticoid precursors and adrenal androgens. The most challenging differential diagnosis is bilateral adrenal adenomas, which can seriously mimic PBMAH both in terms of biological and radiological presentation. However, a careful review of imaging should enable visualization of an internodular hyperplasia (yet presumably inconstant) in PBMAH, which is absent in bilateral adenomas. In addition, a high 17-OHP value provides an argument for PBMAH against bilateral adenomas, in which 17-OHP is expected to be normal.

Aberrant Regulation of Steroidogenesis

In Vitro and In Vivo Initial Concept of Ectopic/Aberrant Hormone Receptors in CS

The first study revealing the presence of multiple specific hormone receptors in adrenal tumors was reported by Schorr et al in 1971. They studied the implication of adenyl cyclase in regulating ACTH action in normal rat adrenal glands and in corticosterone-producing rat adrenocortical carcinoma tissue membranes. In both normal and tumoral tissues, adenyl cyclase was stimulated by ACTH (184, 185); however, in tumoral tissue, in contrast to normal adrenal membranes, adenyl cyclase was also stimulated by epinephrine, norepinephrine, thyrotropin (thyroid-stimulating hormone), luteinizing hormone (LH), and follicle-stimulating hormone, in a dose-dependent manner, but not by angiotensin II, vasopressin, glucagon, insulin, growth hormone, parathyroid hormone, or calcitonin (185). The authors suggested that this abnormal response was secondary to the expression of ectopic hormone receptors in adrenal tumors.

The first in vivo identification of the pathophysiological role of ectopic hormone receptors in humans resulted from the investigation by Hamet et al of a patient with unilateral cortisol-secreting adrenal adenoma with unusual low fasting morning plasma levels that increased only after meals (186). The authors suggested the term food-dependent CS, but the precise mechanism was not yet identified. Five years later, in 1992, glucose-dependent insulinotropic peptide (GIP)-dependent CS was described simultaneously, in 2 patients with PBMAH, by Lacroix et al (187) and Reznik et al (188). The ACTH-independent aberrant responsiveness of cortisol secretion following physiological postprandial secretion of GIP was identified (187, 188). Later, the ectopic expression of nonmutated GIP receptors (GIPR) in unilateral adenoma or PBMAH tissues of patients with GIP-dependent CS was demonstrated by several groups (189-192). The molecular pathogenesis was partially elucidated only in 2017 by Lecoq et al, with the demonstration of somatic duplication and rearrangements in chromosome region 19q13.32 containing the GIPR locus in 2 GIP-dependent adrenocortical adenomas but not in PBMAH (192). Very recently, the genetic cause of GIP-dependent PBMAH with CS was elucidated with the identification of biallelic germline and somatic pathogenic variants in the *KDM1A* gene in most patients (55, 56) (see "Genetic Aspects" section) (Fig. 3 lower panel).

Aberrant G Protein–Coupled Hormone Receptors in Adrenal CS

Following the initial description of the concept of ectopic GPCR as potential regulators of cortisol secretion in adrenal hyperplasia and tumors, systematic clinical exploration of expression of various aberrant receptors in patients with CS in both PBMAH and unilateral adrenal adenoma was conducted

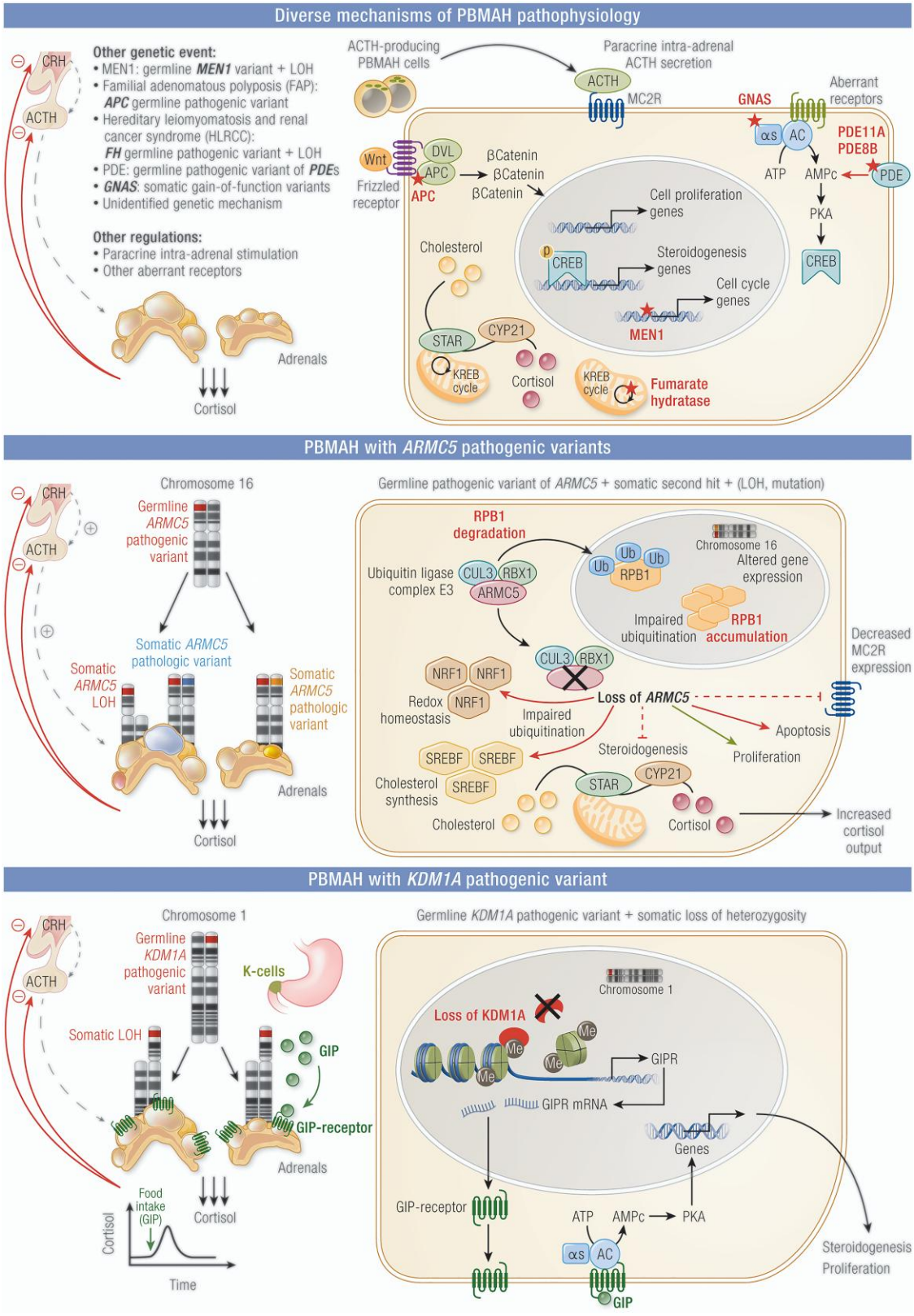


Figure 3. Diverse molecular and genetic mechanisms of primary bilateral macronodular adrenal hyperplasia (PBMAH). In PBMAH, the progressive development of bilateral adrenal hyperplasia and macronodules can be heterogeneous and result in variable degree of cortisol excess. PBMAH can rarely be associated with various syndromic genetic etiologies, including MEN1, FAP, HLRCC, or germline or somatic variants of other genes such as *PDE*, *GNAS* (upper panel). The resulting altered proteins can interfere with the normal adrenocortical cell regulation by ACTH binding to its MC2R and signaling on the GNAS-PKA-CREB regulation of steroidogenesis; when cortisol secretion becomes sufficiently increased the negative feedback of cortisol inhibits CRH and pituitary ACTH secretion. Despite pituitary ACTH suppression, the ectopic expression of ligands (including paracrine production of ACTH in PMAH cells) and several G protein–coupled receptors underlies the aberrant regulation of steroidogenesis. It is presumed that other yet-unidentified genetic

(continued)

by several groups (193). These studies extended our knowledge on the presence and the roles of not only of GIPR, but of various other G protein–coupled aberrant hormone receptors in primary adrenal CS patients. Abnormal regulation of cortisol can result from the aberrant adrenal expression of several hormone receptors, particularly GPCRs and their ligands. We will focus here on studies in PBMAH (143, 153, 194, 195) (Fig. 3, upper panel).

GIP receptor in PBMAH with CS

Patients with GIP-dependent and overt CS often present a specific phenotype that is characterized by low fasting plasma cortisol levels that increase following meal intake; when ACTH is suppressed by sufficient chronic cortisol excess, and GIP levels are low fasting, there is no stimulus for cortisol secretion. Cortisol levels increase following physiological GIP secretion by entero-endocrine K cells located in the duodenum and proximal jejunum following a mixed meal, oral glucose (but not intravenous glucose), lipid-rich and protein-rich meals (187) (Fig. 3, lower panel). The cortisol response was positively correlated with GIP levels (187). Moreover, somatostatin analogs that inhibit gastrointestinal hormones can decrease cortisol response to various meals (187, 188, 196). As simultaneous aberrant adrenal expression of GIPR and luteinizing hormone/chorionic gonadotropin receptor (LHCGR) may occur in PBMAH tissues in the same patient, then cortisol levels may not be as low during fasting period, as several stimuli may be functional in the same tissue (197). GIP-dependent CS has been reported both in patients with PBMAH (55, 56, 72, 77, 155, 171, 187, 188, 190, 196-205) (Table 2) and in patients with unilateral adenomas (186, 189, 191, 192, 206, 207). Overall, at least 39 cases of GIP-dependent CS were described in PBMAH patients, occurring mainly in women (Table 2).

Adrenal nodules may develop asynchronously in rare PBMAH cases as described by N'Diaye et al, in a 33-year-old woman with GIP-responsive CS (200). She presented with 2 predominant right adrenal macronodules of 2.5 and 3 cm that were confirmed by pathology to be PBMAH with internodular hyperplasia also expressing ectopic GIPR and a small 0.8×0.6 cm contralateral left nodule at imaging that maintained mild cortisol GIP-responsive secretion after a right adrenalectomy (200).

GIP-dependent cortisol production results from the ectopic expression of nonmutated GIPR in adrenocortical zona fasciculata (72, 189-191), which can be detected in the early phases of adrenal hyperplasia (72, 200). Groussin et al studied GIPR

Figure 3. (Continued)
alterations are responsible for the bilateral nature of adrenal proliferation and nodular hyperplasia. The most frequent germline inactivating variants identified in 25% of apparently sporadic and 80% of familial forms of PBMAH are present in the Armadillo Repeat Containing 5 (*ARMC5*) gene located on chromosome 16p11.2, (middle panel). Systematically, the germline event is combined with somatic copy-neutral loss of heterozygosity (LOH) of chromosome 16p or point mutations (missense, nonsense, frameshift, or splicing events) of *ARMC5*, compatible with a tumor suppressor gene model; each adrenal nodule may harbor unique distinct somatic mutation (middle panel). Inactivation of ARMC5 results in decreased apoptosis, cell proliferation, and reduction in MC2R and several steroidogenic enzymes, explaining relative inefficiency of steroidogenesis where increased cortisol results from large increase in cell mass rather than overactive individual cells. ARMC5 belongs to a RING Ubiquitin Ligase E3 complex which also includes CUL3 and RBX1 and regulates RPB1 degradation, the largest subunit of RNA polymerase II (PolII). Loss of ARMC5 leads to accumulation of RBP1 and enlarged PolII pool that regulate expression of several genes leading to cell proliferation and altered steroidogenesis. In the cytosol, ARMC5 forms other E3 ubiquitin ligase complexes binding either to sterol regulatory element-binding transcription factor (SREBF) regulating cholesterol synthesis/steroidogenesis and nuclear respiratory factor 1 (NRF1), to regulate redox homeostasis. Germline pathogenic variants of the lysine demethylase type 1A (*KDM1A)* gene was identified in patients with sporadic or familial GIP-dependent PBMAH and CS (lower panel). An additional 2-hit somatic deletion of the chromosome 1p region including the *KDM1A* locus in the adrenal tissues, results in the loss of KDM1A expression in the adrenal lesions. Loss of KDM1A is believed to lead to persistent histone methylation, increased expression of GIPR in PBMAH cells. In such patients, the physiological increase of GIP secretion by intestinal K cells leads to ectopic GIPR activation and postprandial increase of cortisol; when cortisol production becomes sufficiently increased, the suppression of CRH and ACTH can result in reduced serum cortisol concentrations in the fasting state (lower panel).

Practical tip/synthesis:

- Figure 3 summarizes the various regulatory molecular and genetic mechanisms leading to PBMAH development.
- Similar to other causes of primary adrenal etiologies of Cushing's syndrome, cortisol excess suppresses CRH and pituitary ACTH (upper panel).
- In a high proportion of PBMAH tissues, ectopic or eutopic hormone receptors regulate cortisol secretion by interacting with the G-protein, adenyl cyclase, and PKA pathway normally mediating ACTH regulation of steroidogenesis.
- In addition, ectopic expression of ACTH occurs in some PBMAH cells and contributes to regulation of steroidogenesis.
- In several genetic syndromes (MEN1, APC, HLRCC), patients can rarely develop PBMAH as their altered proteins or effectors (menin, B-catenin, fumarate hydratase) can also interfere with the cAMP, PKA pathway (upper panel).
- In approximately 20% to 25% of apparently sporadic and 80 of familial cases of PBMAH germline pathogenic variants of *ARMC5* are identified (-middle panel)
- Germline *ARMC5* mutations result in adrenal hyperplasia and second somatic events or loss of heterozygosity in chromosome 16 are required to develop adrenocortical macronodules and PBMAH.
- ARMC5 is an adapter of a RING Ubiquitin Ligase E3 complex and can bind different substrates, such as RPB1, the largest subunit of RNA polymerase II, SREBF which are transcriptional activators involved in cholesterol synthesis and NRF1, a transcriptional regulator involved in redox homeostasis.
- ARMC5 inactivation leads to reduced apoptosis, cell proliferation, and relative deficiency of MC2R and several steroidogenic enzymes, explaining the relative inefficiency of steroidogenesis and elevation of 17-OH progesterone following partial deficiency of 21 hydroxylase.
- Increased cortisol levels result from the large number of adrenal cells with relatively reduced individual cell steroidogenic capacity.
- In patients with GIP-dependent PBMAH germline of lysine specific demethylase 1A (*KDM1A*) and somatic loss of its 1p chromosomal location underlie development of this disease (lower panel).
- Carriers of germline *KDM1A* pathogenic variants do not develop PBMAH unless a second somatic hit in *KDM1A* occurs in adrenocortical cells.
- Loss of the KDM1A protein alters the methylation of histones resulting in ectopic expression of GIPR in adrenocortical cells and progressive development of adrenal nodular hyperplasia.
- In the fasting state, as pituitary ACTH is suppressed and GIP is low, patients may have low cortisol levels.
- After physiological postprandial increase of GIP, cortisol increases abnormally as the result of activation of G-protein/adenyl cyclase/PKA pathway by activation of ectopic GIPR.
- *KDM1A* mutation can also result in combined foci of myelolipoma mixed with PBMAH tissue.
- Patients affected by *KDM1A* genetic variation can also develop MGUS or multiple myeloma.

Table 2. Cases of patients with food/GIP-dependent PBMAH with overt Cushing's syndrome or mild cortisol secretion

		Sex	Age	CS or MCS	Aberrant response *in vivo*	Other aberrant response *in vivo*	Apparently sporadic (S) or familial (F)	Medical treatment	*Germline DNA KDM1A HGVS nomenclature DNA*	*KDM1A HGVS consequence*	*ACMG classification*	*Tumoral DNA*
1	Lacroix, 1992 (187)	F	48	CS	Mixed meals, oral glucose, GIP	No	S	No				
2	Reznik, 1992 (188)	F	49	CS	Mixed meals, oral glucose, GIP	No	S	Octreotide: transient				
3	Lebrethon, 1998 (198)	F	46	CS	Mixed Meals, GIP	No	Carney	No				
4	Pralong, 1999 (199)	F	36	CS	Mixed meal	No	S	No				
5	N'Diaye, 1999 (200) (patient 6 (192) patient 10 (55)	F	33	CS	Mixed meals, GIP	No	S	No	c.811C > T[a]	(p.Arg271Ter)	Stop gained-Pathogenic	1pLOH
6	Croughs, 2000 (201)	F	60	CS	Meals, Oral glucose	No	S	Octreotide: partial remission 5 months				
7	Gerl, 2000 (202)	F	40	CS	Mixed meals, oral glucose	No	S	No				
8	Doppman, 2000 patient 12 (77), Bourdeau, 2004 (208) (patient 9 (192) patient 7 (55)	F	43	CS	Mixed meals	Partial vasopressin	S	No	c2441_2445del	(p.Gln815AspfsTer14)	Frameshift-pathogenic	1pLOH

(*continued*)

Table 2. Continued

		Sex	Age	CS or MCS	Aberrant response *in vivo*	Other aberrant response *in vivo*	Apparently sporadic (S) or familial (F)	Medical treatment	*Germline DNA KDM1A HGVS nomenclature DNA*	*KDM1A HGVS consequence*	*ACMG classification*	*Tumoral DNA*
9	Groussin, 2002 (203)	F	48	CS	Meals, oral glucose	No	S	No				
10	Groussin, 2002 (203)	F	48	CS	Meals, oral glucose	No	S	No				
11	Bourdeau, 2004 (208)	M	24	CS	Mixed meal	No	S	No				
12	Bertherat, 2005 (197), Louiset 2006 (209) (patient 10 (192) patient 11 (55)	F	45	CS	Mixed meal	GnRH-TRH	S	No	c.386delA[a]	(p.Asn129ThrfsTer80)	Frameshift-pathogenic	1pLOH
13	Bertherat, 2005 (197), Louiset 2006 (209) (patient 11 (192) Patient 12 (55)	F	54	CS	Mixed meal	GnRH/ hCG-TRH	S	No	c.386delA[a]	(p.Asn129ThrfsTer80)	Frameshift-pathogenic	No event detected
14	Messidoro, 2009 (204)	F	57	CS	Mixed meal, GIP	No	S	No				
15	Preumont, 2011 (196) (patient 7 (192) (patient 4 (55)	F	55	CS	Mixed meal, oral glucose	Terlipressin; glucagon	S	Octreotide 6 months/ pasireotide 3 months transient	c.1309G > T[a]	(p.Glu437Ter)	Stop gained-pathogenic	1pLOH
16	Karapanou, 2013 (205) patient 17 (55)	F	42	CS	Oral glucose and Mixed meal	No	S	Long-acting somatostatin analogue octreotide long-acting release (LAR) for 3 months	c.1327C > T[a]	(p.Gln443Ter)	Stop gained-pathogenic	Not available

(*continued*)

Table 2. Continued

		Sex	Age	CS or MCS	Aberrant response *in vivo*	Other aberrant response *in vivo*	Apparently sporadic (S) or familial (F)	Medical treatment	*Germline DNA KDM1A HGVS nomenclature DNA*	*KDM1A HGVS consequence*	*ACMG classification*	*Tumoral DNA*
17	Albiger, 2015 (210) Patient 16 (55)	F	35	CS + androgens	Mixed meal, oral glucose	hCG	S	Octreotide: partial	c.838G > C[a]	(p.Gly280Arg)	Missense-Likely pathogenic	Not available
18	Albiger, 2015 (210)	F	55	CS	Mixed meal	No	S	No				
19	Lecoq, 2017 patient 8 (192) (patient 5 (55)	M	35	CS	Mixed meal		S	No	c.1737_1738insGA[a]	(p.Asp580GlufsTer11)	Frameshift-pathogenic	1pLOH
20	Lecoq, 2017 (patient 12) (192) (patient 6 (55)	F	43	CS	Mixed meal		S	No	c.2361T > G[a]	(p.Tyr787Ter)	Stop gained-pathogenic	1pLOH
21	Lecoq, 2017 (patient 13) (192) Patient 2 (55)	F	42	CS	Mixed meal		F	No	c.352-1G > A[a]	Intronic	Splice acceptor-Pathogenic	1pLOH
22	Lecoq, 2017 (patient 14) (192)	F	46	MCS	Mixed meal		S	No				1pLOH
23	Lecoq, 2017 (patient 15) (192)	F	25	MCS	Mixed meal		S *MEN1*	No				1pLOH
24	Larose, 2019 (72) (patient 9 (55)	F	61	CS	Mixed meal, OGTT, GIP	hLH IV	S	Pasireotide transient	c.811C > T[a]	(p.Arg271Ter)	Stop gained-Pathogenic	No event detected
25	Chasseloup, 2021 (patient 1) (55)	M	54	CS	Mixed meal		F	No	c.1848dupG[a]	(p.Val617GLyfsTer9)	Frameshift Pathogenic	1pLOH

(*continued*)

Table 2. Continued

		Sex	Age	CS or MCS	Aberrant response *in vivo*	Other aberrant response *in vivo*	Apparently sporadic (S) or familial (F)	Medical treatment	*Germline DNA KDM1A HGVS nomenclature DNA*	*KDM1A HGVS consequence*	*ACMG classification*	*Tumoral DNA*
26	Chasseloup, 2021(55) (patient 3) Vaczlavik, 2022 (SP1) (56)	F	47	CS	Mixed meal		F	No	c.352-1G > A[a]	Intronic	Splice acceptor-Pathogenic	1p LOH
27	Chasseloup, 2021 (patient 8) (55)	F	30	CS	Mixed meal		S	No	c.952C > T[a]	(p.Gln318Ter)	Stop gained-pathogenic	1pLOH
28	Chasseloup, 2021 (Patient 13) (55)	F	42	CS	Mixed meal		S	No	c.903_905delTAA[a]	(p.Ile304del)	In frame deletion Likely pathogenic	Not available
29	Chasseloup, 2021 (Patient 14) (55)	F	41	CS	Mixed meal		S	No	c.925T > C[a]	(p.Ser309Pro)	Missense-Likely pathogenic	Not available
30	Chasseloup, 2021 (patient 15 (55)	F	34	CS	Mixed meal		S	No	c.1545_1548delCAAG	p.(Cys515TrpfsTer6)		Not available
31	Vaczlavik, 2022 P6 (56)	F	45	CS	Mixed meal		S	No	c.1796-1_1808dup GGATGTGAAGTGAT[b]	(p.Ile603MetfsTer5)	Frameshift insertion Pathogenic	1p LOH
32	Vaczlavik, 2022 P8 (56)	F	55	CS	Mixed meal	posture	S	No	No germline *KDM1A* mutation	No germline *KDM1A* mutation	No germline *KDM1A* mutation	1p LOH
33	Vaczlavik, 2022 P18 (56)	F	42	CS	Mixed meal		F	No	c.1774_1775insG[b]	(p.Gln592ArgfsTer10)	Frameshift insertion Pathogenic	1p LOH
34	Vaczlavik, 2022 P24 (56)	F	64	CS	Mixed meal		S	No	c.386delA[b]	(p.Asn129ThrfsTer60)	Frameshift deletion Pathogenic	1p LOH
35	Vaczlavik, 2022 P29 (56)	F	42	CS	Mixed meal		S	No	c.2155delA[b]	(p.Ser719ValfsTer4)	Frameshift deletion Pathogenic	1p LOH
36	Vaczlavik, 2022 p36 (56)	F	31	CS	Mixed meal	GnRH	S	No	c.1912C > T[b]	(p.Gln638Ter)	Stop gained Pathogenic	1p LOH
37	Vaczlavik, 2022 SP2 (56)	F	40	CS	Mixed meal		S	No	c.1771C > T[b]	(p.Arg591Ter)	Stop gained Likely pathogenic	1p LOH

(*continued*)

Table 2. Continued

	Sex	Age	CS or MCS	Aberrant response *in vivo*	Other aberrant response *in vivo*	Apparently sporadic (S) or familial (F)	Medical treatment	*Germline DNA KDM1A HGVS nomenclature DNA*	*KDM1A HGVS consequence*	*ACMG classification*	*Tumoral DNA*
38 Vaczlavik, 2022 SP3 (56)	F	32	CS	Mixed meal		S	No	c.2391_2393delCTT[b]	(p.Phe798del)	In frame deletion Pathogenic	1p LOH
39 Vaczlavik, 2022 Sp4 (56)	F	59	CS	Mixed meal		S	No	c.2117_2125del[b]	(p.Leu706_Gly709delinsArg)	In frame deletion-insertion Pathogenic	1p LOH

Includes only the aberrant peak cortisol increase >50% above baseline values.
Abbreviations: ACMG, American College of Medical Genetics and Genomics; CS, Cushing's syndrome; GIP, glucose-dependent insulinotropic peptide; HGVS, Human Genome Variation Society; MCS, mild cortisol secretion.
[a]NM_001009999.3.
[b]NM_015013.4.
Practical tip/synthesis:

- Table 2 summarizes the published cases of patients with GIP-dependent PBMAH.
- In such patients, cortisol levels may be low during fasting period with and increase after food intake.
- Rarely, other aberrant receptors such as LHCG receptor can be also expressed in the same adrenal lesions masking fasting low cortisol levels.
- No *ARMC5* pathogenic variant were found in any patient with GIP-dependent PBMAH.
- Almost all cases of GIP-dependent PBMAH studied were affected by dual germline and somatic alterations of the *KDM1A* gene and its 1p chromosomal region.
- Carriers of *KDM1A* pathogenic variants can also present with adrenal myelolipoma within their PBMAH, MGUS, or multiple myeloma.
- Genetic screening of first-degree relatives of affected GIP-dependent PBMAH patients should be offered.

expression by reverse transcriptase polymerase chain reaction (PCR) in 16 adrenocortical adenomas, 14 adrenocortical cancers, and 8 PBMAH tissues and found GIPR overexpression in 4/8 PBMAH, 1/16 adenoma, and none in adrenocortical cancers (203). Interestingly, the fasting plasma cortisol levels were above 276 nmol/L in all patients except in 2 patients, the first with adenoma and the second with PBMAH; in both cases, adrenal GIPR was overexpressed. Moreover, 3 additional PBMAH cases showing increased adrenal expression of GIPR had fasting plasma cortisol levels above 276 nmol/L, reinforcing that GIPR expression may not always be associated with a low fasting plasma cortisol level (203). In a Brazilian study of 55 patients with adrenocortical tumors (38 adenomas, 17 carcinomas), GIPR mRNA levels determined by real-time PCR were higher in adrenocortical carcinomas than in adenomas from both pediatric and adult groups (207). Coexistence of adrenal myelolipoma and PBMAH may be found in GIP-dependent CS; however, in contrast to PBMAH tissue, myelolipoma do not express GIPR (72).

Activation of GIPR by GIP increases cyclic adenosine monophosphate (cAMP) production, DNA synthesis, and steroidogenic action in a dose-dependent manner in GIP-dependent cortisol-secreting tissues (191, 198). Bovine adrenal cells transfected with the GIPR receptor and transplanted under the renal capsule of adrenalectomized immunodeficient mice led to the development of an enlarged and hyperproliferative adenomatous adrenocortical tissue (211). This tissue was ACTH independent, but GIP-dependent and secreted cortisol, inducing hypercortisolism (211). These data provided important insight demonstrating that GIPR overexpression itself was sufficient for adrenocortical tumor development. No mutations of coding or promoter regions of *GIPR* were found in tissues of patients with GIP-dependent CS; transcription factors (Sp1 and Sp3) necessary for *GIPR* expression did not present specific abnormalities (154, 212). Two studies evaluated the expression profiling of GIP-dependent PBMAH tissues and found altered expression of probe sets related to the WNT pathway, cellular proliferation, and adhesion (208, 213).

In adrenal tissues from 14 patients with GIP-dependent adrenal CS and 1 patient with GIP-dependent aldosteronism, GIPR expression in all 3 unilateral adenomas and 11 PBMAH samples occurred through transcriptional activation of a single allele of the *GIPR* gene (192). This monoallelic pattern of adrenal GIPR expression remains unexplained but could be related to transcriptional bursting because of stochastic fluctuation between transcriptionally active and inactive states (214). No abnormality was detected in proximal *GIPR* promoter methylation, but somatic duplications in chromosome region 19q13.32 containing the *GIPR* locus in the adrenocortical lesions were found in resected adrenal from 3 patients. In 2 adenoma samples, the duplicated 19q13.32 region was rearranged with other chromosome regions, whereas a single tissue sample with PBMAH had 19q duplication only. The juxtaposition of *cis*-acting regulatory sequences such as glucocorticoid response elements in the newly identified genomic environment was driving abnormal expression of the translocated *GIPR* allele in cells of one adenoma (192). No germline *ARMC5* mutations have been identified in GIP-dependent CS (55, 56, 215). However, very recently germline pathogenic variants were identified in the *KDM1A* gene to be the main genetic events in

GIP-dependent CS secondary to PBMAH but not adenomas (see below and Fig. 3 lower panel) (55, 56).

Vasopressin receptors in PBMAH

Vasopressin regulation of steroidogenesis in PBMAH was revealed by an ACTH-independent increase of cortisol levels in patients following upright posture or exogenous vasopressin agonists (48, 193-195, 197, 216-218) (Table 3). In addition, fluctuations of endogenous levels of vasopressin using water and hypertonic sodium loading infusion resulted in parallel changes of cortisol (194). Vasopressin agonists (arginine- or lysine-vasopressin or terlipressin) are the most prevalent hormonal stimuli triggering aberrant cortisol responses in vivo in patients with PBMAH and CS (15, 48, 195, 197, 218-230) (Table 3). Vasopressin-dependent cortisol secretion is mainly regulated by the adrenal arginine-vasopressin (AVP) receptor type I (AVPR1), which is expressed at higher or similar levels to controls (48, 50-52, 217, 222-229). In contrast, in some PBMAH tissues with modest response to vasopressin, no AVPR1 was expressed and in vitro response to vasopressin appeared to be mediated by AVPR2 (228).

Oral administration of the V1-AVPR antagonist OPC-21268 for 8 days decreased urinary free cortisol levels in a 49-year-old man with PBMAH (217). Although it did not affect plasma cortisol levels in vivo, cortisol secretion was completely suppressed by OPC-21268 in vitro in this case (217). Dispersed cells from a number of PBMAH tissues were stimulated by vasopressin (48, 50, 51, 195, 197, 217, 222, 224, 225, 227).

Among 33 patients with CS, there were 42% of responders to the vasopressin-loading test, including 8 of 10 (80%) PBMAH patients and 6 of 23 (26%) patients with unilateral adenomas (229). Aberrant vasopressin cortisol response was reported in PBMAH patients with modest cortisol secretion as well (31, 230). Interestingly, Suzuki et al observed that patients with vasopressin response had lower plasma cortisol than nonresponders at morning and midnight time, and following both overnight dexamethasone suppression of 1 and 8 mg as well (229).

The mechanism of exaggerated steroidogenic response mediated by vasopressin remains unclear, but it is hypothesized that adrenal eutopic AVPR1 may have increased activity within the pathological adrenal tissues (31, 50, 195, 217, 222, 223, 225, 226, 228, 230). AVPR1 protein was similarly expressed, as assessed by immunohistochemistry, in spongiocytes and compact cells of PBMAH tissues with or without *ARMC5* pathogenic variants (156) (Table 3).

A PBMAH patient presented with orthostatic hypotension, despite increased cortisol response to vasopressin; a prolonged vascular vasoconstrictive response to AVP suggested abnormal AVPR1 responsiveness in several tissues (222). The ectopic expression of mRNA for AVPR2 and AVPR3 was found in adrenal tissues from PBMAH patients but confirmation of protein expression was infrequent (50, 52, 223, 226, 228) (Table 4). Its significance is unclear as *in vivo* response to desmopressin was not performed (50, 223, 226) and desmopressin did not stimulate cortisol secretion in vitro (228).

Serotonin receptors in adrenal CS

In the human adrenal gland, serotonin (5-hydroxytryptamine; 5-HT) is released by subcapsular mast cells that stimulate corticosteroid production through a paracrine manner involving 5-HT receptor type 4 (HTR4) located in the zona glomerulosa. 5-HT is more efficient to stimulate aldosterone than cortisol secretion in vitro and administration of HTR4 receptor agonists normally leads to a physiological increase in plasma aldosterone levels without any change in plasma cortisol concentrations (9). In 1999, Lacroix et al described a 63-year-old woman with LH/hCG responsive cortisol-secreting PBMAH where the HTR4 agonists cisapride and metoclopramide produced, respectively, a 4.8- and 2.6-fold plasma cortisol elevation above baseline values (237). Aberrant response to HTR4 agonists was described more frequently in PBMAH with CS (51, 197, 238-240) and modest CS (31, 240) than in unilateral adrenocortical adenoma (241, 242). A family including 2 sisters presented with mild CS secondary to PBMAH showed increased plasma cortisol after terlipressin and metoclopramide administration (51). One of them underwent unilateral adrenalectomy; analysis of the resected adrenal hyperplastic tissue using reverse transcriptase PCR revealed expression of the HTR4 receptor isoforms (a), (b), (c), (i), and (n), and of AVPR1 and AVPR2 (51) (Table 3). Aberrant regulation of cortisol by serotonin in addition to other GPCRs such as gonadotropin-releasing hormone (GnRH), thyrotropin-releasing hormone (TRH), and vasopressin were reported (31, 197, 240).

Overexpression of the eutopic HTR4 was demonstrated in tissues of patients with cortisol-secreting PBMAH or unilateral adenoma responsive to HTR4 agonists (51, 239-241). Specific HTR4 antagonists (GR113808) can inhibit steroidogenesis in vitro but have not been available yet for in vivo treatment (51, 240). HTR4 protein by immunohistochemistry (IHC) was expressed at higher levels in PBMAH nodules of patients carrying *ARMC5* variants compared to those of patients without *ARMC5* mutations (156).

Louiset et al demonstrated in vitro the ectopic expression of HTR7 in cultured adrenal hyperplasia cells from 2 patients with PBMAH and CS (209). Activation of HTR7 enhanced T-type calcium current involving modulation of membrane channels in addition to the cAMP signaling pathway (209).

Moreover, in vitro, 5-HT had an inhibitory action on renin production but stimulated cortisol secretion through the HTR7 in cells from an adrenocortical carcinoma cosecreting renin and cortisol (238). More recently, Lemestre et al demonstrated that prolonged activation of the cAMP/ protein kinase A (PKA) signaling pathway by plasma or intra-adrenal ACTH may induce aberrant serotonergic stimulatory loop in the adrenocortical cells that may contribute to cortisol hypersecretion as well in PBMAH (243).

Catecholamine receptors in adrenal CS

The initial description of cortisol aberrant regulation by ectopic β-adrenergic receptors (ADRB) was in a 56-year-old PBMAH patient with CS in whom cortisol and aldosterone increased during elevations of endogenous catecholamines (upright posture, insulin-induced hypoglycemia, and exercise) (216); infusion of the $beta_1$/$beta_2$ agonist isoproterenol stimulated steroidogenesis in this patient, but not in normal subjects (194, 216) (Table 4). In vitro, isoproterenol increased adenylyl cyclase activity in a dose-dependent manner in the adrenal membranes of this patient, but not in an adrenal gland from a patient with Cushing's disease (216). In addition, high-affinity binding sites compatible with ADRβ1- or ADRβ2 were efficiently coupled

Table 3. Aberrant regulation of cortisol by vasopressin in patients with PBMAH

References	Sex, age, overt Cushing's syndrome (CS) or mild cortisol secretion (MCS)	Aberrant response in vivo	Other aberrant response in vivo	Apparently sporadic (S) or familial (F)	In vitro study confirmation
Makino, 1989 (219)	M 51y, CS	CRH-LVP	Insulin hypoglycemia	S	No
Horiba, 1995 (220)	M 45y, CS; M 37y CS	LVP	No	S	DC
Lacroix, 1997 (222)	F 36y, CS	AVP and water loading	No	S	DC, PCR
Yamakita, 1997 (221)	M 73y, CS polyposis coli	AVP	No	S	*APC* gene mutation
Lacroix, 1997 (216)	M 56 y, CS	AVP, posture,	Isoproterenol	F	β-adrenergic R binding
Iida, 1997 (224)	M 55y, CS	LVP	No	S	DC
Daidoh, 1998 (217)	M 48y	AVP posture	insulin	S	CD inhibition by AVPR1 antagonist
Mircescu, 2000 (194)	5F 1 M 33-64 y 6CS	Posture: 2 AVP: 3	1 β-adrenergic,	S	1 DC AVP AVPR1
Bourdeau, 2001 (31)	M 53y, MCS; F 54y, MCS	AVP	No	S	No
Miyamura, 2002 (48)	F 68y, CS	AVP	catecholamines	F	PCR
Mune, 2002 (223)	M 60y, MCS (patient 4)	AVP	Cisapride, Metoclopramide	S	PCR high AVPR1 in 4 PBMAH cases (217, 221) low levels AVPR2/AVPR3
Tatsuno, 2004 (230)	M 47y, MCS M 49y, MCS; F 62 y; F 57y, MCS; M 52y, MCS	AVP AVP	No No	S S	DC, PCR AVPR1 No
Bourdeau, 2004 (208)	M 59y, CS	AVP	No	S	AVPR not studied microarray
Lee, 2005 (50)	F 46y, CS; F 58y, CS	Posture AVP	No	F	PCR AVPR1/2/3 No desmopressin test
Bertherat 2005 (197) Louiset 2008 (228)	F 34y CS patient 1 H1 patient H2 patient	Terlipressine posture	Cisapride Metoclopramide	S	DC stimulus AVP not desmopressin AVP in cells by IHC AVPR1 and AVPR2 PCR/IHC AVPR2 response to AVP
Suzuki, 2008 (229)	8 PBMAH patients	AVP	No	S	No
Joubert 2008 (225)	6 BMAH 3 MCS, 3 CS	Terlipressin+ in 77%	No	S	DC response to AVP, PCR AVPR1, IHC AVP + AVPR1
Hsiao, 2009 (143)	11 PBMAH with CS	Posture: 4/11 36% AVP 5/11: 45%	ND	S: 8 F: 3	Not detailed
Gagliardi, 2009 (52) (231)	M61y, CS, M60y, CS, M65y CS M75y, CS, M49y, MCS, F46y, CS	AVP AVP	No No	F (*ARMC5*) F (*ARMC5*)	PCR AVPR1/AVPR2, IHC AVPR1 No
De Groot, 2010 (226)	M 60y, CS	AVP	No	S	DC vasopressin and several ligands, PCR higher AVPR1 and lower AVPR2 (and several GPCR) IHC AVPR1
Libé, 2010 (153)	32 PBMAH: 10 CS, 22 MCS	Posture: 14/31(45%) AVP/terlipressine: 6/8 (75%)	Yes	S	No
Miyata, 2013 (227)	M 53y, CS	AVP	No	S	No differential steroidogenic enzymes by IHC in PBMAH cell types

(*continued*)

Table 3. Continued

References	Sex, age, overt Cushing's syndrome (CS) or mild cortisol secretion (MCS)	Aberrant response in vivo	Other aberrant response in vivo	Apparently sporadic (S) or familial (F)	In vitro study confirmation
Hofland, 2013 (195)	35 PBMAH, 22 CS, 13 MCS	Posture 13/26 (50%) Salt loading 1/6 (17%) AVP 6/21 (29%)	several	S	CD: 2/4 AVP PCR: AVPR1 higher in PBMAH
Suzuki, 2015 (232)					
Albiger, 2015 (210)	W 56y, CS	AVP	No	S	No
	W 53y, MCS	Terlipressin and desmopressin	No	S	No
	M 77y, CS	Terlipressin	No	S	No
	W 59y, MCS	Terlipressin	Glucagon and clonidine	S	No
Plockinger, 2017 (172)	22y F. Transient PBMAH during pregnancy	AVP limited response	HCG	S	No AVP expression in PBMAH. AVPr1 and AVPR2 in hyperplastic cells proximal to adrenal medulla
Conceceicao, 2020 (156)	6 PBMAH wild-type *ARMC5* 10 PMAH *ARMC5* variants	Not done	Not done	10 germline	Similar AVPR1 mRNA and protein expression in spongiocytes and compact cells in wild-type and *ARMC5* variants

Includes only the aberrant peak cortisol increase >50% above baseline values.
Abbreviations: AVP, arginine-vasopressin; DC, in vitro stimulation of steroidogenesis by the aberrant ligand in dispersed cells preparations; F, female; IHC, confirmation of aberrant receptor or ligand by immunohistochemistry; LVP, lysine-vasopressin; M, male; P, in vitro stimulation of steroidogenesis by the aberrant ligand during perifusion of adrenal tissues; PCR, in vitro overexpression of mRNA of the aberrant receptor demonstrated by PCR or in situ hybridization.
Practical tip/synthesis:

- Tables 3 and 4 summarize the published cases of patients with vasopressin or catecholamine responsive PBMAH.
- ACTH-independent increase of cortisol following vasopressin administration is the most frequent aberrant response in PBMAH patients.
- Cortisol levels may be stimulated by upright posture, hypertonic saline infusion, or insulin-induced hypoglycemia, acute stress.
- Cortisol response to vasopressin is mostly mediated by AVPR1.
- Combined aberrant response to other stimuli including AVPR1, serotonin HTR4, and B-adrenergic regulation.
- Desmopressin, an AVPR2 agonist, has not been shown yet to stimulate steroidogenesis in PBMAH.
- Aberrant response to catecholamines and vasopressin can be present in familial PBMAH with *ARMC5* germline mutations.

Table 4. Cases of patients with beta-adrenergic pathology proven PBMAH with overt Cushing's syndrome or mild cortisol secretion

References	Sex	Age	Overt CS or mild (MCS) cortisol secretion	Aberrant response in vivo	Other aberrant response in vivo	Apparently sporadic (S) or familial (F)	Medical treatment	Germline DNA *ARMC5* HGVS nomenclature DNA	*ARMC5* HGVS consequence	ACMG classification	Tumoral DNA
Lacroix, 1997 (216)	M	56	CS	ITT, Isoproterenol	Posture, AVP	F[a]	Propranolol: remission	c.327_328insC	(p.Ala110ArgfsTer9)	Frameshift-pathogenic	c.2029G > T (p.Glu677Ter)
Daidoh, 1998 (217)	M	49	CS	Hypoglycemia	AVP	S	OPC-21268 for 8 days				
Mircescu, 2000 (194)	F	50	CS	Isoproterenol	Posture, AVP	S	Propranolol short term				
Imohl, 2002 (233)	M	44	CS	Adrenergic test	No	F	Beta-blocker				
Miyamura, 2002 (48)	M	49	CS	Isoproterenol	AVP	S	No				
Mazzuco, 2007 (234)	W	64	CS	Hypoglycemia	Terlipressin; glucagon	S	Propranolol: remission				
Oki, 2009 (235)	M	61	MCS	Isoproterenol	No	S	Propranolol				
Rhee, 2014 (236)	M	50	CS	Isoproterenol	AVP	S	No				
Bourdeau, 2016 (288)	M	59	CS	Isoproterenol	No	F[a]	Nadolol improvement	c.327_328insC	(p.Ala110ArgfsTer9)	Frameshift-pathogenic	ND
	F	65	MCS	Posture	AVP	F[a]	Nadolol: improvement	c.327_328insC	(p.Ala110ArgfsTer9)	Frameshift-pathogenic	ND
	F	56	MCS	Isoproterenol	AVP	F[a]	Propranolol: improve	c.327_328insC	(p.Ala110ArgfsTer9)	Frameshift-pathogenic	ND
	M	46	MCS	Isoproterenol	AVP	F[a]	Nadolol: improvement	c.327_328insC	((p.Ala110ArgfsTer9)	Frameshift-pathogenic	ND
	F	44	CS	Isoproterenol	AVP	F[a]	Nadolol: failure	c.327_328insC	(p.Ala110ArgfsTer9)	Frameshift-pathogenic	Not found
	F	42	MCS	Isoproterenol	AVP	F[a]	Nadolol: improvement	c.327_328insC	(p.Ala110ArgfsTer9)	Frameshift-pathogenic	ND
	F	59	MCS	Isoproterenol	AVP	F[a]	Nadolol: improvement	c.327_328insC	(p.Ala110ArgfsTer9)	Frameshift-pathogenic	ND
	M	44	MCS	Isoproterenol	AVP	F[a]	Nadolol: improvement	c.327_328insC	(p.Ala110ArgfsTer9)	Frameshift-pathogenic	ND

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AVP, arginine-vasopressin; HGVS, Human Genome Variation Society; ITT, insulin tolerance test; ND, not done.
[a]Cases from Lacroix (1997) and Bourdeau (2016) are from the same family. Includes only the aberrant peak cortisol increase >50% above baseline values.

to steroidogenesis in PBMAH tissues, but not in controls, indicating the ectopic nature of this receptor (216).

In a number of PBMAH patients, there were combinations of various receptors in addition to ADRB, including AVPR1 or HTR4 (Table 4). Aberrant β-adrenergic-driven cortisol secretion was also found in families with PBMAH and were present in patients with mild stages of hypercortisolism (31, 48, 88, 233) (Table 4); in a large PBMAH French Canadian family, aberrant combined β-adrenergic and V1-vasopressin regulation of cortisol was demonstrated in 9 individuals including 7 with mild and 2 with clinical CS (88). A heterozygous germline *ARMC5* mutation was identified in the index case that segregates with the disease in the family. In another PBMAH patient, ADRB2 was overexpressed in adrenal tissues in which salbutamol stimulated cortisol secretion (234). Ectopic catecholamine responsiveness was described only in PBMAH so far and not in unilateral adrenocortical adenoma (218). However, the frequency of ectopic catecholamine responsiveness may be underestimated because the clinical protocol for the search of aberrant regulation of cortisol include only upright posture tests and few investigators have performed more specific tests using β-adrenergic agonists such as isoproterenol infusion (218). There are a few examples of patients in whom treatment of beta-adrenergic responsive CS with beta-blockers such as propranolol or nadolol resulted in complete or partial remission (88, 216, 233-235) (Table 4).

Luteinizing hormone/human chorionic gonadotropin receptor in adrenal CS

Hypercortisolism due to aberrant luteinizing hormone/chorionic gonadotropin receptor (LHCGR) was first identified in a 53-year-old woman with PBMAH and CS who had transient CS during her 4 pregnancies and then constant CS after menopause due to permanent LH elevation (237). The patient's cortisol secretion was stimulated by GnRH, luteinizing hormone (LH), and chorionic gonadotropin (hCG) and by drugs that activate serotonin HT4 receptors. Long-term suppression of LH secretion by the administration of leuprolide acetate every 4 weeks led to complete reversal of CS in this patient until her death from another condition 20 years later (237). Other cases of aberrant receptors for LH/hCG have been reported in both PBMAH and adrenocortical adenoma associated with mild or overt CS (172, 197, 205, 210, 244-247). A number of women with PBMAH and CS responsive to GnRH, LH, or hCG had concomitant aberrant responses, most frequently vasopressin and cisapride/metoclopramide (31, 172, 237, 242, 245, 246). Testosterone secretion was regulated by LHCGR in a patient with an adrenal adenoma (248) and another with PBMAH (37).

The LHCGR is expressed in human zona reticularis and the deeper layer of the zona fasciculata (249, 250). It was shown to co-localize with the cytochrome P450 side chain cleavage enzyme, supporting the concept that LHCGR-positive cells are steroidogenic (249). Moreover, hCG stimulates DHEAS secretion in adrenal fetal but not adult cells (251). LHCGR mRNA expression was demonstrated in PBMAH adrenal tissue from patients with GnRH/LH dependent CS (245, 246). In vitro, hCG but not GnRH stimulated cortisol secretion in PBMAH cells from a patient responsive to GnRH and hCG in vivo (197). LHCGR expression levels were similar in adrenocortical carcinomas and adenomas from pediatric patients but were significantly lower in carcinomas than in adenomas in adults (207).

The most detailed in vitro studies were conducted in adrenal tissues from a patient with transient bilateral adrenal hyperplasia and severe CS during 2 pregnancies (urinary free cortisol reached 30 times upper limit of normal) but normalizing within a few weeks after parturition (172). After adrenalectomy, histopathology demonstrated steroidogenically active adrenocortical hyperplasia and ectopic cortical cell clusters in the medulla that co-expressed CYP11B1/CYP11B2 and AVPR1A/AVPR2. HCG stimulation in vivo and of cultured hyperplastic adrenal cells activated the adenylyl cyclase pathway and significantly increased the production of glucocorticoids, androgens, and corticosterone but not aldosterone (172). LHCGR was localized in subcapsular, zona glomerulosa, and hyperplastic cells. LHCGR transcription factors GATA4, ZFPM2, and proopiomelanocortin (POMC) mRNA expression was upregulated in this hyperplastic tissue compared to control adrenal glands. GATA4 with its cofactors ZFMP2 and SF1 are involved in sex determination, gonadal development, and function and are not normally expressed in adult adrenal glands. As the adrenal cortex and the gonads originate from a common stem/progenitor cell population in the adrenogenital ridge, some gonadal differentiated cells can migrate to the adrenal glands. In this case, pregnancy-induced CS was presumably due to hCG-stimulated transformation of LHCGR-positive undifferentiated subcapsular cells (adrenocortical progenitors) into LHCGR-positive hyperplastic cortical cells. Then the aberrant expression of LHCGR appeared to be an early event which persisted when transient adrenal hyperplasia regressed between pregnancies (172). Interestingly, similarly, in animal models, chronically elevated serum LH following gonadectomy induced adrenocortical benign hyperplasia and tumorigenesis (252, 253).

Other aberrant GPCR in adrenal Cushing's syndrome

In vitro studies indicated the expression of other receptors in human adrenocortical benign and malignant tumors including thyrotropin, follicle-stimulating hormone, and interleukin-1 (15). Cortisol response to glucagon or presence of ectopic glucagon receptors was reported in patients with subclinical or overt CS (153, 194, 197, 244, 254, 255). Transcriptome microarray analyses of PBMAH revealed overexpression of receptors for motilin (MLNR) in 3/18 PBMAH, gamma-aminobutyric acid (GABBR1) in 5/18 PBMAH, and α2 adrenergic (ADRA2A) in 13/18 PBMAH tissues (256). ADRA2A expression was confirmed at protein levels in PBMAH tissues. Moreover, the ADRA2A agonist clonidine induced cortisol secretion that was blocked by the ADRA2A antagonist yohimbine in PBMAH cell cultures (256).

Systematic Clinical Screening Protocol for the Identification of Aberrant Receptors

Protocols were developed to screen patients with adrenal CS to identify aberrant receptors (15, 143, 150, 153, 194, 195, 257) (Table 5). The strategy is to modulate the plasma levels of diverse hormone (endogenous or exogenous) ligands for the potential aberrant receptors. All tests are performed following an overnight fast and with the patient in a supine position for at least 1 hour. For patients with modest cortisol secretion, the studies are conducted under suppression with 1 mg dexamethasone every 6 hours, beginning 48 hours before the tests and is maintained up the end of the screening in order to avoid any effect of ACTH on steroidogenesis.

Table 5. Main studies of systematic clinical screening of aberrant regulation of cortisol in PBMAH patients

	Overt Cushing's syndrome (CS)	Mild Cushing's syndrome	Aberrant responses	Mixed meal/ GIP	Vasopressin	Posture	Cisapride/ metoclopramide	GnRH	TRH	GHRH	Glucagon
Mircescu, 2000 (194)	6	NA	100% (6/6)	33% (2/6)	33% (2/6)	33% (2/6)	16% (1/6)	16% (1/6)	NA	NA	NA
Hsiao, 2009 (143)	14	NA	64% (9/14)	8.3% (1/12)	45.5% (5/11)	36.4% (4/11)	NA	16% (1/6)	33.3% (1/3)	18.2% (2/11)	NA
Libé, 2010 (153)	10	22	87% (28/32)	12% (3/25)	75% (6/8)[a]	45% (14/31)	46.6% (14/30)	NA[b]	NA[b]	NA	47% (8/17)
Hofland, 2013 (195)	22	13	77% (27/35)	11% (4/35)	54% (12/22)	48% (13/27)	24% (7/29)	NA	10% (3/30)	16% (5/31)	15% (4/27)
TOTAL:	52	35	80.5% (70/87)	11.5% (9/78)	53.2% (25/47)	44% (33/75)	33.8% (22/65)	16.7% (2/12)	12.1% (4/33)	16.7% (7/42)	27.3% (12/44)

Includes only the aberrant peak cortisol increase >50% above baseline values.
Abbreviations: NA, data are not available to determine this parameter.
[a]Terlipressin: (triglycyl lysine-vasopressin).
[b]From Libé et al, 2010 (153) data for GnRH and TRH were combined and not included in the current table (GnRH/TRH: 5/31 (16%).
Practical tip/synthesis:

- Table 5 summarizes the systematic in vivo dynamic testing which identified high prevalence of aberrant GPCR in PBMAH tissues, regulating cortisol secretion in ~80% of patients with PBMAH.
- The most frequent aberrant responses are to AVPR1A, HTR4, ADRB1, and LHCGR agonists.
- Several aberrant GPCR can be expressed in the same tissues.
- Microarrays have indicated that several other GPCRs can be expressed.
- Cosecretion of other steroids such as aldosterone can occur.
- Thus, cortisol secretion is independent from pituitary ACTH, but not autonomous as dysregulated by several aberrant GPCR and their ligands.

The screening protocol is performed over 3 days and includes sequentially a posture test to screen for receptors to angiotensin II, vasopressin, or catecholamines; a standard mixed meal to assess the presence of GIP or other gastrointestinal hormone receptors; and an ACTH 1-24 test (250 µg IV) as a reference. The intravenous administration of GnRH 100 µg evaluates responses to GnRH, LH, and follicle-stimulating hormone; intravenous TRH 200 µg screens for modulation by TRH, thyrotropin, or prolactin. On the last day, sequential intramuscular administration of glucagon 1 mg, intramuscular vasopressin 10 UI and 10 mg orally metoclopramide (HTR4 agonist) are performed. Serial measurements of ACTH, cortisol, and other steroid hormones are performed at 30- to 60-minute intervals for 2 to 3 hours following the intervention. An increment of 25% to 49% from baseline steroid levels (no ACTH increase) is defined as a partial response, while >50% increase is considered a positive response; the test should be repeated to confirm reproducibility of the response to the specific stimulus (7, 15, 148).

When a positive response is identified, further tests may be performed to better define the hormone and the specific receptor that is involved; for instance, if a positive response was detected following a mixed meal, the following tests would be conducted to document the presence of a GIP receptor: stimulation by oral glucose, lipids and proteins, non-stimulation by IV glucose, inhibition by octreotide, and finally stimulation with GIP perfusion (187, 188).

Such in vivo protocols showed that a high percentage of patients with unilateral cortisol-secreting adenomas or PBMAH displayed aberrant regulation of cortisol secretion, which was modulated by a wide diversity of aberrant GPCR. This included the expression of ectopic receptors that are not expressed at significant levels in normal zona fasciculata cells or the increased expression or coupling to steroidogenesis of eutopic receptors that can lead to abnormal cortisol production by mimicking the cellular events that are triggered normally by MC2R (15, 148, 218, 257). Four clinical studies have systematically screened patients with PBMAH for the presence of aberrant expression of GPCR in overt and mild cortisol excess (143, 153, 194, 195). Aberrant cortisol response to at least one stimulus was shown in 80% (70/87) of PBMAH patients (Table 5). AVPR1 and HTR4 agonists were the most prevalent hormonal stimuli triggering aberrant responses in vivo with a prevalence of 54% and 35%, respectively (143, 153, 193-195).

Apart from clinical research, the main motivation to perform clinical screening for the presence of aberrant GPCR is the possible opportunity of nonconventional specific medical therapy for controlling hypercortisolism, particularly if aberrant regulation by β-adrenergic or LH receptors is identified (see "Therapy of PBMAH" section). However, in the majority of adrenal lesions, excessive steroid production is regulated by several aberrant receptors together with other factors such as locally produced ACTH (see below). Thus, targeting only one aberrant receptor is not an efficient therapeutic strategy in these cases (258). However, screening for food-dependent regulation in PBMAH has now become essential to identify patients susceptible to carry *KDM1A* mutations (see "Genetic Aspects" section).

Paracrine Production of ACTH and Other Ligands in PBMAH or Adrenal Tumors

Aberrant hormone receptors were also found to exert their activity following their activation by the paracrine secretion of

ACTH or other ligands for those receptors in PBMAH tissues or unilateral adrenocortical tumors. Ectopic ACTH production was reported in steroidogenic adrenocortical cells in PBMAH tissues (13, 156, 259) (Fig. 3, upper panel).

POMC mRNA was detected in PBMAH samples (13). Corticotropin was detected in steroidogenic cells arranged in clusters that were disseminated throughout hyperplastic adrenal specimens, whereas no ACTH staining was detected in the cortex of the normal adrenal glands or 4 primary cortisol-secreting adenomas (13). Corticotropin levels were higher in adrenal venous blood samples than in peripheral venous samples in 2 PBMAH patients, indicating its adrenal source. In vitro incubation studies revealed that all adrenocortical hyperplasia explants spontaneously released ACTH in a pulsatile manner (13). ACTH secretion was not regulated by CRH, dexamethasone, or the GR antagonist mifepristone. In contrast, tissues expressing aberrant GPCR released ACTH and cortisol during perfusion with GIP, serotonin, or hCG; ACTH receptor antagonists cortistatin and corticotropin 7–38 inhibited cortisol secretion induced by aberrant ligands by 40% in these tissues (13) (Fig. 4). Thus, cortisol secretion is controlled in PBMAH by both aberrant GPCR and ACTH produced within the adrenocortical tissue, amplifying the effect of the aberrant receptors' ligands (Fig. 4). The co-expression in the ACTH-positive cells, but not in the normal adrenal cortex, of insulin-like peptide 3, a Leydig and luteal-cell marker, suggests an abnormal tissue differentiation event occurring during embryogenesis in a common gonadal-adrenal progenitor (13). The ectopic expression of POMC and synthesis of ACTH was recently confirmed in PBMAH tissues (156) and their primary cultures (260); expression of POMC, prohormone convertase type 1 (PCSK1), mature ACTH, and of aberrant GPCR were maintained in primary cultures of cells with or without *ARMC5* mutations (260). Interestingly, stimulation of the cells by ACTH increased POMC expression and ACTH secretion and similarly to normal pituitary corticotroph cells, the transcriptional activity of POMC promoter is dependent on protein kinase A (PKA) mediated regulation of calcium channels (260). The confirmation that paracrine adrenal production of ACTH is central in cortisol regulation of PBMAH will necessitate further clinical studies to examine whether specific MC2R receptor antagonists can reverse cortisol excess of affected patients (14).

Other studies indicate that PBMAH tissues may also produce serotonin, vasopressin, or glucagon suggesting additional paracrine regulatory loops of cortisol secretion and cell proliferation (9, 156, 197, 254, 261).

Evaluation of Severity of Steroid Overproduction and Comorbidities

The comorbidities occurring in patients with PBMAH are the consequences of chronic exposure of target tissues to steroid hormone excess (Graphical abstract). The duration and the degree of hormone excess determine their prevalence and severity; however, individual susceptibility, in particular genetic factors, including glucocorticoid receptor sensitivity, and lifestyle also play a role in the development of comorbidities. Cortisol oversecretion leads to insulin resistance, diabetes mellitus, altered lipid profile, and arterial hypertension, all of which increase cardiovascular risk (262). Aldosterone cosecretion may further increase blood pressure and promote left ventricular hypertrophy and vascular damage (41, 262, 263). Besides cardiovascular disease, hypercortisolism impairs bone health, promoting osteoporosis and increased risk of fractures (262). A systematic assessment of metabolic and skeletal comorbidities in patients with PBMAH is essential to offer appropriate management of the adrenal disease itself (Fig. 2 and "Therapy of PBMAH" section) and of associated multisystem comorbidities (26, 264). Patients with PBMAH and severe CS can also present with the same diverse comorbidities of infections, neuropsychiatric disturbances, coagulopathy, sarcopenia, and frailty as those with other etiologies of CS (262); we will restrict our discussion to the complications that are essential to stratify PBMAH patients with mild cortisol excess for the indication of therapeutic interventions.

Cardiometabolic Comorbidities

In a recent meta-analysis and large cross-sectional study comparing comorbidities between patients with nonfunctioning adrenal tumors (morning cortisolemia <50 nmol/L after 1-mg dexamethasone at bedtime) and patients with adrenal tumors responsible of producing mild cortisol excess, the prevalence of cardiometabolic abnormalities was higher in the latter (27, 265). However, patients with so-called nonfunctioning adrenal tumors still had a higher prevalence of comorbidities than the general population, suggesting an effect of mild steroid overproduction undetected by usual diagnostic criteria (27, 265, 266). Cardiometabolic consequences of cortisol excess include mainly diabetes mellitus, arterial hypertension, and dyslipidemia.

Diabetes Mellitus

The pathophysiology of diabetes mellitus (DM) in CS is linked to both altered insulin sensitivity and insulin secretion. The effects of cortisol on glucose homeostasis have been comprehensively summarized in a previous review (267), and their detailed description are beyond the scope of the present article. Briefly, glucocorticoid excess leads to increased gluconeogenesis in the liver by stimulating glucogenesis enzymes, in particular glucose-6-phosphatase. Increased lipolysis and proteolysis provide substrates for hepatic gluconeogenesis. Increased free fatty acid promotes insulin resistance decreasing glucose uptake in skeletal muscles and other tissues (268, 269). In addition, lipotoxicity and glucotoxicity alter beta cell functions and decreases insulin secretion.

The prevalence of DM in patients with overt CS ranges from 20% to 45% (269-277). Prediabetes is found in 10% to 30% of cases; thus, half of patients with CS present with some abnormalities of glucose metabolism. This prevalence may be underestimated, as most studies are only based on fasting glucose concentrations but do not always investigate glucose homeostasis after an oral glucose tolerance load. Generally, the severity of hypercortisolism correlates with the presence of insulin resistance and DM, but genetic susceptibility and lifestyle also contribute to the development of the metabolic phenotype. In addition to heterogeneous biochemical assessment, these elements also account for differences in the reported prevalence of glucose intolerance or diabetes in CS in general.

Only a few studies reported the prevalence of glucose intolerance and DM specifically in patients with PBMAH. The reported prevalence is highly variable, ranging from 20% to 47%. However, in a study published including 98 patients with PBMAH, the prevalence of DM was 33%, comparable with that observed in Cushing's disease (32). More recently, in a

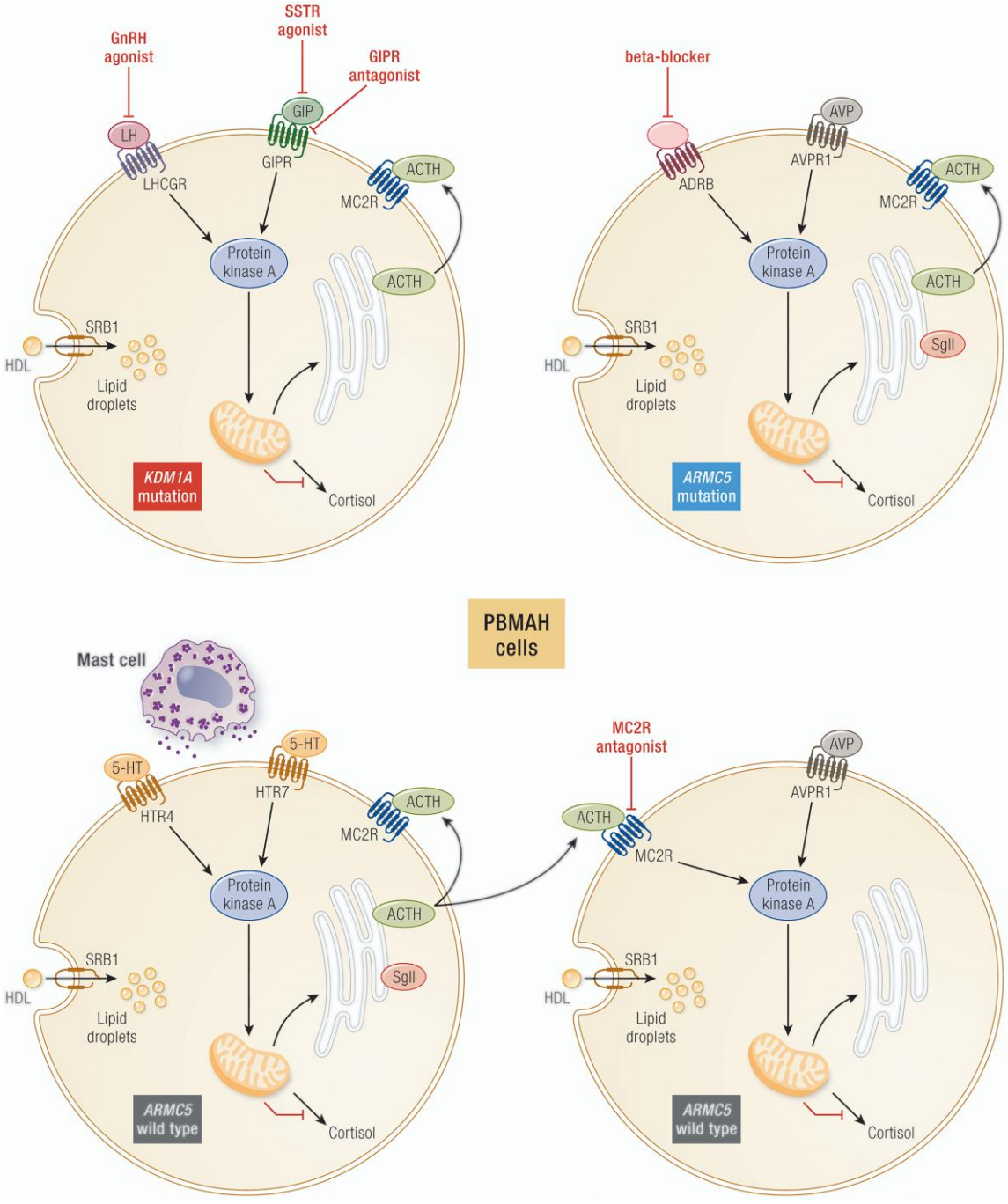


Figure 4. Aberrant and paracrine regulation of ACTH and cortisol in primary bilateral macronodular adrenal hyperplasia. Ectopic production of ACTH occurs in some PBMAH cells, which also express a diversity of functional aberrant GPCR. In some PBMAH patients, the presence of *KDM1A* pathogenic variants specifically result in ectopic expression of glucose-dependent insulinotropic peptide receptor (GIPR) or rarely also overexpression of luteinizing-chorionic gonadotropin hormone receptors (LHCGR). In other patients, the presence of *ARMC5* pathogenic variants can be associated with overexpression of arginine-vasopressin (AVP) receptor type I (AVPR1), and β-adrenergic receptors (ADRB), but a direct role of *ARMC5* variants on aberrant receptors expression has not been clearly elucidated yet. In other patients without *ARMC5* variants, diverse aberrant receptors such as serotonin type 4 (HTR4) and 7 receptors (HTR7) or AVPR1 can be abnormally expressed. Activation of these GPCR by their ligands, including serotonin (5-HT) from increased mast cells in PBMAH tissues, both stimulate cortisol release directly and by increasing ACTH secretion; locally secreted ACTH stimulates cortisol production by activating its melanocortin type 2 receptor (MC2R). Specific GPCR antagonists or inhibition of their ligand production can inhibit cortisol secretion. In contrast, ACTH and cortisol are not regulated by CRH or glucocorticoid negative feedback. Abbreviations: HDL, HDL-cholesterol; SRBI, scavenger receptor B1.

Practical tip/synthesis:

- Regulation of cortisol secretion by PBMAH cells is independent from pituitary ACTH, but not autonomous as regulated by complex autocrine/paracrine mechanisms.
- A diversity of aberrant ectopic or eutopic aberrant GPCR can activate the G-protein-cAMP-PKA pathway to increase cortisol secretion.
- Autocrine/paracrine production of ligands such as ACTH or serotonin (5-HT) occurs in PBMAH cells or tissues.
- Aberrant GPCR stimulate local ACTH production creating an autocrine-paracrine loop of regulation of cortisol secretion.
- Antagonists of various aberrant GPCR or of their ligands can reduce excess cortisol production.

multicentric study including 352 European patients, the prevalence of impaired glucose tolerance was 19% and DM 38% (62), which is similar to the prevalence observed in a monocentric study including 124 PBMAH patients in China (278).

Some complementary information may be gained from the recent literature on adrenal incidentalomas. A meta-analysis investigating the natural history of adrenal incidentalomas with and without mild cortisol excess reported a prevalence of DM of 28% in patients with mild cortisol excess and of 14% in patients with so-called nonfunctioning adrenal tumors. The prevalence of prediabetes was 50% and 11%, respectively (265). However, patients with bilateral adrenal incidentalomas, which could frequently be, in fact, patients with PBMAH, were not analyzed separately. Similarly, in the EURINE-ACT patients with unilateral or bilateral benign adrenal tumors, DM was more prevalent in patients with mild cortisol hypersecretion than in patients without cortisol hypersecretion, although clearly overt PBMAH patients were a priori excluded (27); however, it is probable that PBMAH with mild cortisol secretion constituted a high proportion of patients with bilateral incidentalomas.

Treatment of DM in patients with PBMAH and CS should follow similar clinical practice as in type 2 DM (270), in addition to cortisol-lowering strategies discussed in the "Therapy of PBMAH" section.

Arterial Hypertension

Arterial hypertension contributes to increased cardiovascular morbidity and mortality observed in CS and PBMAH (Graphical abstract). The pathogenesis of high blood pressure in CS is multifactorial including increased mineralocorticoid activity related to cortisol excess, modification of the sympathetic nervous system, and increased vasoconstriction (262).

The prevalence of high blood pressure varies largely among different studies ranging from 25% to 93% (262). Five studies assessed the prevalence of arterial hypertension specifically in patients with PBMAH (Table 6), reporting a prevalence of high blood pressure close to 70% (32, 62, 127, 169, 278). The prevalence of arterial hypertension in patients with PBMAH seems comparable to that observed in adrenocortical adenomas and in CD (127). However, data from the European Registry on Cushing's syndrome ERCUSYN including 481 CS patients should be interpreted prudently, as only 4 patients with PBMAH were included (279). Similarly to abnormalities of glucose metabolism, the prevalence of arterial hypertension is increased both in patient with adrenal lesions with mild cortisol excess (64%) and in patients with so-called nonfunctional adrenal tumors (58%), as compared to the prevalence of 30% reported in general population (27, 265, 266).

In some patients with PBMAH, cortisol secretion can be associated with some degree of mineralocorticoid hormone secretion (262, 263). Besides aldosterone-producing cells in the zona glomerulosa, clusters of cells expressing aldosterone synthase are also observed in the subcapsular region of the adrenal glands (see pathology section) and are not subjected to the same regulatory mechanisms. Aldosterone cosecretion in PBMAH very likely arises from similar clusters rather than from zona glomerulosa cells (25, 263). Aldosterone cosecretion in PBMAH can contribute to the severity of arterial hypertension and worsen hypokalemia.

Treatment of arterial hypertension in CS, should follow the general guidelines for management of high blood pressure, in addition to adrenal directed strategies to control hypercortisolism (Fig. 2). However, some steroidogenesis inhibitors such as metyrapone and osilodrostat (see therapy section) can increase blood pressure by accumulation of precursors such as deoxycorticosterone with mineralocorticoid activity (280), probably more often in Cushing's disease due to increased ACTH stimulation than in PBMAH. Correction of high blood pressure after remission of CS is not always possible as CS leads to vascular remodeling. Indeed, 25% to 54% of patients still display arterial hypertension after normalization of cortisol production (262).

Dyslipidemia

Dyslipidemia is found in 12% to 72% of CS patients and is characterized by elevated triglyceride and LDL-cholesterol concentrations. A well-known cardiovascular risk factor, it promotes atherosclerosis (262). Even though dedicated studies in PBMAH patients are lacking, reported lipid profiles seem to be comparable between different etiologies of CS

Table 6. Prevalence of diabetes mellitus and arterial hypertension in patients with PBMAH with CS

References	Number of patients studied	Diabetes mellitus	Arterial hypertension
Debillon et al, 2015 (169)	15 patients, all with overt CS	30%	67%
Espiard et al, 2015 (32)	98 patients, 10% nonsecreting PBMAH, 47% with mild cortisol secretion and 43% with overt CS	34%	69%
Albiger et al, 2017 (127)	72 patients, 25% with nonsecreting PBMAH, 28% with mild cortisol secretion and 47% overt CS	24%	67%
Bouys et al, 2022 (62)	352 (73% with mild cortisol secretion from bilateral incidentalomas and 27% of overt CS)	38%	73%
Wang et al, 2022 (278)	124 (55% with overt CS)	47%	91%

Abbreviations: CS, Cushing's syndrome; PBMAH, primary bilateral macronodular adrenal hyperplasia.
Practical tip/synthesis:

- Table 6 summarizes the limited number of studies which examined the prevalence of diabetes and hypertension in patients with PBMAH and Cushing's syndrome.
- Even mild cortisol excess in PBMAH is associated with increased prevalence of arterial hypertension and glucose intolerance compared to normal populations.
- Severity of cortisol excess is believed to be mostly responsible for comorbidities irrespective of its etiology.
- Cosecretion of aldosterone may contribute to severity of hypertension and therefore cardiovascular comorbidities.

(281). A recent meta-analysis including 1101 patients with adrenal incidentalomas identified a similar prevalence of dyslipidemia in patients with mild cortisol excess (33%) and in patients with nonfunctioning adrenal tumors (34%) (265). In 1305 patients of the EURINE-ACT cohort with adrenal incidentalomas, prevalence of dyslipidemia did not differ between participants with so-called nonsecreting tumors (28.8%) and those with various degrees of cortisol excess (15.7%–35.9%) (27).

Treatment of CS-associated dyslipidemia should be based on current guidelines of dyslipidemia management. Treatment of CS can improve the lipid profile in some patients. However, lipid abnormalities remain frequently present after remission of hypercortisolism; as increased cardiovascular and mortality risks persist even after remission of CS, it seems appropriate, although not demonstrated in clinical trials, that therapy of dyslipidemia should follow guidelines for patients with high risk factors, such as those with modified Framingham score >20% (282).

Skeletal Consequences

Dual-energy x-ray absorptiometry (DXA) is commonly used to assess mineral bone density. According to the current WHO criteria, the term *osteoporosis* for postmenopausal women and men older than 50 years is defined by a T-score value ≤ −2.5 SD in at least one site (spine, total hip, and femoral neck). In premenopausal women and in men younger than 50 years of age *low bone mineral density* designates a T-score or a Z-score values ≤ −2.0 SD (283). Osteoporosis is generally found in 22% to 57% of patients with CS, exposing them to high risk of fractures (262, 282). However, increased bone fragility is also observed in CS patients without decreased bone mineral density, reflecting the altered microarchitecture of the bone tissue related to cortisol excess (262, 282); it is thus also recommended to perform dorsal and lumber spine imaging to identify compression fractures.

The effect of glucocorticoids on bone has been comprehensibly reviewed (284-286). Briefly, glucocorticoids decrease osteoblastogenesis and induce osteocyte apoptosis that account for the loss of bone strength, occurring before the significant loss of bone mineral density and the above-mentioned discrepancy between bone mineral density and the risk of fracture (284). Further, cortisol excess increases the lifespan of osteoclasts, therefore promoting bone resorption (284, 285). Moreover, the muscular atrophy associated with the catabolic effect of glucocorticoid excess, also affect muscle trophic effect on bone.

Very few studies assessed bone mineral density in patients with PBMAH. In the recent multicentric study including 352 PBMAH patients from France and Germany, the prevalence of osteoporosis in PBMAH was similar in patients with *ARMC5* harboring genetic variants (27%) and in patients without *ARCM5* variants (24%) (62). In patients with adrenal incidentalomas, the risk of facture is increased in those with mild dysregulated cortisol secretion. It has been suggested that the rate of vertebral fragility fracture is increased in patients with bilateral adrenal incidentalomas (possibly early PBMAH) by comparison with unilateral incidentaloma regardless of the degree of cortisol hypersecretion and bone mineral density (287). About half of patients presenting one or more fractures in patients with mild cortisol excess and adrenal masses presented with normal or only slightly decreased bone density (288, 289). Assessment of bone microarchitecture in addition to dual-energy x-ray absorptiometry could improve the estimation of fracture risk (289).

The treatment of osteoporosis and of bone fractures should follow the current guidelines in addition to the attempt to normalize cortisol secretion (289, 290).

In summary, in patients with PBMAH associated with overt CS, the comorbidities are comparable to those observed in other etiologies of CS and globally correlated with the degree of hypercortisolism (262, 284). In patients with PBMAH and mild cortisol secretion, assessment of comorbidities is crucial as it guides the choice of therapeutic management (Fig. 2). Whether these comorbidities are higher in PBMAH with mild cortisol secretion than in unilateral adenomas is suggested, but work is still required for it to be clearly demonstrated.

Pathology

Adrenal Histology

Adult adrenal glands are divided into 2 different portions: the central adrenal medulla, which secretes catecholamines, and a peripheral portion, the adrenal cortex, which produces adrenal steroids (291). The average weight of the normal adult adrenal glands is around 6 grams and dimensions are approximately 5 cm in length, 3 cm in width and 0.6 cm in thickness; nodules are mostly noted as an increased size of the adrenal thickness (57). The cortex is subdivided into layers from the outer to the inner portion of the adrenal. Layers are responsible for the different hormonal secretions and present different histological structures: the zona glomerulosa, where mineralocorticoids are synthesized; the zona fasciculata, where glucocorticoids are produced; and the zona reticularis, the source of adrenal androgens. The zona glomerulosa is composed of spherical cells, the zona fasciculata is characterized by parallel fibers divided by vertical columns of cells, and the zona reticularis is made of fibrous tissue organized in sheets interlaced with groups of cells (291). The adrenal glands are covered by a fibro-elastic tissue called the capsule.

Histological Features of PBMAH

In primary macronodular hyperplasia, the adrenal glands are enlarged and usually have a combined weight of more than 60 g that can reach up to 200 g (12, 292, 293). Nodules are larger than 1 cm in diameter. Nodules smaller than 1 cm are observed in micronodular hyperplasia, and often associated with pigmented fasciculata (in most cases, due to lipofuscin depots) and referred as primary pigmented nodular adrenocortical disease (PPNAD) and can be a part of Carney complex (12, 294). The absence or limited presence of internodular pigmentation in micronodular hyperplasia is also described and is referred as Isolated Micronodular Adrenocortical Disease (i-MAD) (294).

On macroscopic examination of PBMAH, the nodules appear yellow with high lipid content and the adrenals have a lobulated or multinodular aspect (12, 295). On microscopic evaluation, irregular cortical nodules include 2 different cell types depending on lipid content: small compact eosinophilic cytoplasm cells with lower lipid content (Fig. 5A); and larger fasciculata-like cells rich in lipid content with clear cytoplasm, called spongiocytes (Fig. 5A). Mitotic events are rare in both cell types. Spongiocytes are positive for 3β-hydroxysteroid

dehydrogenase and show poorly developed smooth endoplasmic reticulum. Small compact cytoplasm cells are positive for CYP17A1 (17α-hydroxylase). Finally, CYP11A1 (P450 side chain cleavage), CYP21A2 (21-hydroxylase), and CYP11B1 (11-hydroxylase) are also positive in most cells but their level of expression is decreased (295, 296, 299, 300). This expression profile could be a sign of altered steroidogenesis and explain the mild dysregulated cortisol secretion frequently associated with PBMAH despite enlarged adrenals (292, 293, 295, 296). In some patients with PBMAH, glucocorticoid excess can be accompanied by other hormone secretion. Some patients present with mineralocorticoid secretion associated with cortisol excess. Clusters of cells expressing CYP11B2 without CYP11B1 can be observed outside the zona glomerulosa (280). When patients present with hypersecretion such as estrogen hypersecretion, LHCGR overexpression is often reported. In addition, higher CYP19A1 (aromatase) staining in one patient with *ARMC5* mutation and elevated estrogen secretion has been reported in the setting of PBMAH (297).

The internodular tissue can be either atrophic or display diffuse nodular hyperplasia without any residual normal adrenal cortex leading to less clear boundaries between nodule and internodular tissue (143). An internodular adrenal cortex atrophy can be the sign of mosaic pattern of *GNAS*-activating mutations occurring in some adrenal cells leading to a constitutive activation of the cAMP pathway, and consequently to hyperplasia. The cells not carrying the activating mutation become atrophic because of suppressed corticotropin concentrations and in contrast to the hyperplasia of mutated cells (Fig. 5B). Activating mutations of Gsα have been found in nodules of adult patients who do not present other features of McCune-Albright syndrome (298).

Distinction Between PBMAH and Bilateral Adenomas

As cortisol-secreting bilateral adenomas with a diameter over 1 cm and PBMAH can appear similar in imaging, histology can differentiate between these entities. Most adrenal cortical adenomas are homogeneous and have well-defined lesions. They can display a pseudocapsule in some cases. Adrenocortical adenomas are enriched in vacuolated cells with a clear cytoplasm and rich in lipid content and a variable number of compact cells. Cells are ZF-like and organized in an alveolar pattern (299). The internodular tissue is characteristically atrophic (Table 1) (12). As PBMAH can also sometimes display internodular atrophy, the difference between bilateral adenomas and PBMAH can be difficult. In bilateral adrenocortical adenomas, both cell types are usually immunoreactive to 3β-HSD and 17α-hydroxylase (12, 29), whereas in PBMAH, only spongiocytes display immunoreactivity for 3β-hydroxysteroid dehydrogenase and the small compact cells are positive for CYP17A1 (17α-hydroxylase) (296, 300).

Other Immunochemical Markers in PBMAH

PBMAH is usually easily recognized by pathologists using routine hematoxylin-eosin staining (12). However, in some situations, staining for steroidogenic enzymes described above or other markers can be useful. First, immunochemistry features can be used as signs of the genetic etiology. In patients with *ARMC5* pathogenic variants, staining of ARMC5 can be decreased in the hyperplastic tissue compared to the nonhyperplastic areas (54) (Fig. 6); however, this is not always found (156), and may depend on specific genetic alteration. Patients carrying *ARMC5* pathogenic variants also have inefficient steroidogenesis and display decreased staining for CYP11A1 and other enzymes (54, 156) (Fig. 3). In GIP-dependent PBMAH secondary to *KDM1A* germline genetic variation associated with adrenal loss of heterozygosity (LOH) in 1p, 2 studies have shown that these events lead to the loss of the KDM1A nuclear staining in foci of the hyperplastic tissue (55, 56) (Fig. 6). It is also possible that the presence of myelolipoma foci among the hyperplastic tissue can be a histological trace of *KDM1A* loss as detailed below.

Immunochemistry can also depict adrenocortical ACTH production. In normal conditions, only chromaffin cells display ACTH immunoreactivity. In 2013, Louiset et al demonstrated intra-adrenal production and paracrine secretion of ACTH by the adrenal lesions (13). Corticotropin-positive cells have been detected specifically in cluster of hyperplastic adrenocortical cells. A recent study confirmed that ACTH was similarly expressed in spongiocytes and compact cell cytoplasm in PBMAH tissues from patients with or without *ARMC5* pathogenic variants (156).

Both the Wnt/βcatenin and the PKA/cAMP pathways are important drivers of adrenal disease; nuclear CTNNB1 staining was found to be higher in macronodules than in micronodules. PKA subunits were strongly expressed in adrenal nodules, but their expression was lower in macronodules (301).

Adrenal glands also display immunoreactivity against inhibin alpha-subunit, a glycoprotein member of the transforming growth and differentiation factors, mainly in the zona reticularis (302). It has been shown that adrenal hyperplasia, adrenal cortical adenoma, and carcinoma can be positive for inhibin A regardless of hormonal secretion. However, pheochromocytoma and other tumors are usually negative (303). In PBMAH, inhibin A immunostaining can be increased in the hyperplastic adrenal tissue (304).

Besides steroidogenic cells, the adrenal cortex contains other cell types, such as stem cells, adipocytes, immune cells, neurons, and endothelial cells, with evidence that these different cell types constitute a microenvironment contributing to the pathophysiology of adrenal remodeling and steroid overproduction in adrenal hyperplasia and adrenal tumors (305).

Possible Association With Myelolipoma

Myelolipomas are lipomatous tumors consisting of both adipose tissue and myeloid cells (306) (Fig. 5C, 5D, and 5F). Three lines of extra medullary hematopoiesis components should be identified to define myelolipoma foci: megakaryocytes, erythroid cells, and granulocytic cells (Fig. 5E (red-circle), Fig. 5G (red circle). Pathologists can identify these cell types using routine hematoxylin staining, and therefore confirmation using immunohistochemical staining is not always necessary. Without these 3 components of myelopoiesis, pathologists can refer to these foci as myeloid dysplasia. Recent studies have shown an association of myelolipoma or myeloid metaplasia with ectopic GIP-receptor expression bearing *KDM1A* pathogenic variants (55). As KDM1A is a regulator of hematopoietic differentiation (307), such development of myelolipoma is likely the consequence of KDM1A functional loss in the adrenal tissue (55, 56).

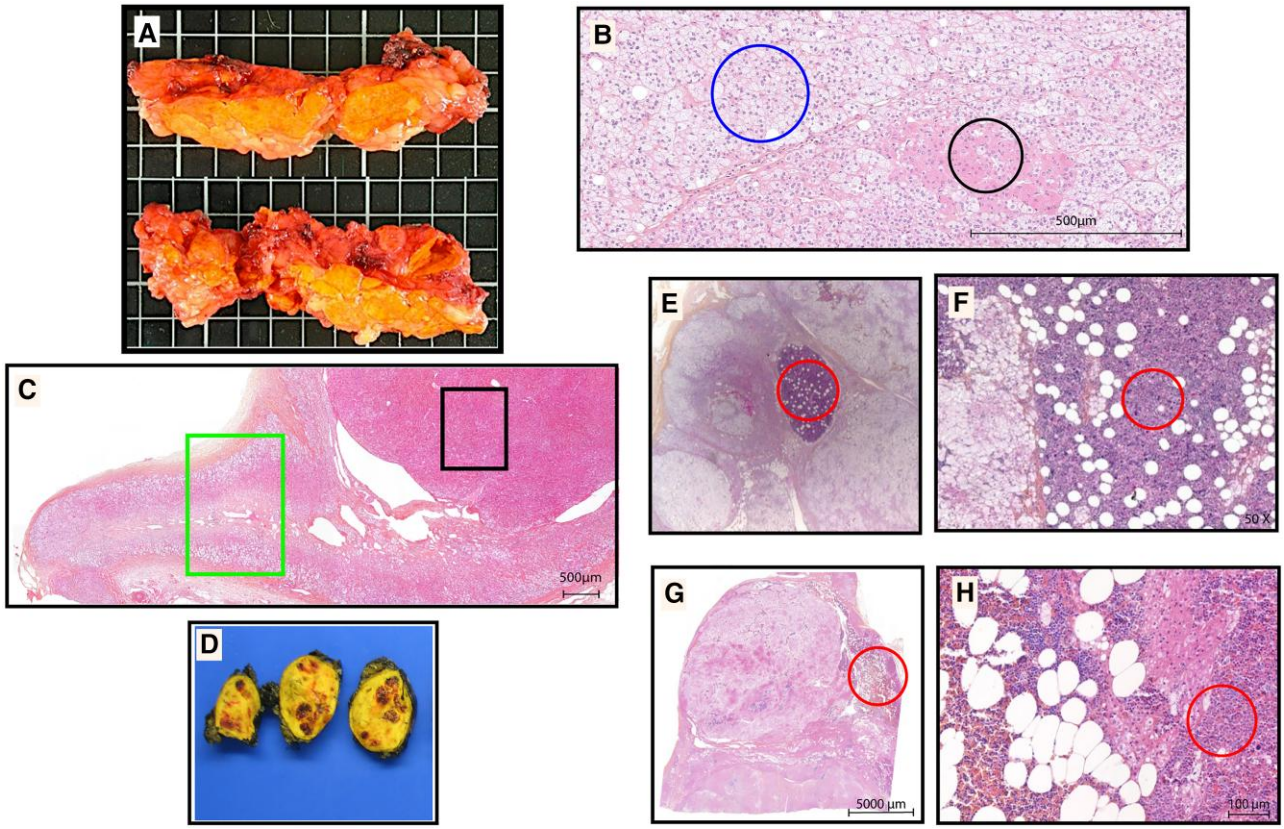


Figure 5. Macroscopic and histological aspects of PBMAH. Macroscopic and histological aspect of primary bilateral macronodular adrenal hyperplasia: (A) Macroscopic examination of PBMAH: the adrenals have a lobulated or multinodular aspect. (B) histology from a patient with PBMAH showing 2 cell types: spongiocytes with clear cytoplasm (blue circle) and compact cells (black circle) (Hematoxylin Eosin Saffron staining). (C) Adrenal histology from a patient with PBMAH and somatic *GNAS* mutation showing nodular zone (black square) with adjacent cortical atrophy (green square). (D) Adrenal macroscopic aspect showing foci of adrenal myelolipoma in dark brown and cortical hyperplasia (yellow) (patient not mutated for *KDM1A*). (E) Histology issued from the left adrenal of a patient carrying a *KDM1A* germline pathogenic variant without LOH (published and reproduced with permission from (55, 72) displaying multiple PBMAH nodules and a small area of myelolipoma (red circle). (F) Right adrenal of the patient displayed in (E): large areas of myelolipoma are present with scattered islands of adrenocorticortical cells. (G) Histology from a patient carrying a *KDM1A* germline pathogenic variant and somatic LOH. The adrenal cortex displays classic PBMAH features interlaced with myelolipoma foci (red circle). (H) Myelolipoma foci of a patient carrying *KDM1A* germline mutation and somatic LOH, displayed in (G). The 3 cell types (megakaryocytes, erythroid cells, and granulocytic cells) are present (red circle) (patient displayed in (G) and (H) was previously published in (55).

Practical tip/synthesis:

- Adrenal glands of patients with PBMAH and Cushing's syndrome are bilaterally enlarged with a combined weight >60 g and up to 200 g.
- Adrenals have a lobulated or multinodular aspect of yellow color in relation with high lipid content.
- PBMAH tissues are composed of multiple lipid-rich clear cells >1 cm nodules intermingled with areas of eosinophilic cells.
- Altered levels of distinct steroidogenic enzymes can be found in each cell type using immunohistochemistry (IHC).
- IHC can reveal expression in PBMAH cells of various proteins such as ACTH, aberrant receptors, or markers of genetic variants.

The rate of abnormal adrenal secretion from myelolipoma is difficult to estimate as patients presenting with such adrenal lesions are not always screened for hormonal hyperfunction. For example, in a series of 321 patients with myelolipomas, only 41.3% of patients benefited from hormonal evaluation. Three out of 92 had dysregulated cortisol production and 9 out of 74 screened patients had primary aldosteronism attributed to ipsilateral or contralateral adenoma (308). Similar numbers are found in another series of 150 patients with myelolipoma, among whom only 20 patients received hormonal evaluations, leading to diagnosis of dysregulated cortisol production in 3 and primary aldosteronism in 1 (309). The rate of endocrine dysfunction is thus probably underestimated in patients with myelolipoma. Myelolipomas are not always confirmed by histopathological analysis. It is therefore possible that the few reported cases presenting hormonal hyperfunction were in fact adrenal hyperplasia with interlaced myelolipoma foci (310). Su et al reported 3 hormonally active myelolipomas and 1 nonactive and showed that the adrenal cortex was in fact hyperplasic in patients with myelolipomas presenting hormonal hyperfunction (311). Later, a case of GIP-dependent CS presented a similar histology with myelolipoma tissue interlaced with strands of PBMAH (72) and this is one of the initial tissues in which exome sequencing identified a *KDM1A* mutation (55).

Genetic Aspects

Syndromic Etiologies and Genes

Although PBMAH is most often sporadic, it may rarely be associated with certain genetic syndromes, including multiple endocrine neoplasia type 1 (MEN1), familial adenomatous

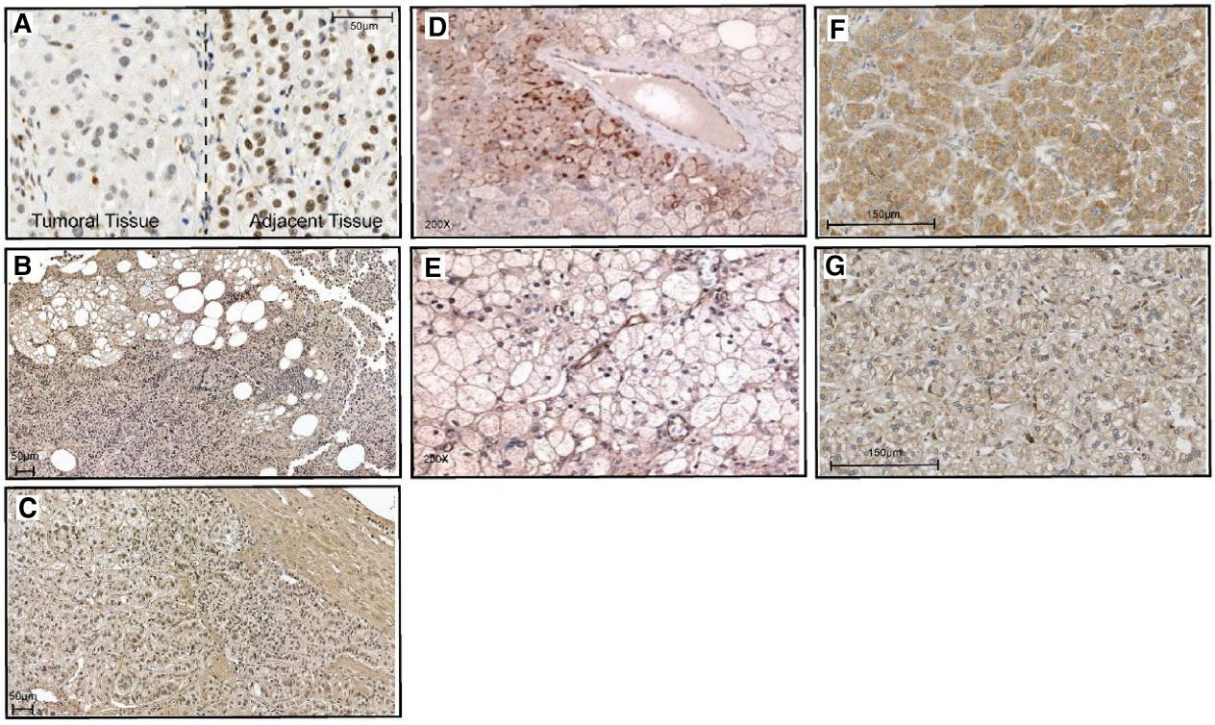


Figure 6. Immunohistochemistry aspects of PBMAH. (A) Immunohistochemistry of KDM1A in a patient with PBMAH and *KDM1A* pathogenic variant: most cells showed weaker nuclear KDM1A staining in the tumoral tissue than in the adjacent nontumoral adrenal gland. (B) Immunohistochemistry of KDM1A in a patient with PBMAH and *KDM1A* pathogenic variant (patient published in (55, 72)): foci of myelolipoma displayed decreased KDM1A nuclear staining. (C) Immunohistochemistry of KDM1A in a patient with PBMAH and *ARMC5* pathogenic variant (patient published in (55)): KDM1A nuclear staining appears normal compared with patients with *KDM1A* pathogenic variant as displayed in (A) and (B). (D) GIPR IHC of the right adrenal gland from a patient with *KDM1A* pathogenic variant and PBMAH displayed in (B) (published in (55, 72)): endothelial staining and focal moderate membranous staining in the adrenocortical cells. (E) GIPR IHC of the left adrenal gland from a patient with *KDM1A* pathogenic variant and PBMAH displayed in (B) (published in (55, 72)): only endothelial staining is seen and with very minimal staining is seen in adrenocortical cells. (F) Immunohistochemistry of ARMC5 from a patient with PBMAH without *ARMC5* pathogenic variant. (G) Immunohistochemistry of ARMC5 from a patient with PBMAH and *ARMC5* pathogenic variant displaying a decreased staining (previously published in (54)).

polyposis (FAP) or hereditary leiomyomatosis and renal cell cancer (HLRCC) (53, 143, 312) (Fig. 3, upper panel).

MEN1

MEN1 is an autosomal dominant disease caused by mutations in the *MEN1* gene (OMIM 613733 located on chromosome 11q13.1). *MEN1* is a tumor suppressor gene that codes for menin, a protein that plays a role in cellular differentiation and proliferation (42, 313). The most common clinical manifestations of MEN1 are parathyroid hyperplasia/adenomas with an estimated penetrance of 90%, followed by enteropancreatic tumors that are diagnosed in 30% to 70% of cases and pituitary adenoma with a penetrance of 30% to 40% (42). In addition to these most frequent tumors, patients with MEN1 can display tumors in other tissues such as bronchial carcinoid, facial angiofibromas, collagenomas, thyroid tumors, lipomatous tumors, meningiomas, breast tumors, and adrenal tumors (42, 314).

The reported incidence of adrenocortical lesions in patients with MEN1 varies between 20% to 73% depending on the definition of adrenal involvement and the type of imaging used (42, 315, 316). Adrenal lesions found in MEN1 are most frequently nonfunctional lesions and include adenomas, hyperplasia, multiple adenomas, nodular hyperplasia, cysts, or carcinomas. Fewer than 10% of patients have hormonal hypersecretion, being mainly primary hyperaldosteronism and CS.

In 2012, the Groupe d'étude des Tumeurs Endocrines database described a cohort of 715 patients with MEN1 (45). Within the whole cohort, adrenal enlargement was reported in 20.4% of cases, but 10.1% of patients had adrenal tumors defined by an adrenal lesion >1 cm. Among these patients, 12.5% had bilateral disease, which may include presumably patients with PBMAH (45) described previously as MEN1 (143). In 1996, Burgess et al reported that adrenal lesions were found in 12 out of 33 (36%) patients from a same kindred with MEN1 (317). Among them, 5 had bilateral adrenal lesions and 2 had a diagnosis of PBMAH that was confirmed at histology, but hormonal investigations were not reported. Cases of PBMAH associated with MEN1 in patients carrying germline MEN1 pathogenic variants were reported (318). In contrast, a number of cases of patients with PBMAH and CS were found to have clinical manifestations of MEN1 (hyperparathyroidism, insulinomas, and/or pituitary adenomas) without *MEN1* mutations (319, 320). For example, in 2011, Yoshida et al described a 60-year-old man with PBMAH and subclinical hypercortisolism with concomitant parathyroid tumors, multiple insulinomas, and nonfunctioning pituitary adenoma; no germline genetic variants were found in the MEN1 gene, but GIPR was highly expressed in the PBMAH adrenal compact cells by immunohistochemistry, suggesting GIP-dependent cortisol secretion, but his cortisol regulation was not explored in vivo before the adrenalectomy (321).

Familial adenomatous polyposis

FAP is an autosomal dominant inherited disorder (adenomatous polyposis coli [*APC*] gene) characterized by the development of hundreds to thousands of adenomatous polyps of the colon and rectum, predisposing to colorectal carcinoma if not surgically treated. It may be associated with extracolonic manifestations, including adrenocortical tumors (322, 323). In a cohort of patients with FAP, the prevalence of adrenal involvement was 16% (48/311) and 23% of these masses were bilateral (43). A number of cases of patients carrying *APC* germline mutations were described with pathology proven PBMAH (143, 221) (Fig. 3 upper panel). FAP is caused by a germline mutation in the tumor suppressor gene *APC* (OMIM 611731, located on chromosome 5q22.2) that leads to Wnt/β-catenin signaling activation (324). Interestingly, Bourdeau et al studied the gene expression profiling of PBMAH and described overexpression of genes related to the Wnt/β-catenin pathway signaling in PBMAH tissues (155). Gaujoux et al described a patient with FAP and PBMAH with abnormal β-catenin localization and biallelic inactivation of *APC* with somatic and germline genetic alterations (325). Biallelic inactivation of *APC* was found in 2 other patients with adrenocortical tumors carrying germline *APC* mutations but not in sporadic adrenocortical cancers (325).

More recently, Vouillarmet et al described the case of a 26-year-old man with primary aldosteronism and modest nonsuppressible cortisol following overnight 1-mg DST (326); bilateral macronodular adrenal hyperplasia was found at imaging. Following the demonstration of lateralized aldosterone secretion, the resected adrenal showed 3 nodules, one of them expressing aldosterone synthase and harboring a somatic *KNCJ5* mutation. Further gastrointestinal investigations led to the diagnosis of FAP, which was confirmed by the identification of a germline *APC* gene mutation. A study of the somatic DNA from the aldosterone-producing adenoma and a second adrenal nodule revealed biallelic *APC* inactivation demonstrated by LOH in both nodules supporting a sequence of 2 genetic events involving both *APC* and *KCNJ5* genes, at least in the specific aldosterone-secreting adenoma within the PBMAH tissue of this patient (326). More recently, abnormal expression of the GIP, 5-HT7 and LH receptors were observed in one *APC*-mutated adrenocortical tumor, but regulation of steroidogenesis by those receptors was not examined in vivo (327).

Hereditary leiomyomatosis and renal cancer syndrome

Hereditary leiomyomatosis and renal cancer syndrome (HLRCC) is an autosomal dominant disorder that is caused by a germline mutation in the tumor suppressor gene fumarate hydratase (*FH*, OMIM 136850, located on chromosome 1q43) involved in the Krebs cycle. It can manifest with leiomyomas (cutaneous and uterine), uterine leiomyosarcomas, renal cancer, and rarely PBMAH (44) (Fig. 3, upper panel). Matyakhina et al reported a patient with HLRCC carrying a germline inactivating FH mutation that had PBMAH associated with mild cortisol secretion. The study of the PBMAH tissue showed allelic losses of the 1q42.3-43 *FH* locus in addition to the germline *FH* mutation supporting a causal role of *FH* gene alterations in the development of PBMAH in this patient (44). In a study including 255 HLRCC patients, 20 patients had adrenal lesions (7.8%) and 3 were bilateral (15%). In all the 10 patients that underwent surgery, pathology examination revealed unilateral adenomas or PBMAH (328).

The *ARMC5* Gene

Identification of *ARMC5* inactivation in PBMAH

In 2013, whole genome sequencing and single-nucleotide polymorphism (SNP) arrays led to the identification of germline heterozygous inactivating variants of the armadillo repeat containing 5 (*ARMC5*) gene, mapped at 16p11.2, in 18 (54.5%) of 33 patients presenting with PBMAH treated by adrenalectomy (54). Systematically, the germline event was combined with another somatic heterozygous deleterious change of *ARMC5*, either copy-neutral loss of heterozygosity (LOH) of chromosome 16p or point mutations (missense, nonsense, frameshift, or splicing events), leading to a bi-allelic inactivation of *ARMC5*, compatible with a tumor suppressor gene model (329) (Fig. 3, middle panel). In a single patient, each adrenal nodule may harbor unique distinct somatic mutation (54, 330, 331); in 1 case, 17 different mutational events were identified in the 20 nodules studied (330) (Fig. 3, middle panel). Since the initial report in 2013, progress has been made through multiple genetic studies of PBMAH series and case reports, allowing for a better understanding of the phenotype associated with *ARMC5* alteration, its frequency, as well as the pathophysiological understanding of its involvement in adrenocortical tumorigenesis (53).

Clinical studies: mutation rate

Germline *ARMC5* mutations were first identified in almost 55% of a very selected population of PBMAH patients with severe CS needing adrenalectomy, but in more heterogeneous PBMAH patients, whether operated or not, this rate appears to be lower, approximately 20% to 25% according to further series of apparently sporadic patients showing more various phenotypes of PBMAH (8, 10, 32, 62, 127, 263). However, a recent Japanese series of 14 patients showed 71% of *ARMC5* mutation carriers and identified a probable hotspot specific to the Japanese population (NM_001105247.1: c.1855C>T:p.Arg619*), but the surgical status of the 14 patients was not mentioned (33). *ARMC5* mutations are responsible for around 80% of clearly familial cases of PBMAH (10, 11, 53, 88, 231, 232, 263, 332, 333). *ARMC5* pathogenic variants are spread all over the coding sequence of the gene, and more than 100 different germline variants have been reported to date, with just a few clearly identified hotspots (215). In patients with bilateral adrenal incidentalomas, germline *ARMC5* mutations were very rarely found (83, 334).

Genotype/phenotype correlation

The high frequency of *ARMC5* pathogenic variants in patients with a severe form of PBMAH requiring surgical therapy suggests that ARMC5 is responsible for a more pronounced phenotype, and it has been demonstrated by some series in the past few years: *ARMC5*-mutated index case patients have a more severe CS than wild-type patients according to 24-hour UFC, morning plasma cortisol after 1-mg DST, and midnight plasma or salivary cortisol, associated with lower ACTH (8, 32, 62, 127). They also display larger adrenals, more nodules (32, 62) and more frequent metabolic complications such as diabetes and arterial hypertension (32, 62). Further, they are more often treated, either by adrenalectomy

or medical treatment (32, 62), and when operated, the surgery is more often bilateral (62). However, this more distinct condition is not constant in all *ARMC5* mutated patients: indeed, according to a recent retrospective series involving 52 PBMAH index case patients with *ARMC5* pathogenic variants and 300 PBMAH wild-type patients, more than 40% of *ARMC5* patients had a normal 24-hour UFC value (62), which explains why some ARMC5 patients may not present with obvious clinical signs of CS, as previously described (10, 83, 334). Mild excess of cortisol secretion is the most frequent presentation in patients with PBMAH (7), particularly in wild-type patients (75%) and even in patients with *ARMC5* variants, but in a more balanced manner (57%) (62). In familial cases, related mutation carriers often present a less severe form of PBMAH than the index case, rarely with unilateral smaller lesions and an incomplete penetrance, although possible high penetrance that probably also depends on patient age since in large families some investigated relatives are younger than 40 years (10, 88). The mechanisms underlying this phenotypic disparity remain unknown and further familial cases are needed, to investigate the hypothesis of modifier genes. It is, however, possible that the germline *ARMC5* mutation produces the initial phase of bilateral hyperplasia, but the successive number of second somatic mutations are required to generate sufficient number or size of macronodules (overall PBMAH cell mass) to produce overt CS (215) (Fig. 3, middle panel). Index cases with overt CS constitute a selected population with more severe disease than patients without *ARMC5* mutations requiring surgery. However, cases detected following systematic genetic screening are likely to be identified during the whole spectrum of the slow evolution of the disease.

ARMC5 and aberrant receptors

The aberrant expression of GPCRs in PBMAH, leading to an increased secretion of cortisol in response to various hormonal stimuli, has been largely studied in the past 30 years (see "Aberrant G Protein–Coupled Hormone Receptors in Adrenal CS"). Cortisol responses to upright posture and vasopressin or metoclopramide administration (due to the abnormal expression of adrenergic receptors, vasopressin receptors, and serotonin receptors, respectively) have been reported in patients with *ARMC5* pathogenic variants with prevalence similar to that found in wild-type patients (32, 54, 88). An interesting finding is that *ARMC5* pathogenic variants have never been reported in GIP-dependent PBMAH related to the GIPR ectopic expression, which is consistent with the recent identification of *KDM1A* inactivation as the molecular cause of the peculiar presentation, and the apparent mutually exclusive nature of *KDM1A* and *ARMC5* mutations (55, 56).

ARMC5 in primary aldosteronism

In 2015, a systematic screening for *ARMC5* mutations in 56 patients investigated for primary aldosteronism (PA) identified 6 (10.7%) African American patients with germline pathogenic variants (160); however, in the light of the constantly evolving knowledge about variant classification, some of those variants may be, nowadays, classified as benign. In addition, the influence of *ARMC5* haplotypes on the renin-angiotensin-aldosterone system in African American individuals has been considered recently (335). In contrast, no *ARMC5* germline pathogenic variant were identified in an Italian series of 39 patients presenting with primary aldosteronism and bilateral adrenal masses (336), nor in 16 patients from 4 large families of familial hyperaldosteronism type II (FH-II) (337). At this point, the currently available data do not allow the establishment of a clear relationship between *ARMC5* mutations and primary aldosteronism, and further studies including larger series of primary aldosteronism patients will be necessary to clarify this possibility.

Associated lesions in patients with PBMAH

The causal role of genetic alterations of *ARMC5* in other tumors than PBMAH was initially demonstrated in a German report describing a family with PBMAH carrying a germline *ARMC5* pathogenic variant. *ARMC5* analysis of a meningioma of a woman from this family with concomitant PBMAH and multiple intracranial meningiomas showed the presence of a different somatic damaging somatic *ARMC5* variant supporting the two-hit hypothesis of tumorigenesis in its pathogenesis (338). Meningiomas were also found in 2 South Korean sisters with PBMAH and ectopic expression of vasopressin AVPR1 and AVPR2 receptors (50) and a French Canadian woman carrying a germline *ARMC5* pathogenic variant concomitantly with familial cortisol-secreting beta-adrenergic/vasopressin responsive PBMAH (88). Concomitant intracranial meningiomas with PBMAH were reported in 3 out of 7 members of an *ARMC5* Brazilian family (10), while they are diagnosed much less frequently in the general population. More recently, a number of other nonfamilial case reports of patients with PBMAH carrying *ARMC5* germline pathogenic variants having meningiomas were described (339, 340).

In 2020, Jojima et al studied the case of a 65-year-old woman with PBMAH and meningioma (340). In contrast to previous reports, this patient presented initially with neurological symptoms due to a meningioma and the diagnosis of PBMAH was performed later on. Interestingly, the woman carried a germline pathogenic variant of *ARMC5* and somatic loss of heterozygosity (LOH) of the *ARMC5* wild-type allele within her meningioma (340). This report further supports the two-hit mechanism of tumorigenesis in *ARMC5*-related meningiomas as well as found in PBMAH tissue (340), but in this case LOH instead of somatic *ARMC5* variant as described previously (338). Altogether, these data bring evidence for the involvement of *ARMC5* in meningiomas, but further studies with systematic cerebral imaging of both *ARMC5* patients and controls would be needed to assess precisely the penetrance of meningiomas among patients carrying *ARMC5* pathogenic variants prior to conclude to a multiple neoplasia syndrome.

In addition to meningiomas, other tumors were reported in patients with PBMAH carrying *ARMC5* germline pathogenic variants such as acromegaly (231), pancreatic serous microcystic adenoma, pinealoma and, primary hyperparathyroidism (231) that may suggest that *ARMC5* may be causative for multiple tumor syndrome. Although most of these tumors were not studied to search for a second somatic *ARMC5* hit, the previous demonstration of the wide expression of *ARMC5* in brain, pituitary gland and several other tissues support the possibility that *ARMC5* pathogenic variants may be responsible for additional tumors beyond PBMAH and meningiomas (14, 341).

Interestingly, both PBMAH and primary hyperparathyroidism may be found in MEN1 syndrome raising the question of possible overlap between clinical manifestations of germline *ARMC5* and *MEN1* carriers (342). In 2020, Damjanovic et al addressed this question inversely by performing germline *ARMC5* genetic analysis in patients with sporadic neuroendocrine tumors (spNETs) or MEN1 (343). Interestingly, 76 out of 111 patients (68.4%) carried *ARMC5* germline variants; however, only 2 were predicted as likely pathogenic or pathogenic and they were both found in 2 patients with classical MEN1 syndrome carrying germline *MEN1* pathogenic variants as well. The presence of ARMC5 haploinsufficiency or biallelic inactivation in spNETs and MEN1 related tumors led the authors to conclude that *ARMC5* variants may play a modifier role in the phenotype of patients with spNETs or MEN1 (343).

Further work is needed to determine if these associations are coincidental or causal and to better characterize the full spectrum of *ARMC5*-related neoplasias in addition to meningiomas and PBMAH.

ARMC5 functional studies

Genomics studies. Recently, the integrated genomic classification of benign adrenocortical lesions led to the identification of a common pattern of gene expression of *ARMC5*-mutated PBMAH, characterized by a gonadal transcriptomic signature, notably related to the relatively high expression of FOXL2 and CYP19A1, encoding the aromatase, as well as a miRNome specific clustering, a global hypermethylation in CpG islands, and frequent copy-neutral LOH in chromosome 16p (344, 345), as previously described (54). This specific pattern has been further corroborated by an integrated genomic analysis of 52 PBMAH lesions from 36 patients (56). The CYP19A1 overexpression has been clinically observed in a male patient with PBMAH and hyperestronemia associated with gynecomastia (297).

In vitro studies. The first clue of the pathophysiological involvement of ARMC5 in adrenocortical tumorigenesis was the demonstration of a decreased apoptosis in human adrenocortical cancer H295R cells transfected with *ARMC5* mutants compared with those transfected with wild-type *ARMC5* (32, 54). Despite a more pronounced CS in most *ARMC5*-mutated patients, cortisol secretion was less efficient in H295R cells with *ARMC5* silencing by siRNA, associated with decreased levels of expression of enzymes involved in steroidogenesis (54). Both decreased expression of steroidogenic enzymes genes and increased proliferative capacity have been confirmed in nonmutated PBMAH cell cultures after *ARMC5* silencing (260). Conversely, the overexpression of ARMC5 in both mutated and nonmutated PBMAH cell cultures led to a decrease in steroidogenesis and apoptosis (260) (Fig. 3, middle panel). Altogether, despite a decreased steroidogenic capacity of each cell (following reduced expression of steroidogenic enzymes), we can hypothesize that the increased adrenal volume, at least partially due to the impaired apoptosis, leads to a global overproduction of cortisol (346). ARMC5 protein is ubiquitously expressed (341) and contains a N-terminal armadillo repeat domain and a C-terminal BTB domain. Cavalcante et al demonstrated an interaction between ARMC5 and the E3-ubiquitin ligase cullin3 through its BTB domain, leading to ARMC5 ubiquitination and further degradation by the proteasome (347). This interaction is impaired by the presence of an *ARMC5* mutation in its BTB domain, leading to an altered degradation of ARMC5 by the proteasome, which could be involved in cell cycle dysregulation (347).

Three recent articles provide important pieces of evidence on the role of ARMC5 in this cullin-RING E3 ubiquitin ligase complex: beyond the interaction with cullin3, ARMC5 is an adaptor of this complex, and can bind different substrates. The first study revealed that ARMC5 previously believed to be a cytosolic protein can also shuttle to the nucleus, form homodimers, and anchor a multiple subunit RING-type ubiquitin ligase (E3) specific for RPB1 the largest subunit of DNA-dependent RNA polymerase II (Pol II) (348). Mutations of *ARMC5* in PMBAH tissues or its deletion in mice compromised the function of this E3 by either reducing its binding with RPB1 or with CUL3, leading to reduced RPB1 ubiquitination (demonstrated by in vitro assay), a huge accumulation of RPB1 and enlarged Pol II pool size. The loss of ARMC5 in mice adrenal glands resulted in differential expression of 1486 genes, the majority (93.5%) were upregulated including antiproliferative genes Pcdh8 and Tfcp2l1, while others are oncogenes such as Mafa and Taf4b and were also overexpressed in *ARMC5* variant PBMAH tissues; in contrast the rate-limiting steroidogenic enzyme StAR expression was reduced in (348).

Two other studies identified that in the cytosol, ARMC5 forms other E3 ubiquitin ligase complexes binding to sterol regulatory element-binding transcription factors (SREBF), which are transcriptional activators involved in cholesterol synthesis (349), and nuclear respiratory factor 1 (NRF1), a transcriptional regulator involved in redox homeostasis (350). Once bound, these substrates were ubiquitinated and further degraded by the proteasome. But a mutant ARMC5 protein, consecutive to a genetic variation affecting its BTB (cullin binding) or Armadillo (substrate binding) domains, leads to impaired ubiquitination of these substrates and thus, to their stabilization and accumulation, which can in turn affect transcription, steroidogenesis and redox homeostasis. These observations bring to light several new functional roles for ARMC5, which could serve either in the nucleus or cytosol as an adaptor for other substrates to be further identified. Further studies will be required to identify how loss of ARMC5 leads to specific modifications of transcription, post-translational alterations of the effectors responsible for the relatively slow process of benign proliferation characteristic of PBMAH in contrast to the more rapid or aggressive proliferation in other adrenal tumors.

In vivo studies. Murine models of *Armc5* deficiency have been generated and studied. In mice, the complete knockout (KO) of *Armc5* (*Armc5*-/-) leads to early embryonic death in most cases by preventing gastrulation (351), but this depends on the genetic background of the mice (352). KO embryos and living pups are smaller than their wild-type counterparts. Aged KO mice have increased corticosterone levels and show an adrenal hyperplasia without nodules. Besides, KO mice have impaired T-cell proliferation and differentiation, compromising immune response (352). Heterozygous *Armc5* deficient mice (*Armc5*+/-) show a transient decrease of corticosterone levels at 1 year of life, consistent with the decreased expression of steroidogenic enzymes, followed by a further normalization, and in one-third of them a consecutive

hypercorticosteronemia at 18 months of age, associated with some changes in adrenal cortex architecture, but do not display adrenocortical hyperplasia nor nodules; Hu et al also found increased glucocorticoids levels in older KO mice with larger but non-nodular adrenals (352). These findings suggest that *Armc5* haploinsufficiency is not sufficient to induce adrenocortical tumorigenesis (351).

KDM1A Inactivation in GIP-dependent PBMAH

In 2 recent studies from our groups, the *KDM1A* gene was identified as a gene involved in GIP-dependent PBMAH with CS (55, 56) (Fig. 3, lower panel). In one study, we reported germline pathogenic or likely pathogenic variants in *KDM1A* in all 17 (100%) patients studied with familial or apparently sporadic GIP-dependent PBMAH with CS (55). Most patients also displayed a deletion of the chromosome 1p region including the *KDM1A* locus in their adrenal tissues, resulting in the loss of KDM1A expression in the adrenal lesions (55). Further, *KDM1A* pathogenic or likely pathogenic variants were identified in 9 out of 10 (90%) familial or apparently sporadic patients with GIP-dependent PBMAH with CS, associated with 1p deletions in 6 (100%) studied patients (56) (Fig. 3 lower panel). *KDM1A* variants were distributed over the whole gene. Sequencing of the entire span of the gene is therefore required for diagnostic purposes. None of patients harboring *KDM1A* variants had *ARMC5* genetic variation or any other genetic event in genes associated with adrenal tumorigenesis. Taken together, our studies revealed that GIP-dependent PBMAH is a genetic disease caused almost in all cases by germline inactivating pathogenic variants of *KDM1A* with a loss of heterozygosity (LOH) of the second *KDM1A* locus in the adrenal lesions, suggestive of a tumor suppressor gene model of tumorigenesis.

In contrast, none of the studied patients with GIP-dependent adrenocortical adenomas harbored *KDM1A* pathogenic variants (55) suggesting that they arise from a different molecular mechanism such as chromosome rearrangement as previously reported (192).

KDM1A, also called *LSD1*, encodes the lysine demethylase type 1A (353). Histone tails are subjected to covalent modifications that affect chromatin structure and the recruitment of regulatory factors consequently affecting transcription (354). Methylation of histone lysine residue can be associated with either activation or repression of transcription. Interestingly, KDM1A is involved in several physiological processes and has an important role in the developmental differentiation of endocrine cells. KDM1A is a key factor in the differentiation of pituitary somatotroph lineage and of pancreatic cells (355, 356). However, the role of KDM1A in the adrenal has been unknown so far.

In the setting of PBMAH, *KDM1A* acts as a tumor suppressor gene with germline pathogenic variant associated with somatic LOH. In affected patients, mRNA and KDM1A protein levels were lower in affected patients compared to PBMAH without evidence of food-induced cortisol production. The global gene expression and more generally the genomic profiles of affected patients were profoundly different from those presenting *ARMC5* mutation or from those in whom no genetic events were identified (55, 344). From a histological point of view, BMAH with KDM1A functional loss displayed foci of myeloid and lipomatosis metaplasia, or myelolipoma (55) and increased percentage (>30%) of eosinophilic cells (56) (see "Pathology" section).

Functional studies validated the implication of KDM1A in GIP-dependent PBMAH. In vitro pharmacological inhibition and silencing of KDM1A using siRNA led to an increased GIPR expression in human adrenocortical H295R cells. Also, the knockout of *KDM1A* by Crispr-Cas9 was responsible for an increase in GIPR expression (55) (Fig. 3, lower panel). The exact mechanism of regulation of GIPR expression by KDM1A is not fully understood. KDM1A was first functionally characterized as a lysine demethylase (353). However, accumulating evidence indicates that KDM1A is involved not only in epigenetic regulation of gene expression but also in the regulation of other cellular pathways by several mechanisms including protein-protein interactions, protein stability, regulation of subcellular localization, or promoter binding (354).

Our studies did not dissect the exact molecular mechanism by which KDM1A loss results in aberrant GIPR transcriptional activation in the adrenal tissue. In addition, whether *KDM1A* genetic inactivation, which seems mutually exclusive with *ARMC5* genetic variation, per se increases adrenal cell proliferation and leads to adrenal lesion development remains an open question.

KDM1A was previously described as a gene predisposing to familial multiple myeloma (357). In our studies, we reported 2 patients belonging to a family with occurrence of multiple myeloma or monoclonal gammopathy of unknown significance (MGUS) in several members (55, 56). Other neoplasia in patients and their relatives were also described without clear association so far. Nonetheless, clinicians should be aware of those possible associations especially with multiple myeloma during follow-up of patients with GIP-dependent PBMAH with CS with germline *KDM1A* pathogenic variants. In the largest family studied to date, penetrance of PBMAH development was relatively low in germline *KDM1A* mutation carriers as second somatic event in adrenal tissues is required to result in GIP-dependent PBMAH (55); however, a larger number of families will need to be studied to determine more precisely the adrenal disease prevalence.

In summary, *KDM1A* pathogenic variants are the genetic cause of GIP-dependent CS with PBMAH (Fig. 3, lower panel). Genetic variation in *KDM1A* should be screened for in patients with PBMAH associated: 1) with low fasting plasma cortisol concentrations increasing after meal intake; and 2) with histological traces of myeloid metaplasia or myelolipoma in their adrenals. As of today, sequencing of *KDM1A* in PBMAH patients without these 2 characteristics does not seem founded as none of the patients without evidence for food-dependent cortisol production harbored *KDM1A* genetic variation. However, larger cohorts of patients need to be analyzed before a more straightforward recommendation can be made. Patients presenting with PBMAH with overt or subclinical CS should therefore be well biochemically phenotyped, and especially low fasting cortisol concentration should attract attention. Of note, in case of bilateral incidentaloma, screening of CS by a 1-mg DST exclusively could misdiagnose patients with food-dependent PBMAH, since their fasting plasma cortisol is low. We now recommend that ectopic GIPR should be examined in every patient with PBMAH, as *KDM1A* mutations would be likely present with high risk for familial cases and associated malignancies. This can be screened simply by measuring serum cortisol

before and 2 hours after an oral 75-g glucose load or a mixed meal. A reproducible more than 50% increase in cortisol would warrant genetic testing for *KDM1A* mutation (Fig. 2).

In patients with an identified pathogenic variant, plasma protein electrophoresis should be performed for the possible increased risk of multiple myeloma and MGUS (357). In first-degree relatives, *KDM1A* germline pathogenic variants should be genetically screened. In kindreds carrying *KDM1A* variants, hypercortisolism should by searched for by careful clinical examination and biochemical assessment including combination of 24-hour urinary free cortisol excretion with fasting (morning) and postprandial serum cortisol concentration and plasma protein electrophoresis.

Genetic Aspects: Involvement of the cAMP/PKA Pathway

Constitutive activation of the cAMP/PKA pathway, whether by activating mutations of positive regulators or inactivating mutations of negative regulators, may lead to adrenocortical tumorigenesis and steroid production (346).

Two concomitant mutations of *MC2R*, encoding the ACTH receptor, have been reported in a single case of PBMAH, leading to a loss of the capacity of ligand binding and a constitutive activation of the receptor (358).

Post-zygotic gain-of-function mutations of *GNAS* are observed in McCune-Albright syndrome (MAS) potentially associated with nodular adrenal hyperplasia and CS with internodular adrenal atrophy, arising from the fetal adrenal tissue and occurring in early infancy (359). Somatic activating variants of *GNAS*, not found in germline DNA, were described in some cases of apparently isolated PBMAH, without any other feature of MAS (143, 298) (Fig. 3, upper panel). The 2 different variants found in the 4 patients affected the same codon 201 (NM_000516.7: c.601C>A:Arg201Ser in 1 patient, NM_000516.7:c.602G>A: p.Arg201His in 3 patients, respectively). It is still unclear whether this corresponds to localized somatic pathogenic variants in the adrenal tissue or to a later genetic event in *GNAS* during embryogenesis, leading to a low mosaicism and thus to a very localized form of MAS (360).

The phosphodiesterases (PDEs) form a superfamily of 12 different forms of enzymes involved in the degradation of the cyclic nucleotides cAMP and cGMP. The cAMP-specific PDEs act as negative regulators of the cAMP/PKA pathway. Germline variants of the *PDE11A* gene, encoding a PDE able to degrade both cAMP and cGMP, have been reported more frequently in PBMAH patients than in control subjects (361) (Fig. 3, upper panel). Some of these variants have been proven to be responsible for an impaired enzymatic activity in vitro (362). A murine model of *Pde11a* KO shows a surprising continuing expression of *Pde11a* in most tissues but presents an adrenocortical phenotype associating adrenal hyperplasia and impaired dexamethasone suppression (363), suggesting that *PDE11A* might play a role in PBMAH development.

Similarly, germline variants of *PDE8B*, encoding a cAMP-specific PDE, have been reported in rare cases of PBMAH (364), after being identified in a 2-year-old girl presenting with isolated micronodular adrenal dysplasia and severe CS and her father with a more attenuated form of the disease (365).

Germline duplication of the *PRKACA* gene, encoding the catalytic subunit of the PKA, have been primarily identified in 4 patients presenting with PPNAD and 1 patient with PBMAH diagnosed at the age of 3 (366). *PRKACA* duplication has been further observed in a mother and her child both presenting with PBMAH (367).

Genetic and Clinical Screening of Kindreds

At least 18 PBMAH families have been identified as carriers of heterozygous germline pathogenic variants in the *ARMC5* gene, affecting between 2 and 16 members of the family (10, 11, 52, 82, 88, 127, 232, 332, 333, 338, 368). The most common *ARMC5* germline variants found in familial PBMAH were frameshift and nonsense (32, 369). Data on familial *KDM1A* PBMAH is limited. Up to now, 2 families with a mother and a daughter affected by PBMAH were described (55, 56). Another family included a brother and a sister with GIP-dependent PBMAH and 5 family members with multiple myeloma or monoclonal gammopathy of undetermined significance (55); among them, 3/5 underwent germline genetic testing that revealed the familial *KDM1A* variant segregation.

ARMC5 and *KDM1A* genetic screening

Considering the high phenotypic heterogeneity of PBMAH, including in mutated *ARMC5* patients, genetic screening for *ARMC5* variant should be offered to all patients presenting with bilateral adrenal masses larger than 1 cm, associated with at least mild dysregulated cortisol secretion, defined by a plasma cortisol after 1 mg DST above 50 nmol/L, as recently proposed (62), and to all first-degree relatives of identified probands harboring an *ARMC5* pathogenic variant. The interpretation of *ARMC5* genetic variations should be cautious and made according to the American College of Medical Genetics/Association for Molecular Pathology (ACMG/AMP) guidelines (370), especially for missense substitutions, in order to not misclassify those patients.

These patients should be engaged in shared decision making for genetic testing. Genetic counseling should be offered for a clear understanding of the medical, psychological, and familial implications of genetic contribution to PBMAH and written consent should be obtained. A detailed past medical and familial history of PBMAH should be performed and a search included for adrenal diseases and CS in addition to clinical manifestations associated with multiple endocrine neoplasia type 1 (MEN1) syndrome, familial adenomatous polyposis (FAP), or hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome. Targeted next-generation sequencing (NGS) is now the recommended approach by enabling testing of several relevant genes in a single panel. NGS generates a large set of data and careful interpretation of an identified variant is mandatory and should follow the Standards and Guidelines for the Interpretation of Sequence Variants as described previously (370).

KDM1A genetic analysis should be performed in PBMAH patients suspected of having GIP-dependent cortisol excess (Table 7, Fig. 2). Screening for the presence of ectopic GIPR should be proposed to all patients with PBMAH by measuring serum cortisol before and 2 hours after an oral 75-g glucose load or a mixed meal. A reproducible more than 50% increase in cortisol would support this diagnosis and lead to genetic testing for *KDM1A* pathogenic variant. Although clinical and biochemical phenotypes may guide genetic analysis in

patients before surgery, for patients who already underwent bilateral adrenalectomy and in whom clinical screening for ectopic GIPR was not or cannot be performed, both *ARMC5* and *KDM1A* genetic testing should be offered.

Complementary investigations for *ARMC5* and *KDM1A* carriers

ARMC5 pathogenic variants carriers should undergo brain imaging to exclude a coexisting meningioma. Carriers of *KDM1A* pathogenic variants should be screened biochemically for GIP-dependent stimulation of cortisol if not already performed, and complete blood count and serum protein electrophoresis should be performed to detect potential associated multiple myeloma or MGUS (55).

Familial screening for first-degree relatives

Considering that PBMAH has an autosomal dominant pattern of inheritance, all first-degree adult relatives of PBMAH index cases carrying a pathogenic variant should be offered genetic testing for the specific variant found in their relative index cases (Table 7). Late age of onset or the lack of family history of the disease do not exclude a germline pathogenic variant and may reflect lack of diagnosis of PBMAH or low penetrance of the disease in the other family members.

All *ARMC5* pathogenic variant adult carriers should have a detailed questionnaire and physical examination. Hormonal evaluation should include 1-mg DST; the frequency of repeat screening has not been determined, but it seems reasonable to perform this test every 2 years if levels are well below the 50 nmol/L cutoff and annually if adrenal lesions are present. We recommend that adult relatives carrying a pathogenic *ARMC5* variant undergo adrenal imaging and brain imaging at baseline. Once again, no published study can inform us on the frequency of repeated imaging if the initial imaging is normal and the 1-mg DST is also normal; considering the slow progression of PBMAH, a 5-year interval in adrenal imaging appears reasonable unless clinical or biochemical alterations occur.

In adult *KDM1A* pathogenic variant carriers, in addition to questionnaire and examination, hypercortisolism should be assessed with fasting and postprandial plasma cortisol concentration, as 1-mg DST could be falsely low because of overnight fasting. All carriers should undergo plasma protein electrophoresis (Table 7).

In apparently sporadic cases where no germline pathogenic variant was identified in the index case, the possibility of familial PBMAH is not completely excluded as it may be secondary to a yet-unidentified genetic cause of PBMAH. Keeping this in mind, the first-degree family members of PBMAH index cases should undergo careful clinical examination and biochemical screening using a 1-mg dexamethasone test to exclude modest hypercortisolism in particularly those older than 50 years; in specific cases already presenting new onset diabetes, obesity, and or arterial hypertension, more extensive biochemical investigation may be required (Table 6). The best long-term surveillance for kindred of patients without an identified PBMAH genetic driver event remains to be determined based on multicenter large prospective systematic screening studies.

In summary, genetic screening for *ARMC5* and *KDM1A* should now be offered for most PBMAH patients. The identification of a causal germline pathogenic variant allows earlier diagnosis and therapy of the disease in unsuspected cases in families, thus avoiding the deleterious comorbidities of hypercortisolism. Further work is needed to better characterize genotype/phenotype correlations in larger cohorts of patients with PBMAH and to extend the spectrum of germline causal genetic variants of PBMAH. In addition, the frequency and interval of biochemical and imaging screening to identify progressive development of PBMAH in asymptomatic variant carriers remain to be better determined, as such cohorts will be studied prospectively.

Therapy of PBMAH

Indications for Therapy of PBMAH Patients With Excess Cortisol Secretion

A challenging aspect in evaluating PBMAH patients with mild cortisol secretion is the selection of those who will require treatment. The degree of cortisol excess and resulting comorbidities are determinant to decide which patient needs a specific therapy for hypercortisolism. It is well-established that PBMAH patients with overt CS and elevated UFC need to be treated (24, 81, 371). For patients with mild dysregulated cortisol excess and normal UFC levels, decision making is, however, more complex; while management has followed recommendations for adrenal incidentalomas, it has not been validated specifically for PBMAH patients (26, 264). In patients with normal UFC, normal ACTH, cortisol <138 nmol/L (<5 mcg/dL) following 1-mg overnight DST, and absence of hypercortisolism-induced comorbidities, monitoring without adrenal surgery is a fair option (Fig. 2). In these patients, further surgical therapy should be based on the progression of hypercortisolism comorbidities such as arterial hypertension, diabetes, obesity, dyslipidemia, osteoporosis, and neuropsychological manifestations (26, 264). However, recent studies reporting progressively increased mortality rates, mainly related to infection and cardiovascular events in patients with an adrenal incidentaloma and modest cortisol excess, have influenced the management of patients with PBMAH (27, 28, 372, 373). A systematic review and meta-analysis of 4121 patients with benign adrenal incidentalomas (14.5% bilateral, representing 21.8% of those with modest cortisol secretion) found a prevalence of hypertension (64%), obesity (41%), dyslipidemia (34%), type 2 diabetes (28%), and cardiovascular events (6%) in patients with mild autonomous cortisol secretion (MACS) that is remarkably higher than expected for Western populations (265). The European Society of Endocrinology guidelines recommended that surgical treatment should be considered in an individualized approach based on the patient's age, general condition, cortisol-related comorbidities, degree of cortisol excess, and patient preference, as adverse outcomes were reported in patients with plasma cortisol >138 nmol/L (>5.0 mcg/dL) after a 1-mg DST (26). This was supported by the cross-sectional EURINEACT study of 1305 patients with adrenal incidentalomas (67% women); greater prevalence and severity of hypertension in those with post–1-mg DST cortisol > 138 nmol/L and CS (UFC above normal) compared with patients with nonsecreting tumors (DST < 50 nmol/L; adjusted prevalence ratios [aPRs], 1.15 and 1.37, respectively) as well as higher prevalence of requirement for 3 or more antihypertensive medications (aPRs, 1.31 and 2.2, respectively) (27). Diabetes was more prevalent only in CS compared with nonsecreting tumors (aPR, 1.62), and insulin therapy was

Table 7. Suggested investigations for individuals with PBMAH and their first-degree kindreds/relatives

Baseline investigation at genetic diagnosis	Carrier of germline *ARMC5* pathogenic variants	Carrier of germline *KDM1A* pathogenic variants	No identified germline genetic causes
Clinical diagnosis	PBMAH non-GIP-dependent CS	PBMAH GIP-dependent CS	PBMAH
Hormonal investigation	1-mg dexamethasone suppression test	Fasting morning cortisol level Serum cortisol before and 2 hours after an oral 75-g glucose load or a mixed meal.[a]	1-mg dexamethasone suppression test to consider; particularly if patient >50 yo, present with obesity and/or diabetes and/or high blood pressure
Adrenal imaging	Yes	Yes	Yes, if abnormal hormonal investigation
Other investigations	Brain imaging	Plasma protein electrophoresis	none

[a]A more than 50% reproducible increase in cortisol suggests the diagnosis of GIP-dependent cortisol secretion. If positive, complete with urinary free cortisol, plasma ACTH.

Practical tip/synthesis:

- PBMAH is more frequently hereditary than previously believed.
- In rare syndromic etiologies, such as MEN1 or familial polyposis, presence of adrenal lesions should be screened by adrenal imaging.
- First-degree relatives of patients with PBMAH should be offered screening for early identification of development of this disease.
- Table summarizes our recommendations for such screening based on the presence or not of identified genetic variants.
- Every patient with PBMAH should be screened for possible food-dependent aberrant response as germline *KDM1A* variant carriers may be predisposed to other diseases such as MGUS or multiple myeloma.
- Recommendations for age and frequency of repeated screening will become better defined by long-term studies of large cohorts of patients and families with PBMAH.

required more frequently in persons with DST > 138 nmol/L and CS than in those with nonsecreting tumors (aPRs, 1.89 and 3.06, respectively). Patients with bilateral adrenal lesions (probably a high proportion with PBMAH and mild cortisol excess) presented with almost double the incidence of morning cortisol post DST > 138 nmol/L and severity of cortisol excess; only patients with bilateral tumors and cortisol post DST > 138 nmol/L had an increased metabolic burden. Using multisteroid profiling, increasing urinary glucocorticoid excretion progressively suppressed plasma ACTH, serum DHEAS levels, and urinary androgens, which correlated with the degree of cortisol excess (27). This study may have underestimated cardiometabolic risk of mild dysregulated cortisol secretion as the so-called nonfunctional tumor control group already had much higher prevalence of hypertension (64%) and diabetes (28%) than expected in a normal population; as so-called nonsecreting tumors do secrete small amounts of cortisol in vitro, it remains to be determined what is the real impact of very low cortisol secretion by unilateral incidentalomas or PBMAH (266). A retrospective cohort study of 1048 patients with adrenal incidentalomas in Sweden identified increased mortality hazard ratios of 2.30 for patients with post–1-mg DST cortisol levels of 83 to 137 nmol/L and 3.04 for cortisol levels of 138 nmol/L or more compared with those with cortisol levels less than 50 nmol/L (28); thus, further studies may better identify increased risks at cortisol levels between 50 and 138 nmol/L after DST (266). Thus, surgical therapy should be suggested if there are signs of disease progression during follow-up, such as suppression of ACTH, increased UFC, or impact of hypercortisolism on various target organs (26, 264) (Fig. 2).

Available Treatments for PBMAH

Medical therapy

Adrenal enzyme inhibitors such as ketoconazole and metyrapone can be efficient options before adrenalectomy or when severe hypercortisolism has resulted in a life-threatening morbidity (264, 374). Very severe cortisol excess is rare in patients with PBMAH, but in exceptional patients with acute psychosis, pulmonary embolism, uncontrolled arterial hypertension, infection, or myocardial infarction, rapidly decreasing cortisol plasma levels with these medical therapies can be life-saving when surgery needs to be delayed (374). Ketoconazole was used in some case reports of patients with PBMAH either as a bridge while awaiting further therapy (39, 255) or during long-term low-dose therapy (375) (Table 8). Metyrapone was effective in 2 PBMAH patients in the large retrospective UK study (376, 377) as well as in 2 Japanese patients with PBMAH including 1 woman treated for 7 years without development of hirsutism (377, 378). In a PBMAH patient in whom monotherapy with ketoconazole or osilodrostat was inefficient, well-tolerated synergetic combination of ketoconazole and osilodrostat achieved good control of hypercortisolism (379). As the 11-hydroxylase (cyp11B1) inhibitors metyrapone and osilodrostat produce an increase in the immediate precursor 11-deoxycortisol, this may produce clinically relevant crossreactivity with cortisol in both blood and urine concentrations when immunoassays are used for their monitoring. Structure-based assay modalities such as LC-MS should be utilized to monitor blood and urine levels of cortisol.

Dosing can be adjusted according to cortisol diurnal rhythm, with higher doses in the evening than during daytime to reset the cortisol nycthemeral rhythm and reduce the cardiovascular risk marker interleukin-6 (380) (Table 8). Trilostane, a 3β-hydroxysteroid dehydrogenase inhibitor, partially suppressed cortisol production with clinical improvement, but did not prevent PBMAH growth during a 4-year treatment period (383). Rarely, PBMAH patients were treated with mitotane, a multisteroidogenic enzyme inhibitor and adrenolytic agent (384). In patients who would present excess cortisol production in a cyclic manner, steroidogenesis inhibitors should be administered in a block-and-replace strategy with hydrocortisone on 2 to 3 split doses.

In a small series of 4 PBMAH patients with CS, the competitive glucocorticoid (and progesterone) receptor antagonist mifepristone induced clinical remission and reduced excess cortisol comorbidities in a median time of 5 months (Table 8); the most common side effect was fatigue (381). However, it is difficult to adjust therapy, since it can be based on glucose and blood pressure improvement and clinical symptoms, while cortisol levels are not reliable markers of drug efficiency.

Medical therapy for aldosterone excess. In patients with PBMAH with confirmed renin-independent aldosterone excess in addition to cortisol excess, medical therapy with the specific mineralocorticoid receptor antagonists spironolactone or eplerenone are essential to normalize blood pressure, potassium levels, and to normalize renin activity or levels in order to revert the increased morbidity of primary aldosteronism (385, 386) (Table 8). Use of the epithelial sodium channel (ENAC) blocker amiloride, potassium supplements, or other antihypertensive drugs can be required with careful monitoring if mineralocorticoid receptor antagonists alone are not efficient (386). If cortisol excess is sufficient to indicate a surgical approach, significant aldosterone excess may indicate the need to conduct adrenal vein sampling to confirm the dominant source of cortisol and aldosterone excess using blood metanephrine concentrations to correct for blood flow.

Specific pharmacological therapies. Since cortisol secretion is regulated by aberrantly expressed GPCRs in 77% to 87% of PBMAH patients with modest or overt CS (see "Aberrant G Protein–Coupled Hormone Receptors in Adrenal CS") (218), some targeted pharmacological therapies aimed at blocking the activation of the aberrant receptors were attempted (Table 8). In GIP-dependent PBMAH, pharmacological blockade of postprandial release of GIP with the somatostatin receptor analogs octreotide or pasireotide was only transiently effective, requiring an eventual adrenalectomy after a few weeks of medical treatment (72, 188, 196, 387, 388). In the future, targeting ectopic GIPR receptor by its naturally occurring GIP antagonist such as GIP(3-30) NH2 could be an interesting therapeutic option for patients with GIP-dependent CS (382, 389). In patients with PBMAH and CS regulated by aberrant β-adrenergic receptors (catecholamine-dependent CS), β-blockers, such as propranolol or nadolol, can provide long-term control of hypercortisolism in some patients (88, 233-235, 387). However, only biochemical improvement is obtained in other patients, without clinical improvement (210). If β-adrenergic receptor (ADRB) antagonists are unable to completely normalize UFC or if their use is limited by side effects such as bradycardia, unilateral adrenalectomy is suggested with maintenance of β-blockers to control production from contralateral gland (88, 193, 216). This approach was also successful in one case of PBMAH with combined CS and primary aldosteronism regulated by β-adrenergic and V1-vasopressin agonists in whom atenolol was efficient during 10 years until escape required adrenalectomy (390). In PBMAH patients with LH/hCG-dependent CS, administration of long-acting leuprolide acetate—a GnRH agonist – can suppress endogenous LH and allow control of hypercortisolism for more than 10 years without CS progression (31, 37, 205, 210, 237, 246) or of estrogen excess in another PBMAH case (87); it may be necessary to replace lack of gonadal steroids and monitor bone density at regular intervals, especially in female patients before the age of menopause.

The AVPR1 and HTR4 are the 2 most prevalent aberrant GPCR regulating cortisol secretion in patients with PBMAH, but unfortunately, there are currently no available antagonists for those receptors (193, 218). Similarly, there are no available antagonists for the aberrant ligands in PBMAH tissues such as ACTH, but some novel antagonists are under clinical trials for other indications and may become quite useful therapies in this setting in the future. Since in most PBMAH tissues, excessive steroid production is regulated by several aberrant receptors together with locally produced ACTH, targeting may require combined therapies (258) (Table 8).

Surgical Therapy

Bilateral adrenalectomy

Bilateral adrenalectomy has been the recommended therapeutic approach in PBMAH patients with overt CS (7, 81). Laparoscopic bilateral adrenalectomy has been the minimally invasive and safe procedure of choice, although patients with severe hypercortisolism carry higher perioperative morbidity and mortality risks than patients with no or modest cortisol secretion (391). This approach requires lifelong gluco- and mineralocorticoid replacement with current incompletely physiological oral steroid regimens and carries the significant risks of adrenal insufficiency crisis (7, 24, 371, 391-393). A systematic review of 1300 CS patients who underwent bilateral adrenalectomy for various etiologies in 37 studies found it improved symptoms and comorbidities in the majority of CS patients; surgical morbidity was 18% for postoperative complications and 3% for mortality (391). Adrenal crisis incidence was heterogeneous in 6 studies ranging from 9% to 64% (391). Two studies with the largest number of PBMAH patients reached similar conclusions, namely that bilateral adrenalectomy is a safe, effective, and long-lasting option for patients with CS (394, 395).

Unilateral adrenalectomy

To avoid lifelong steroid dependence following bilateral adrenalectomy, some authors started in the 1990s to perform single adrenal resection (Fig. 7) as a surgical alternative for PBMAH patients, particularly those with less severe CS (Table 9) (77, 152, 200, 216, 396). In the first 4 case reports, patients achieved remission, while 3 patients experienced adrenal insufficiency, 1 transient for 17 months, and the other 2 were still present after 12 and 64 months of follow-up; no patient progressed to hypercortisolism requiring a contralateral adrenalectomy during follow-up. Other case reports and small-sized series including from 2 to 124 PBMAH cases totaling 286 patients in 31 publications treated with unilateral adrenalectomy are summarized in Table 9 (30, 51, 85, 169, 171, 210, 221, 233, 278, 320, 387, 397-408). In other studies, unilateral adrenalectomy was also performed in 20 other cases with clinical improvement, but these are not included in the table since detailed biochemical and follow-up data were insufficiently provided (263, 409, 410). Among patients who underwent unilateral adrenalectomy, some expressing aberrant receptors (GPCRs) were also treated with specific pharmacological therapies (Table 9 and next section). Overall, initial CS remission was achieved in 222 patients (77%). Among them, 83 patients (29%) experienced a

Table 8. Current or potential future medical therapies for PBMAH

Target	Drug	Dose	Studies in PBMAH	Potential adverse effects	Particular issues
Adrenal steroidogenesis inhibition	Ketoconazole	200–1600 mg/d PO, dosing BID/TID Potential use low-dose bedtime in mild cortisol secretion?	Limited case reports (39, 255) or low dose longer-term (375)	GI disturbances, hypogonadism in males, adrenal insufficiency	• *EMA approved for treatment of endogenous CS, off-label use in US* • Needs gastric acid (avoid PPIs) • Risk for serious hepatotoxicity; mostly transient but regular monitoring required • Careful review of other medications for potential drug-drug interactions
	Metyrapone	500 mg- 6 g/d PO; dosing q 6–8 hours Potential use low-dose evening in low cortisol secretion?	Limited case reports (376) or long-term use (377, 378). Low dose in evening in mild cases (380)	Increase in androgenic and mineralocorticoid precursors; hirsutism, hypertension, hypokalemia, adrenal insufficiency	• *EMA approved for treatment of CS* • Possible cross reactivity with 11-deoxyxortisol in cortisol immunoassays
	Osilodrostat	2–60 mg/d PO dosing BID	Single case in combination with ketoconazole (379)	Increase in androgenic and mineralocorticoid precursors; hirsutism, hypertension, hypokalemia, adrenal insufficiency	• *FDA approved for patients with CD* • *EMA approved for treatment of CS* • Not yet widely available
Glucocorticoid receptor antagonist	Mifepristone	300–1200 mg/d PO dosing daily with meal	One series 4 PBMAH cases (381)	GI disturbances, hypokalemia, arthralgia, peripheral edema, hypertension, vaginal bleeding, adrenal insufficiency	• *FDA approved for hyperglycemia with CS* • No laboratory markers of efficacy • Careful review of other medications for potential drug-drug interactions is essential
Mineralo corticoid receptor antagonists	Spironolactone Eplerenone	25–400 mg/d PO dosing daily 50–400 mg/d PO dosing BID	Aldosterone cosecretion Hypokalemia from large cortisol excess	Hyperkalemia, renal insufficiency, hypotensiom	• Spironolactone: anti-androgenic activity results in gynecomastia, impotence in males at higher levels
Aberrant receptor or ligand antagonists	Octreotide Pasireotide	100–1500 mg/d SC dosing BID-TID 0.6–1.8 mg/d SC dosing BID	GIP-dependent PBMAH transient efficacy (72, 188, 196, 201, 205, 210)	Hyperglycemia, diabetes, arrhythmias with QT-prolongation, diarrhea, nausea, abdominal pain, cholelithiasis, fatigue	• Short-term efficacy following desensitization of inhibition of GIP release by K cells • Potential future therapy with GIPR specific antagonists such as GIP(3-30)NH2 (382)
	β-blocker	Propranolol 30–120 mg/d PO dosing TID	β-adrenergic-responsive PBMAH short and long-term response (88, 216, 233-235, 387)	Bradycardia, fatigue, hypotension, asthma	• Frequent expression of more than one aberrant receptor may limit efficacy

(*continued*)

Table 8. Continued

Target	Drug	Dose	Studies in PBMAH	Potential adverse effects	Particular issues
		Nadolol 20–120 mg/d PO dosing daily		exacerbation, insomnia, drowsiness, depression	• Can be combined with unilateral adrenalectomy • No demonstration of reduction of PBMAH adrenal size • Combined use of MRA antagonists if aldosterone cosecretion
	Leuprolide acetate depot	3.75–7.5 mg SC dosing; monthly	LH/hCG-dependent PBMAH long-term efficacy to control cortisol/androgen excess (37, 205, 210, 237, 246) or estrogen excess (87)	Hypogonadism, osteoporosis	• Estrogen/androgen replacement
Potential future receptor antagonists	AVPR1 antagonists HTR4/7 antagonists MC2R antagonists GIPR antagonists				• Most frequent aberrant receptors regulating cortisol secretion on PBMAH • Paracrine production of ACTH regulates cortisol secretion and could be reduced by MC2R antagonists
Potential future specific inhibitors of genes products implicated in PBMAH development	Molecules modulating signaling pathways activity of ARMC5, KDM1A, β-catenin, PKA on proliferation and steroidogenesis				• All gene products implicated in PBMAH pathophysiology (Fig. 3 and "Genetic Aspects" section) could be the targets for specific modulation of their action

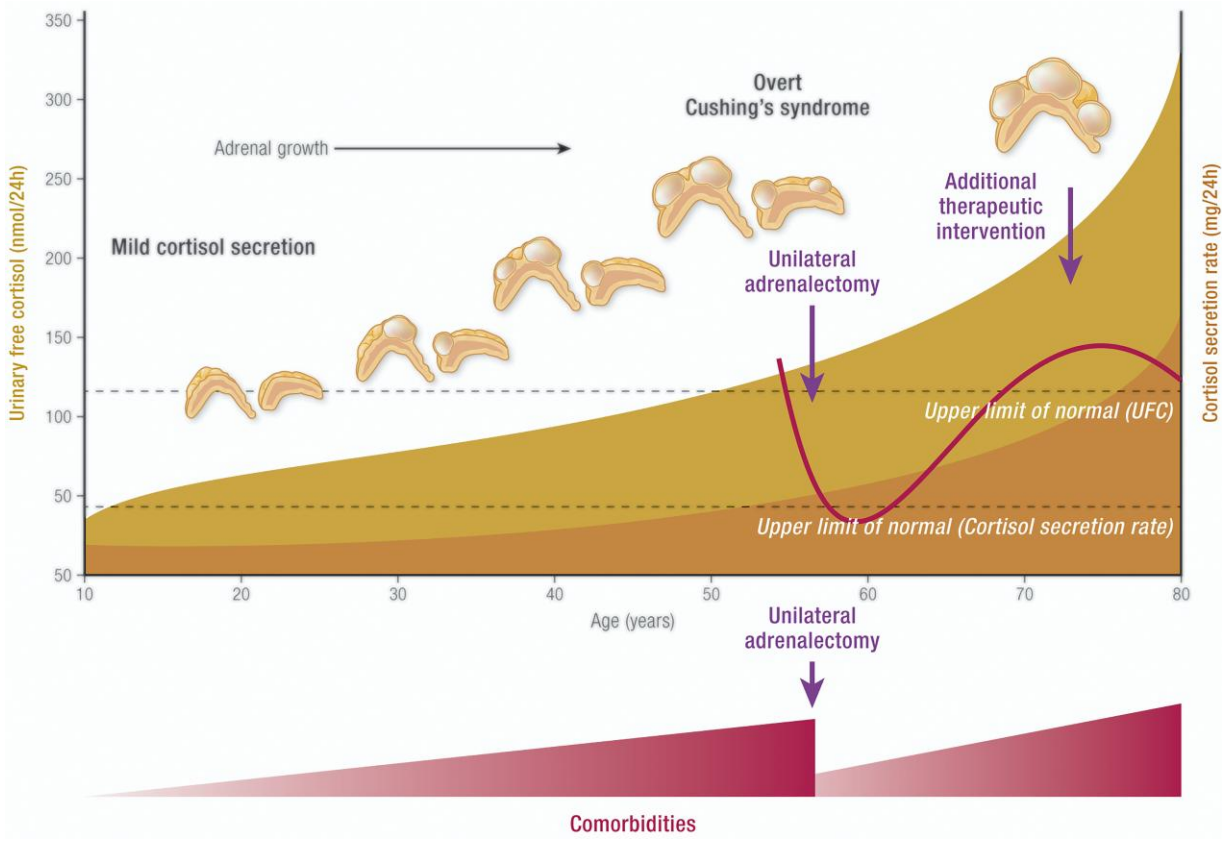


Figure 7. Evolution of PBMAH development on cortisol secretion and comorbidities over time and effect of unilateral adrenalectomy. Slow progressive development of adrenal hyperplasia and macronodules over years may result in progressive increase in cortisol secretion rate (right axis). 24-hour UFC levels (left axis) increase more than secretion rate as excess cortisol saturates blood cortisol binding globulin (CBG) leading to larger increase in free cortisol levels and higher UFC levels. Resection of larger right adrenal gland will decrease cortisol secretion rate proportionally to adrenal cell mass resection, and this can lead to a decrease in UFC levels and clinical improvement. Depending on residual left adrenal gland growth and cortisol secretion over time, UFC levels may remain low, normal or become elevated over time with impacts on excess cortisol comorbidities as proposed by Cristante et al (412). If the residual adrenal increases and cortisol secretion becomes elevated with comorbidities after a first adrenalectomy, further therapeutic intervention with either medical therapy or second surgical intervention (partial or total adrenalectomy) must be discussed with patient and multidisciplinary adrenal advisory board.

Practical tip/synthesis:

- PBMAH develops slowly over decades with bilateral adrenocortical hyperplasia and eventual macronodules formation.
- The course may involve progressive dysregulated cortisol secretion and development of comorbidities (arterial hypertension, diabetes, osteoporosis, cardiovascular events).
- Elevated serum cortisol saturates cortisol binding globulin (CBG), increases free plasma cortisol and urinary free cortisol (UFC) proportionately more than cortisol secretion rate.
- Sufficient cortisol excess (cortisol >140 nmol/L post 1 mg dexamethasone, low ACTH/DHEAS levels, high UFC, and comorbidities) indicates surgical removal of largest adrenal.
- Cortisol secretion rate and comorbidities can be normalized or improved by unilateral adrenalectomy.
- Long-term follow-up is required to monitor potential growth of contralateral adrenal growth and increased cortisol secretion.
- If the residual adrenal produces excess cortisol with increasing comorbidities, medical therapy or surgery (partial or completion of bilateral adrenalectomy) becomes indicated.

recurrence of hypercortisolism, either biochemically alone or associated with clinical symptoms, depending on the studies. Overall, 89 patients (31%) required a completion contralateral adrenalectomy; in the largest study, the median time to second surgery was 22 months in patients with overt CS and of 72 months in those with mild disease following their initial unilateral adrenalectomy (Table 9). As the definitions of both CS remission and recurrence were heterogeneous across the studies and follow-up duration often relatively limited, caution is required when analyzing results.

An important aspect of studies of PBMAH treated with unilateral adrenalectomy is the severity of CS of the patients included. It was previously suggested that unilateral adrenalectomy should be restricted to patients with UFC levels less than 2 to 3 times the upper limit of normal in order to restore normal UFC after unilateral adrenalectomy. However, as pointed out by Chabre et al, the saturation of CBG in hypercortisolism results in a proportionally higher increase in UFC than the true increase in cortisol secretion rate; thus removing 50% of adrenal tissue mass can result in much more than 50% reduction of UFC (Fig. 7) (412). In the studies summarized in Table 9, almost half were categorized as overt CS, while the remaining were classified as moderate or cyclic CS. Thus, despite a high proportion of patients with elevated UFC, the initial remission rate is still high and percentage of recurrence relatively low. To date, no clear factors have been identified to predict unilateral

Table 9. Outcomes of patients with Cushing's syndrome due to PBMAH treated with unilateral adrenalectomy or combined therapy

Authors	No. of patients	Severity of CS[+]	Choice of adrenal to resect	Initial remission n (%)	Adrenal insuffi-ciency n (%)	Recurrence n (%)	Time to recurrence (months after UA)	Completion contralateral adrena-lectomy n (%)	Time to second surgery (months after UA)	Follow-up (months)
Boronat et al, 1996 (396)	1	Overt	Largest	1	—	—	—	—	—	120
Lacroix et al, 1997[#] (216)	1	Overt	Largest	1	1[a]	—	—	—	—	36
Yamakita et al, 1997 (221)	1	Overt	Equal size Right colectomy APC	0	0	1	—	1	3	—
N'Diaye et al, 1999[#] (200)	1	Overt	Largest and other criteria[+]	1	1	—	—	—	—	12
Doppman et al, 2000 (77)	1	Moderate	Uptake on NP-59 scan	1	1	—	—	—	—	64
Imohl et al, 2002[#] (233)	1	Moderate	Largest	1	1	—	—	—	—	30
Lamas et al, 2002 (397)	4	Overt 1 Moderate 3	Largest	4 (100%)	2 (50%)[b]	—	—	—	—	78.8 (30-137) Mean (range)
Ogura et al, 2003 (398)	1	Mild	Largest	1 (100%)	—	—	—	—	—	24
Sato et al, 2006 (320)	1	Moderate	Largest	1	1	—	—	—	—	8
Albiger et al, 2007[#] (171)	1	Overt	Largest and other criteria[+]	1	1[c]	—	—	—	—	20
Vezzosi et al, 2007[#] (51)	1	Mild	Largest	1	1[d]	—	—	—	—	7
Iacobone et al, 2008 (399)	7	Overt 5 Moderate 2	Largest	6 (86%)	2 (29%)[e]	—	—	1 (14%)	7	53 (27-68) Median (range)
Mazzuco et al, 2009[#] (387)	1	Overt	Largest and other criteria[+]	1	—	—	—	—	—	84
Hamajima et al, 2010[□] (400)	1	Moderate	Largest and other criteria[+]	1	1[f]	—	—	—	—	24
Kobayashi et al, 2012 (401)	1	Moderate	Largest	1	—	1	60	1	72	84
Ito et al, 2012 (411)	2	Mild 2	Largest and adrenal venous sampling	2 (100%)	—	—	—	—	—	24 and 90

(*continued*)

Table 9. Continued

Authors	No. of patients	Severity of CS[+]	Choice of adrenal to resect	Initial remission n (%)	Adrenal insuffi-ciency n (%)	Recurrence n (%)	Time to recurrence (months after UA)	Completion contralateral adrena-lectomy n (%)	Time to second surgery (months after UA)	Follow-up (months)
Maghrabi et al, 2013 (403)	1	Mild	Largest	1	1[g]	—	—	—	—	10
Xu et al, 2013 (404)	14	Overt	Largest 13 and other criteria[+] 1	13 (93%)	2 (14%)[b]	—	—	1 (7%)	NS	69 (23-120) Median (range)
Albiger et al, 2015[‡] (210)	12	Overt 3 Moderate 5 Mild 3 Cyclic 1	Largest and other criteria[+]	11 (92%)	2 (17%)[i]	8 (73%)	54 ± 56 (12-180) Mean ± SD (range)	4 (33%)	NS	92 (24-230, 233-235, 237-267) Median (range)
Debillon et al, 2015 (169)	15	Overt 4 Moderate 11	Largest	15 (100%)	6 (40%)[j]	2 (13%)	84 and 96	1 (7%)	108	60 (39-105) Median (range)
Li and Yang., 2015 (405)	15	Overt 13 Moderate 2	NS	15 (100%)	NS	3 (20%)	36	3 (20%)	NS	NS
Juliá-Sanchis et al, 2018 (84)	2	Overt 1 Mild 1	Largest and iodocholesterol scan	2 (100%)	0 (0%)	—	—	—	—	—
Osswald et al, 2019 (30)	25*	Overt Moderate	Largest and other criteria[▪]	21 (84%)	Transient: 10 (50%) Persistent: 1(5%)	3 (14%)[⌂]	NS	3 (12%)	NS	50 Median
Sheikh-Ahmad et al, 2020 (406)	9	Overt 2 Moderate 5 Mild 2	Largest	8 (89%)	3 (33%)[k]	(30) 1 (13%)	NS	2 (22%)	2 and 168	72 (5-168) Median (range)
Tanaka et al, 2019[‡] (407)	1	Overt	Largest and other criteria[+]	1	1	—	—	—	—	3
Higashitani et al, 2020 (85)	1	Overt and renovascular hypertension	Largest, 131I-adosterol scan, FDG PET	1	0	0	—	—	—	ND
Zhang et al, 2020 (86)	39	22/28 overt 7/11 mild	Largest	15 (68%) 6 (86%)	NS	NS	NS	7 overt (32%) 1 mild (14%)	ND	20 (3-81)
Amodru et al, 2021 (379)	1	Overt	Largest and Iodocholesterol scan	1	1 Transient 3 months	—	—	—	—	—
Nishiyama et al, 2021 (408)	1	Overt	Largest	1	0	—	—	—	—	18

(*continued*)

Table 9. Continued

Authors	No. of patients	Severity of CS[+]	Choice of adrenal to resect	Initial remission n (%)	Adrenal insuffi-ciency n (%)	Recurrence n (%)	Time to recurrence (months after UA)	Completion contralateral adrena-lectomy n (%)	Time to second surgery (months after UA)	Follow-up (months)
Wang et al, 2022 (278)	124	68 overt 56 mild	Largest	71% (43/65) with overt CS NS for mild cases	7/116 (6%) transient	72% (47/65) in overt CS 33.3% (17/51) in mild disease	NS	55% 64/116	22 in overt CS 72 in mild disease	28 (1-165)
Overall n (%)	286 (100)	—	—	222 (77%)	47 (16%) Transient 38 (13%) Persistent 9 (3%)	83 (29%)	—	89 (31%)		—

Transient insufficiency: [a]17 months; [b]14 and 60 months; [c]7 months; [d]6 months; [e]7 and 24 months; [f]18 months; [g]3 weeks; [b]length not specified; [i]9 and 36 months; [j]1/6 patient: 24 months; [k]7, 12 and 24 months.
Abbreviation: NS, Not specified.
[+]Overt: elevated UFC and clinical signs of CS; moderate: 1 mg DST cortisol >140 nmol/L, normal UFC; mild: 1 mg DST cortisol 50–140 nmol/L, normal UFC. Severity estimated from description in publications with variable available details.
[‡]Patients with adrenal aberrant receptors
[†]Prevalent side of uptake on adrenal iodine ^{131}-norcholesterol scintigraphy
[□]1-year-old patient with McCune-Albright syndrome
*Long-term follow-up was available for 20 patients
[▪]Cortisol gradients during adrenal vein sampling and iodine-131 or [123 I] iodometomidate scintigraphy
[⌂]However, at follow-up, only 32% were still biochemically controlled
Practical tip/synthesis:

- Bilateral adrenalectomy has been the usual therapy of PBMAH with overt Cushing's syndrome.
- Many case reports and series summarized in table 9 indicate that unilateral adrenalectomy can lead to sustained normalization of UFC levels.
- The largest adrenal gland should be removed; when iodocholesterol scan is available it can assist in confirming the dominant side of cortisol excess.
- Transient or long-term adrenal insufficiency can occur after removal of largest gland and should be monitored and replaced adequately in the postoperative period.
- Long-term follow-up to identify increased secretion or growth from the remaining adrenal should be conducted regularly including with 1-mg dexamethasone tests, basal plasma ACTH levels, and UFC.
- In the largest series to date recurrence was highest (up to 50%) in patients with overt CS, or when contralateral residual adrenal volume was >34 mL.

adrenalectomy effectiveness to control hypercortisolism or to avoid recurrence from the residual adrenal tissue. It was suggested that the best results follow removal of the largest adrenal in cases with asymmetric gland size (169, 210, 278), with a possible positive correlation between the size of the contralateral adrenal and CS persistence (406). In the most detailed and largest study, a contralateral residual adrenal volume >34 mL and higher initial UFC levels predicted eventual recurrence; 55% of their patients required a completion adrenalectomy, particularly those with initial overt CS with a mean UFC 5.8 times above upper limit of normal (278). However, remission and hypocortisolism may also occur in cases with symmetrical involvement. Some clinicians continue to recommend bilateral adrenalectomy for the rare cases with severe CS with large and equal adrenal sizes and high clinical burden.

The most frequent short-term morbidity following unilateral adrenalectomy is adrenal insufficiency. In the first 30 studies in Table 9, approximately one-third of patients suffered from adrenal insufficiency, and the majority were successfully weaned from steroid substitution within variable postoperative delay (from 3 weeks to 60 months), while ~25% did not recover and still needed glucocorticoid supplements at last follow-up; in the more recent largest series, adrenal insufficiency was only reported in 6%, but no detailed ACTH stimulation test was performed (278) (Table 9). Adrenal insufficiency after unilateral adrenalectomy is mostly transient, while it is definitive after bilateral adrenalectomy and can never recover. Second, adrenal insufficiency after bilateral adrenalectomy is complete, with cortisol, aldosterone and adrenal androgen deficiencies (169, 412). However, a recent retrospective study reported a higher mortality in patients treated by unilateral adrenalectomy than in patients treated by bilateral adrenalectomy despite biochemical remission and a lower incidence of adrenal crisis; this could be the consequence of imperfect control of hypercortisolism with possible lack of normal nycthemeral cortisol rhythm (30).

Guidelines from the European Society of Endocrinology (ESE) and the Endocrine Society advocate bilateral adrenalectomy for the treatment of overt CS in PBMAH patients and selective unilateral adrenalectomy of the larger adrenal in selected cases (26, 371). Despite their recommendations and unilateral adrenalectomy being an attractive surgical option, the best choice of therapy is yet to be determined by proper prospective studies. The following principles should be taken into consideration in improving current available treatments: 1) treatment should normalize the global cortisol production to avoid morbidity linked to hypercortisolism; 2) treatment should achieve sufficient cortisol production to avoid adrenal insufficiency; and 3) treatment should reduce the risks of recurrence of hypercortisolism from the residual adrenal (412).

Adrenal-sparing surgery

An alternative conservative surgical procedure was performed by some groups in PBMAH patients with unilateral adrenalectomy on one side and subtotal resection of the contralateral adrenal. Partial adrenalectomy, sparing at least a third of the second adrenal was initially used in patients with multiple endocrine neoplasia type 2 (MEN2) or Von Hippel-Lindau (VHL) and bilateral pheochromocytoma and was later applied to selective resection of aldosteronomas (413-415). Promising results were reported with cortical-sparing adrenalectomy in CS patients from different bilateral adrenal etiologies (409, 416-418). In a study of 42 patients, unilateral adrenalectomy was performed on 16 patients, while 26 patients underwent simultaneous bilateral adrenal surgery, including bilateral adrenalectomy (n = 3), bilateral subtotal adrenal resection (n = 9), and unilateral adrenalectomy + contralateral subtotal adrenal resection (n = 14). The majority of the cohort (31/42 patients) consisted of patients with mild cortisol excess (418). At a median follow-up period of 40 months, the overall remission rate was 92% (including the 3 patients who underwent bilateral adrenalectomy), while all patients treated with cortical-sparing bilateral adrenalectomy achieved eucortisolism postoperatively. Corticosteroid supplementation therapy was required following surgery in 24/39 patients (62%) who underwent cortical-sparing surgery (6/16 patients treated with unilateral adrenalectomy and 18/23 patients treated with less-than-total bilateral adrenal surgery). This percentage dropped to 28% at their last follow-up assessment. Finally, 3 patients treated initially with unilateral adrenalectomy experienced a recurrence. Two proceeded with partial contralateral adrenal resection and both were in remission after second surgery at last follow-up. Two other patients developed enlargement of their contralateral gland following their unilateral adrenalectomy; they remained asymptomatic without biochemical hypercortisolism (418). In 22 patients with PBMAH, Zhang et al performed unilateral adrenalectomy in 14, bilateral in 4, unilateral and subtotal in 2, and subtotal bilateral in 2, but did not provide sufficient detailed follow-up data to compare the outcomes (409).

In a prospective study, PBMAH patients were treated with resection of the largest adrenal gland and a partial sparing contralateral adrenalectomy (419). Among 17 patients, including 10 with modest cortisol excess, 95% achieved hypercortisolism control postoperatively, and 71% experienced recovery of their hypothalamic-pituitary-adrenal axis after a median follow-up period of 41 months. Only 1 patient had a relapse of his hypercortisolism after 30 months, requiring a completion surgery (419). Since many patients had only mild cortisol excess, the risk-benefit ratio of performing a bilateral procedure over unilateral adrenalectomy is not clear. Additional robust studies are needed to clarify the indication for a bilateral conservative surgical approach compared with unilateral adrenalectomy in PBMAH patients with CS considering this higher rate of adrenal insufficiency and the similar rate of remission. Furthermore, hypercortisolism recurrence should be monitored more precisely after resolution of adrenal insufficiency using basal ACTH levels, 1-mg dexamethasone DST, and 24-hour UFC levels at 6- to 12-month intervals with evaluations of comorbidities. Finally, an attempt to control hypercortisolism with CT-guided percutaneous ethanol ablation was unsuccessful in 5 PBMAH patients (420).

Considering the rarity of overt CS secondary to PBMAH, but the high prevalence of those with modest dysregulated steroid production, the selection of the most appropriate therapeutic approach should be individualized, considering comorbidity status, degree of cortisol/aldosterone excess and adrenal gland characteristics on imaging, following a multidisciplinary group discussion. Further prospective multicenter studies with large cohorts will be useful to better define the most appropriate therapeutic recommendations.

Which adrenal should be removed?

Following the choice of unilateral surgery to treat PBMAH patients, the selection of which adrenal to remove arises. Several criteria were considered to identify which adrenal contributes the dominant source of cortisol: adrenal size on imaging, side of maximal adrenal uptake of iodine 131-norcholesterol, iodine 123-iodometomidate scintigraphy, or FDG PET scan, and cortisol lateralization during bilateral adrenal venous sampling (AVS). In most studies using unilateral adrenalectomy for PBMAH patients with CS, the largest adrenal on CT scan analysis was the one removed (Table 11). In the 6 largest studies available, the larger adrenal on imaging was resected with an initial remission rate of 71% to 85% (86, 169, 278, 397, 399, 406). When iodine 131-cholesterol scintigraphy was performed, the adrenal with the dominant uptake was always the largest gland on CT (169). In contrast with its important role in primary aldosteronism, the use of AVS in PBMAH patients has not proven to be more accurate than conventional imaging. This method is associated with risk of inadequate bilateral selectivity using plasma metanephrine as a marker, with success rates rarely over 85% to 90% (416, 421-423). In 3 PBMAH patients with CS, AVS demonstrated bilateral source, not improving the therapeutic decision (85, 416). In another study, AVS indicated bilateral cortisol hypersecretion in all patients without lateralization (422). In a recent study of 16 patients, using basal samples without ACTH stimulation, AVS was useful only in 3 patients with lateralized gradient despite having symmetrically enlarged glands (423). Thus overall, the current literature indicates that the size of the adrenals should be utilized to decide which adrenal should be removed.

Two case reports described the use of volumetry modeling before adrenalectomy for PBMAH: in 2016, a Brazilian team performed the total resection of the largest (left) adrenal associated with a sparing adrenalectomy of the right adrenal, with the help of the 3D-printing of the right adrenal based on the volumetric modeling obtained on CT images (424); in 2021, a Japanese group reported a surgical decision based on a volumetric modeling of PBMAH, leading to a postoperative control of cortisol excess with a 66% decrease of 24-hour UFC compared with the baseline level; however, this case is not included in Table 10 as it does not provide longer-term follow-up data (425). Future prospective studies assessing the performance of volumetric modeling for the decision of unilateral or bilateral adrenalectomy in PBMAH patients would be needed.

Following unilateral adrenalectomy, an ACTH 1-24 test may provide a false-positive response since the remaining hyperplastic tissue may respond abnormally to ACTH (169). In most studies, improvement of cortisol-related comorbidities, including obesity, diabetes, and hypertension, is achieved in patients treated by unilateral adrenalectomy (152, 169). In contrast, a recent retrospective study suggested that unilateral adrenalectomy may lead to insufficient biochemical remission compared to bilateral adrenalectomy as post-DST cortisol or late-night salivary cortisol levels may remain increased in some patients (30). Thus, more stringent evaluations of biochemical remission should be conducted.

Combination of Unilateral Adrenalectomy and Medical Therapy

Patients in whom combined therapy with unilateral adrenalectomy and a specific pharmacological approach targeting a specific aberrant receptor expressed in the PBMAH tissues are described in Table 9. In some cases, combination therapy has been beneficial. In a patient with combined catecholamine/vasopressin-dependent CS and PBMAH, monotherapy with propranolol significantly decreased cortisol secretion, but UFC values remained elevated at twice the upper limit of normal (216). A unilateral adrenalectomy transiently normalized UFC but it increased above normal later, and the addition of β-blocker completely normalized the clinical and biochemical manifestations of CS until now (25 years as of this report) (88). Similarly, in a patient with PBMAH with combined overt cortisol and aldosterone secretion aberrantly regulated by β-adrenergic and V1 vasopressin agonists, atenolol reduced UFC and urinary aldosterone by 50% but they still remained elevated; steroid excess was normalized for 3 months following unilateral adrenalectomy but as it increased above normal, atenolol normalized UFC for 10 years in combination with eplerenone to control primary aldosteronism until escape required contralateral adrenalectomy (390). This approach was less successful in a woman with PBMAH and myelolipomas and GIP-dependent CS; therapy with octreotide or pasireotide were only transiently beneficial, requiring unilateral adrenalectomy; after 3 months of this combination, a contralateral adrenalectomy was required (72).

These examples demonstrate that although targeted pharmacological therapies may induce partial response to hypercortisolism by blocking the aberrant receptors in PBMAH patients, their combination with unilateral adrenalectomy offers the possibility of avoiding bilateral adrenalectomy and long-term steroid supplementation, while resulting in sustained CS remission. In the future, the discovery and utilization of new specific receptor antagonists for serotonin, vasopressin, GIP (GIP[3-30]NH2) (389), ACTH, or other aberrant receptors may enable a broader spectrum of pharmacological options for patients with PBMAH and hypercortisolism, since the prevalence of aberrant receptors is high in this disease (218).

Summary of Recommended Surgical and Combined Therapies for PBMAH

Recent studies show that unilateral adrenalectomy should be considered in PBMAH patients with CS (Fig. 2). Its combination with a specific pharmacological therapy blocking aberrant receptors seems to be an interesting approach and should be explored in future studies. In rare cases of overt CS with severe comorbidities and symmetrical adrenals, bilateral adrenalectomy may be the favored option. Finally, adrenal-sparing surgery seems promising, but further studies with cohorts of patients with a higher degree of hypercortisolism than subclinical CS are needed to assess its full therapeutic potential compared with unilateral adrenalectomy.

Perioperative Management and Postoperative Follow-up

Laparoscopic adrenalectomy has proven its many advantages over the years and is now widely adopted to treat PBMAH. The choice of minimally invasive approach for adrenalectomy (lateral transabdominal, retroperitoneal, or robotic adrenalectomy) should be patient and surgeon-specific. CS patients are at high risk of thromboembolism due to an activated coagulation cascade and impaired fibrinolysis creating a hypercoagulable state (426). The Endocrine Society and the

Pituitary Society guidelines recommend perioperative thromboprophylaxis in such patients for up to 4 weeks following surgery (371, 427). Early postoperative mobilization should also be encouraged particularly in CS patients (371, 426, 427). Furthermore, PBMAH patients should all be considered at risk of postoperative adrenal insufficiency, even after unilateral adrenalectomy (169). Thus, physicians should carefully evaluate for adrenal insufficiency in all patients with early morning cortisol levels and if borderline, also with ACTH 1-24 stimulation test.

Systematic long-term postoperative follow-up is essential (Fig. 2). It should include evaluation for adrenal insufficiency and replacement with exogenous steroids if needed. The requirement for hormonal replacement should be reassessed periodically with morning serum cortisol every 3 months, since a large proportion of adrenal insufficiency will be transient. Education of patients and their families concerning adrenal insufficiency and adrenal crisis after surgery is also mandatory, including medical card or bracelet, stress dose adjustments, and injectable glucocorticoid. Follow-up of PBMAH patients who have undergone unilateral adrenalectomy should also include rigorous monitoring for recurrence of progressive hypercortisolism from contralateral adrenal (Fig. 7) with basal cortisol and ACTH, 1-mg DST, late-night salivary cortisol, and if those are abnormal, with UFC. It is also important to reassess cortisol-related comorbidities, including cardiometabolic, bone health, and neurocognitive effects. While some will significantly improve following a successful treatment, some might not normalize. Of note, persistent cardiovascular risk factors such as arterial hypertension, dyslipidemia, and diabetes after remission are associated with a longer duration of hypercortisolism. While medications may be reduced or even discontinued in many CS patients after appropriate treatment, they are still required at higher prevalence several years after remission; stringent criteria for targets of blood pressure, glucose and lipids should be followed. Moreover, given the immunosuppressive effects of CS, clinicians should be aware of possible flare-up of underlying autoimmune conditions and also recommend age-appropriate vaccinations to this population (24, 371, 427).

Future Perspectives

The numerous discoveries made this last decade in various aspects of PBMAH have demonstrated the pertinence of this condition to making progress in the field of adrenal CS, and at the same time have revealed the heterogeneity of this disease. This underlines the need for further progress both in clinical as well as in translational research.

With a better knowledge and awareness by endocrinologists of PBMAH and the overall progress in the investigation of adrenal incidentalomas, the frequency of PBMAH is clearly higher than thought a decade ago, but precise epidemiology is lacking. Considering that incidentally discovered cases of PBMAH are probably more frequent than the ones diagnosed after investigations of clinical signs of CS, studies of the imaging criteria for PBMAH diagnosis need to be developed. This will also improve the differential diagnosis of bilateral adrenal incidentalomas.

Various terminologies for this disease have been used. Although PBMAH is the most used term since 2013, the recent WHO revision of adrenal tumor pathology suggests using a different terminology. A consensus on this aspect will be important, but probably needs as a prerequisite better description and understanding of the clinical and pathological heterogeneity of the disease. Because most of the patients diagnosed today are not operated, integration of various clinical, imaging, hormonal, molecular, and pathological parameters in the understanding of this heterogeneity and its classification will be needed to improve the definition of the disease.

A clear link between cortisol dysregulated secretion and increased cardiovascular morbidity and mortality has been established in unilateral adrenal benign incidentalomas. Because alteration of cortisol secretion seems to be more frequent in PBMAH, even incidentally discovered, it will be important to analyze in large PBMAH cohorts its association with comorbidities and the long-term outcome, since treatment might be even more important in this population. How steroid profile and metabolomics that can now be investigated in detail by mass spectrometry will correlate with disease classification and comorbidities could help to answer this question.

PBMAH treatment was for a long time limited only to surgical therapy. In terms of surgical treatment, the respective place of bilateral vs unilateral adrenalectomy needs to be defined; how the level of cortisol dysregulation as well as adrenal imaging or AVS can be used in this surgical discussion needs to be investigated. Similarly, the long-term benefit of unilateral adrenalectomy, not only on adrenal function but also comorbidities, needs to be investigated. The benefit of medical therapies (medical treatment of comorbidities, anticortisolic drugs, aberrant receptor targeting, etc) as an alternative to surgery or in patients with mild form of cortisol dysregulation warrants further investigation in large retrospective cohorts and by prospective studies.

The last decade has clearly shown that PBMAH frequently can be a genetic disease. In particular, identification of *ARMC5* and *KDM1A* pathogenic variants allows familial screening for early diagnosis. This will help to understand the natural history of the disease and it will be important to prospectively study the benefits of earlier management of PBMAH in the identified relatives affected by the disease. Here again, the criteria for therapeutic intervention, as discussed above in mild form of the disease, will be important to define. Because today a genetic cause is not identified in all patients, it is likely that other genetic defects are to be identified in the future. Investigating how the various genetic causes correlate with the heterogeneity of the disease will help to better classify PBMAH. Exploring other genetic or environmental factors implicated in the development of the disease and patient's phenotype will be key to understand adrenal CS. The study of the signaling pathways altered by the genetic alterations causes of PBMAH will undoubtedly help to better understand adrenal tumorigenesis and steroid dysregulation. Ultimately, the development of specific treatment targeting these genetic defects (Table 8) will be a triumph in the treatment of this adrenal disease.

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