A Gene-Based Classification of Primary Adrenocortical Hyperplasias

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ABSTRACT
Primary or adrenocorticotropin-independent adrenocortical tumors and hyperplasias represent a heterogeneous group of adrenocortical neoplasms that arise from various genetic defects, either in isolation or familial. The traditional classification as adenomas, hyperplasias, and carcinomas is non-specific. The recent identification of various germline and somatic genes in the development of primary adrenocortical hyperplasias has provided important new insights into the molecular pathogenesis of adrenal diseases. In this new era of personalized care and genetics, a gene-based classification that is more specific is required to assist in the understanding of their disease processes, hormonal functionality and signaling pathways. Additionally, a gene-based classification carries implications for treatment, genetic counseling and screening of asymptomatic family members. In this review, we discuss the genetics of benign adrenocorticotropin-independent adrenocortical hyperplasias, and propose a new gene-based classification system and diagnostic algorithm that may aid the clinician in prioritizing genetic testing, screening and counseling of affected, at-risk individuals and their relatives.

Introduction
Adrenocorticotropin (ACTH)-independent, primary adrenocortical tumors and hyperplasias are neoplasms of the adrenal cortex that arise due to perturbations in various key molecular pathways; for benign tumors cyclic AMP (cAMP)-signaling is the main pathway (Fig. 1) [1]. Traditionally, ACTH-independent adrenocortical tumors and hyperplasias were classified into three major categories as adenomas, hyperplasias, and carcinomas [2]. This classification is non-specific for several reasons. First, it does not factor the various genetic defects and hormone functionality of these lesions. Second, causative genes in the predisposition and/or development of these lesions are increasing in number owing to the rapid adoption of genetic technologies in routine clinical practice and translational research. Third, these lesions may arise in the context of familial or syndromic conditions, which require careful phenotyping and genetic counseling of at-risk individuals or their relatives. Thus, a more robust and specific classification system is required.

The various types of adrenocortical tumors and hyperplasias, their histology and genetics are described in Table 1. Translation-
in adrenocortical tumorigenesis, if not in all, at least in cortisol-producing lesions [5–7]. Other early genetic discoveries included defects of the regulatory subunit type 1-α (R1a) of protein kinase A (PRKAR1A) and protein kinase cAMP-activated catalytic subunit beta (PRKACB) [8] in Carney complex (CNC) [9], phosphodiesterases (PDE11A, PDE8B) in isolated micronodular adrenal hyperplasia (IMAD) [10], potassium voltage-gated channel subfamily J member 5 (KCNJ5) in aldosterone-producing adenomas (APA) [11], and armadillo repeat containing 5 (ARM5) in primary bilateral macronodular adrenocortical hyperplasia (PBMAH) [12]. In this review, we discuss the genetic and molecular mechanisms responsible for the formation of ACTH-independent adrenocortical hyperplasias and propose a new genetic classification given the most recent advances in gene discovery and to aid the clinician in the diagnosis and counseling of these conditions.

Molecular Pathways in Primary Adrenocortical Hyperplasias

The major molecular pathway that is perturbed in primary adrenocortical cortisol-producing neoplasms is the cAMP-signaling pathway (Fig. 1). Briefly, The G-protein coupled receptor for ACTH (ACTHR), also known as melanocortin 2 receptor (MC2R), is a seven-transmembrane receptor that undergoes extensive conformational changes in response to its ligand, ACTH (Fig. 1). This leads to activation of adenylyl cyclase (AC) through the G proteins and the generation of cyclic AMP, activating protein kinase A (PKA), a holoenzyme that consists of a tetramer of two homo- or heterodimers regulatory subunits (R1α, R1β, R2α and R2β), and catalytic subunits (Ca, Cβ, Cγ and PRKX) that are encoded by the respective genes [13]. This in turn enables phosphorylation of PKA targets, including gene expression to mediate cell growth, differentiation and hormone production (e.g., cortisol and its pre-cursors). As shown in Fig. 1, genetic alterations in key genes of the cAMP-signaling pathway leads to the formation of various primary adrenocortical lesions.

Classification of Primary Adrenocortical Hyperplasia

In 2007, a comprehensive classification of ACTH-independent adrenocortical hyperplasia was proposed [2]. On histopathology,
these lesions are grossly divided into micro- (<1 cm) and macro-nodular (>1 cm) disease, a classification that primarily relies on the size of nodules [2]. Although size has biologic relevance [2], reliance on size alone may lead to erroneous classification of these nodules, as smaller or larger nodules can co-exist and/or be seen in either disease states. Additional tissue markers may help in the differentiation of the two processes, including the presence or absence of internodular hyperplasia or atrophy of the surrounding cortex (as in PBMAH) and pigmentation of the nodule or cortex (as in PPNAD) [2]. The most specific pigmentation is lipofuscin, a light to dark brown autofluorescent granularity of adrenal tissue which represents lysosomal residual bodies containing end products of oxidative damage to lipids [14]. Additional characteristics of these lesions includes sparing of the adrenal medulla and the persistence of cells that are probably derived from fetal adrenal precursors in the micronodular forms [15].

Macronodular adrenocortical hyperplasia

PBMAH is the most common subtype of macronodular adrenocortical hyperplasia. This condition is often bilateral and affects the adult population [16]. First described in 1964 [17], PBMAH has been referred to by many names which continue to pose confusion in medical literature, including massive macronodular adrenocortical disease (MMAD), bilateral macronodular adrenal hyperplasia (BMAH), ACTH-independent macronodular adrenocortical hyperplasia (AIMAH), autonomous macronodular adrenal hyperplasia (AMAD), primary macronodular adrenal hyperplasia (PMMAH), ACTH-independent massive bilateral adrenal disease (AIMBAD), and “giant” or “huge” macronodular adrenal disease [18]. Asynchronous involvement of only one adrenal gland in PBMAH is rare [19]. After the recent discovery of a local intra-adrenal secretion of GIPR [31], a second somatic event is required to mediate tumorigenesis and polyclonal nodularity, either 16p loss of heterozygosity or a somatic hit in ARMC5 [31]. Interestingly, each nodule in PBMAH may harbor a ‘private’ disease-causing variant, thus tissues have defects that completely inactivate ARMC5 [31].

Other genetic defects have been implicated in the pathogenesis of PBMAH, including the somatic activating variant of GNAS at codon Arg (201) without features of MAS [22, 32], the germline p.R867G variant of PDE11A in a patient with familial PBMAH [22], the several disease-causing germline variants of PDEBB [33], and germline variants in FH, MEN1, and APC in the context of familial syndromes as detailed below in this review [22, 32]. Very rarely, patients with familial PBMAH may carry germline duplications of PRKACA resulting in copy number gains [34, 35]. Possible disease-causing variants of the MC2R gene have also been reported in PBMAH [36]. A number of genomic/transcript abnormalities have been reported in PBMAH, including losses in 20q13 and 14q23 [7], overexpression of WISP2, BCL2, EZF1, EGF, c-KIT, MYB, PRKACA, and CTNNB1, which implicates various aberrant oncogenic pathways in nodular polyclonality and growth [37]. Chromatin deregulation of DOT1L and HDAC9, implicated in regulating gene transcription and cell proliferation, have also been implicated in the pathogenesis of PBMAH [38].

Other rare subtypes of ACTH-independent macronodular adrenocortical hyperplasia include primary bimorphic adrenocortical disease (PBAD, as seen in MAS) and food-dependent Cushing syndrome (FDCS, also known as GIP-dependent Cushing syndrome). PBAD due to MAS is a congenital disorder that presents in the infantile period with severe hypercortisolism due to nodular adrenocortical hyperplasia with sharply defined zones of cortical atrophy that give the cortex a bimorphic appearance [5, 39]. PBAD is caused by constitutive activation of the cAMP-signaling pathway from postzygotic gain-of-function variants in GNAS, within exon 8 of the Gsα subunit (▶Fig. 1) [7]. Moreover, the differential diagnosis of hypercortisolism in patients with MAS also includes bilateral “atypical” adenomas [40], isolated bilateral hyperplasia [41], and hyperplasia with spontaneous resolution [42] or improvement after unilateral adrenalectomy [43, 44].

In a rare form of FDCS, glucocorticoid excess is driven by aberrant glucose-dependent insulinotropic polypeptide receptor (GIPR) expression that arise from somatic duplications (within the adrenal lesions) in chromosome region 19q13.32 containing the GIPR locus [45]. GIP-dependent PBMAH should be distinguished from FDCS. The differential histopathologic characteristics and features of these lesions are summarized in ▶Table 1.

Micronodular adrenocortical hyperplasia

Of the micronodular variety, PPNAD which is congenital and often bilateral is the most common subtype. PPNAD is either pigmented (c-PPNAD) as seen in CNC, or isolated (i-PPNAD); this condition has also been referred to as non-specific nomenclatures, including micro-nodular adrenal disease and microadenomatosis or primary adren-
### Table 1: Classification and characteristics of primary adrenocortical hyperplasia.

<table>
<thead>
<tr>
<th>Adrenocortical lesions</th>
<th>Genes (locus)</th>
<th>Histopathology</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td>PBMAH</td>
<td>ARMC5 (16p11.2)</td>
<td>Nodules or macroadenomas, &gt; 1 cm, with (type 1) or without (type 2) inter nodular atrophy Hyperplasia with dominant nodule</td>
<td>Middle age, mild hypercortisolism and/or mineralocorticoid excess Associated with MEN-1, FAP, MAS, HLRCS, isolated (AD) Most lesions have aberrant GPCRs (vasopressin, serotonin, catecholamines, GIP, luteinizing hormone) PMAH carry the ability of intra-adrenal production of ACTH with an autocrine/paracrine effect on glucocorticoid or mineralocorticoid production</td>
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<td>MEN1 (11q13)</td>
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<td>Fh1 (1q42.3–43)</td>
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<td>APC (5q22.2)</td>
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<td>PDE11A (2q31.2)</td>
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<td>PDE8B (5q13.3)</td>
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<td>GNAS (20q13)</td>
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<td>PRKACA duplication (19p13.1)</td>
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<td></td>
<td></td>
<td>Distinct adenomas (&gt; 1 cm), with occasional microadenomas and inter nodular atrophy</td>
<td>Infants and very young children MAS</td>
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<td>Isolated or familial aberrant GPCRs (GIPR), low fasting cortisol, hypercortisolism post-meals</td>
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<td>Micro adenomatous (&lt; 1 cm) hyperplasia with pigmentation</td>
<td>Children and young adults Lentiginosis in few cases</td>
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<td>Children, young and middle aged adults Disease at a younger age and a higher frequency of myxomas, schwannomas, and thyroid and gonadal tumors than patients without PRKAR1A variants. In-frame deletion of exon 3 and the c.708 + 1G&gt;T appears to confer a more severe CNC phenotype, while the splice variant c.709 (–7–2)del6 and the initiation alternating substitution c.1A&gt;G/p. M1Vp has been associated with incomplete penetrance of CNC, as seen in i-PPNAD CNC: The hot spot c.491–492delTG is most closely associated with lentigines, cardiac myxoma, and thyroid tumors when opposed to all other PRKAR1A variants Expressed Ri iso mutant protein present with more cases and aggressive CNC-phenotype CNC2: Sporadic disease later in life with a lower frequency of myxomas, schwannomas, thyroid and LCCSCT</td>
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<td>Mostly children and young adults Cyclical hypercortisolism May be associated with a paradoxical rise of glucocorticoid excretion during the Liddle’s test</td>
<td>Isolated or AD</td>
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**PRKAR1A**: Carney complex-associated primary pigmented nodular adrenocortical disease; **PRKACA**: Carney complex; **PDE11A**: Phosphodiesterase 11A gene; **PDE8B**: Phosphodiesterase 8B gene; **PDE11A**: Phosphodiesterase 11A gene; **PRKAR1A**: Protein kinase, cAMP-dependent, regulatory, type I, α gene.

...Review

PNNAD despite its association with other manifestations of CNC. Additionally, i-MAD is regarded as a separate entity from PNNAD, with earlier onset of clinical manifestations, and clinically manifesting in isolation; however,
these two entities share several features including micronodularity, paradoxical rise of glucocorticoid excretion during the Liddle’s test (1 mg overnight and low and high dose dexamethasone suppression tests) [49], and autosomal dominant inheritance in some cases [50, 51]. On pathology, iMAD is characterized by multiple small yellow-to-dark brown nodules surrounded by a cortex with a uniform appearance, which leads to capsular deficits resulting in extra-adrenal cortical excrescences and moderate diffuse cortical hyperplasia [2, 51, 52]. This disease is highly heterogeneous, and disease-causing variants in PRKACB [33, 35, 54] have been implicated in its pathogenesis. Micronodular adrenocortical hyperplasias mainly produce cortisol and/or its pre-cursors, although non-cortisol producing lesions exist (unpublished data) but have not been extensively studied [15].

Familial Syndromes Associated with Primary Adrenocortical Hyperplasia

Several monogenic disorders have been associated with the development of primary adrenocortical hyperplasias (Table 2). The following section briefly enlists these conditions.

<table>
<thead>
<tr>
<th>Familial syndromes</th>
<th>Gene (locus)</th>
<th>Mode of inheritance</th>
<th>Major features</th>
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<tbody>
<tr>
<td>Carney complex</td>
<td>PRKAR1A (17q22–24, CNC1 locus) 2p16 (CNC2 locus, gene unknown) PRKACB (1p31.1, CNC3 locus)</td>
<td>AD</td>
<td>• PPNAD&lt;br&gt; • Cardiac myxomas&lt;br&gt; • Pigmented skin lesions (lentiginosis and blue nevi)&lt;br&gt; • Somatotroph-pituitary adenomas&lt;br&gt; • LCCSCT&lt;br&gt; • Benign thyroid nodules, differentiated thyroid cancer&lt;br&gt; • Melanocytic schwannomas</td>
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<tr>
<td>Multiple Endocrine Neoplasia Type 1</td>
<td>MEN1 (11q13)</td>
<td>AD</td>
<td>• Primary hyperparathyroidism&lt;br&gt; • Pituitary adenomas&lt;br&gt; • Neuroendocrine neoplasms&lt;br&gt; • Skin lesions&lt;br&gt; • Adrenal tumors, including PBMAH</td>
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<td>Familial Adenomatous Polyposis</td>
<td>APC (5q22.2)</td>
<td>AD</td>
<td>• Large pre-cancerous colorectal polyps in the second and third decade of life&lt;br&gt; • Adrenocortical tumors, including adrenocortical cancer and PBMAH&lt;br&gt; • Papillary thyroid carcinomas&lt;br&gt; • Lipomas&lt;br&gt; • Pancreatic carcinomas</td>
</tr>
<tr>
<td>Hereditary Leiomyomatosis and Renal Cell Cancer</td>
<td>FH (1q42.3–43)</td>
<td>AD</td>
<td>• Hereditary leiomyomatosis&lt;br&gt; • Renal cancer&lt;br&gt; • Adrenocortical tumors, including PBMAH</td>
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**Carney Complex (CNC)**

CNC is an autosomal dominant multiple neoplasia syndrome arising from genetic alterations in three loci, PRKAR1A (which codes for R1α subunit of PKA and is known as the CNC1 locus), 2p16 (CNC2), and PRKACB amplification (CNC3 locus) [55]. Most disease-causing variants are caused by inactivating variants in R1α of PKA that are spread along the whole coding sequence [9, 56]. The clinical manifestations of CNC include PPNAD (more than 60 % of patients with CNC have clinically detectable PPNAD), cardiac myxomas, various pigmented skin lesions, growth-hormone excess due to somatotroph-pituitary hyperplasia, benign large cell calcifying Sertoli cell tumor of the testis, thyroid lesions and melanocytic schwannomas. CNC does not predispose to macronodular adrenocortical hyperplasia.

**Multiple Endocrine Neoplasia Type 1 (MEN-1)**

MEN-1 is an autosomal dominant condition that arises from heterozygous disease-causing inactivating germline variants of MEN1 (11q13) [57]. The clinical manifestations of MEN-1 include primary hyperparathyroidism (>95%), skin lesions (>90%), pituitary adenomas (45%), and neuroendocrine neoplasms (>30%). Adrenal enlargement are seen in approximately 20.4 % (146/715) of patients with MEN-1. MEN-1 may predispose to PBMAH (10.1 % of the
Familial Adenomatous Polyposis (FAP)
FAP is an autosomal dominant condition that arise from the tumor suppressor gene APC. The clinical manifestations include large pre-cancerous colorectal polyps, primary adrenocortical lesions including PBMAH, papillary thyroid carcinomas, lipomas, and pancreatic carcinomas. PBMAH is an infrequent manifestation of FAP [22]. It is not known if FAP predisposes to micronodular adrenocortical hyperplasia.

Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCS)
HLRCC is an autosomal dominant condition arising from disease-inactivating variants of the mitochondrial enzyme fumarate hydratase (FH). Clinical manifestations include hereditary leiomyomatosis, renal cancer, and adrenal lesions. HLRCC predisposes to PMAH and/or adrenocortical adenomas that can be non-functional [22, 59, 60], although the first demonstration of FH’s involvement in adrenocortical tumors was in a patient with PBMAH and mostly subclinical hypercortisolism [59].

Carney Triad (CT)
CT is a sporadic condition from an unknown genetic defect with a female predominance. In CT, patients harbor a recurrent aberrant DNA methylation of the promoter region of the SDHC gene [61]. This condition predisposes to hamartomatous lesions in various organs (such as pulmonary chondromas, gastrointestinal stromal tumors, pheochromocytoma, and esophageal leiomyoma [62]. CT is the only known adrenal condition that has among its clinical manifestations both adrenocortical and medullary involvement; in fact, adrenal lesions are the fourth component of this condition, which includes PMAH and/or adrenocortical adenomas that are mostly non-functional [63].

Genetic Classification, Testing and Counseling of Patients with Familial Primary Adrenocortical Hyperplasias
Advances in the field of genetics and genomics has substantially progressed and informed our understanding of the molecular pathogenesis of sporadic and familial forms of primary adrenocortical hyperplasias. In keeping with the growing list of genes implicated in the pathogenesis of these lesions, and to better serve genetic testing and counseling of affected or at risk patients, a gene-based classification in which patients are grouped based on the presence of disease-causing germline variants or other genetic alterations is required (Fig. 2). This classification will highlight the causative genes, which may aid with prioritizing genetic testing and/or counseling of affected family members. Additionally, this approach may decrease the risk of misclassifying familial cases as sporadic. An example of such a classification system includes listing the implicated gene name before the type of hyperplasia, such as ARMC5-PBMAH, MEN1-PBMAH, PRKAR1A-CNC, and PDE11A-iM-
AD (►Fig. 2). As there are yet unidentified molecular cause(s) of these adrenocortical hyperplasias [15], we anticipate a growing number of genes implicated in these disorders over the next decades and a robust, flexible and easy to follow classification system is thus required.

Since most familial forms of adrenocortical hyperplasias are inherited in an autosomal dominant manner, establishing a causative gene is important for understanding the disease mechanisms, mode of inheritance and usefulness of cascade screening. Although the genotype-phenotype correlation is often times unpredictable, providing specific screening and counseling could decrease a patient’s anxiety towards this uncertainty, decrease genetic discrimination and ensure appropriate disease surveillance. Genetic screening may begin as early as infancy in at risk individuals, especially in CNS or iMAD from MAS. A successful patient counseling model should incorporate the patient’s values and attitudes toward their disease, while underscoring the risks and benefits of genetic screening and counseling, psychosocial interventions and service delivery [64].

In the context of ARMC5-PBMAH or PDE11A-iMAD/PBMAH, the penetrance is decreased and first-degree relatives that are carriers may not be affected. Therefore, all first-degree relatives with a known carrier state should be referred for genetic counseling and phenotype-directed biochemical screening per established clinical guidelines. Family screening of ARMC5, as with the other genes as listed in ▶Table 1 and ▶2, will allow early detection of carriers, and prospective follow up. Genetic testing should be offered when an individual is free to refuse or accept the test according to their wishes and moral beliefs. Children should not be tested for these low penetrant genes unless there is clinical evidence of disease.

Conclusions
The identification of several genetic causes of primary adrenocortical hyperplasias, primarily in the cAMP-signaling pathways, have paved the way for large-scale clinical and molecular studies and future research. The traditional classification as adenomas, hyperplasias and carcinomas is non-specific and has added confusion to the nomenclature of these lesions. In this new era of personalized care and genetics, a gene-based classification that is more specific is required to assist in the understanding of their disease processes, hormonal functionality and signaling pathways. Additionally, a gene-based classification carries implications for treatment, genetic counseling and screening of asymptomatic family members.

Author and Contributors
All authors contributed equally to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; and drafting the work or revising it critically for important intellectual content; and final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of Interest
Dr. Stratakis holds patents on the PRKAR1A, PDE11A, GPR101 genes and/or their function and his laboratory has received support from Pfizer Inc on research on GPR101 and acromegaly.

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