Pathophysiology and Histopathology of Primary Aldosteronism

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Abstract

Primary aldosteronism (PA) can be sporadic or familial and classified into unilateral and bilateral forms. Sporadic PA predominates with excessive aldosterone production usually arising from a unilateral aldosterone-producing adenoma (APA) or bilateral adrenocortical hyperplasia. Familial PA is rare and caused by germline variants, that partly correspond to somatic alterations in APAs. Classification into unilateral and bilateral PA determines the treatment approach but does not accurately mirror disease pathology. Some evidence indicates a disease continuum ranging from balanced aldosterone production from each adrenal to extreme asymmetrical bilateral aldosterone production. Nonetheless, surgical removal of the overactive adrenal in unilateral PA achieves highly successful outcomes and almost all patients are biochemically cured of their aldosteronism.

Primary Aldosteronism

Aldosterone is the principal mineralocorticoid hormone produced by the zona glomerulosa cells of the outer layer of the adrenal cortex [1]. It indirectly modulates water retention, blood volume and blood pressure through its central role in the regulation of renal sodium reabsorption and potassium excretion. Aldosterone production is tightly regulated by the renin-angiotensin-aldosterone system [2]. This strict control progressively declines with age to reach an autonomous aldosterone physiology and a potential mechanism of age-related vascular dysfunction [3, 4].

Primary aldosteronism (PA) is the most frequent cause of endocrine hypertension in which plasma aldosterone concentrations are normal or elevated relative to suppressed plasma renin levels [5]. The prevalence of PA is around 5% in patients with hypertension in primary care [6, 7], 10% in hypertension referral centers [8], and up to 20% in patients with resistant hypertension [9]. PA is however underdiagnosed [10, 11] and the true prevalence may be considerably higher than generally contended [12]. Patients with PA have a higher risk of heart and kidney organ damage than patients with essential hypertension [13-17]. These observations underscore the importance to diagnose PA in a timely manner and implement a specific targeted treatment to avert or reverse the aldosterone-specific toxicities.

Sporadic forms comprise the vast majority of PA cases (95-99%), with rare familial forms making up the remainder. Sporadic PA can be unilateral or bilateral, whereas familial forms are always bilateral. Patients with confirmed PA are optimally treated by curative adrenalectomy for unilateral disease, or for life with a mineralocorticoid receptor antagonist for bilateral forms. Thus, unilateral PA must be differentiated from bilateral forms to identify surgically treatable forms and the gland with aldosterone hypersecretion for surgical

resection. The recommended approach is by computed tomography scan of the adrenal glands and adrenal vein sampling which can detect functional overactivity [18].

PA has many different causes that include sporadic aldosterone-producing adenomas (APAs), bilateral adrenal hyperplasia, apparently unilateral adrenal hyperplasia, and in exceptional cases, adrenal carcinomas, and extra-adrenal aldosterone-producing tumors. In addition, in an expanding list of germline variants, four rare familial forms of hyperaldosteronism (FH, familial hyperaldosteronism) have been identified that are classified according to the underlying germline pathogenic variant [19-24]. Recurrent pathogenic somatic variants underlie the pathophysiology of most APAs [19, 25-28]. Of note, genes that carry PA germline variants may also be targets of somatic variants which determine aldosterone overproduction in APAs [19, 28-30].

Immunohistochemistry of adrenals using a highly specific monoclonal antibody against CYP11B2 (aldosterone synthase) [31] demonstrated the wide histopathologic spectrum of PA with a diverse array of aldosterone-producing lesions [32-37]. Evaluation of CYP11B2 immunohistochemistry also helps to direct sequence analysis to the appropriate adrenal region (CYP11B2-guided sequence analysis) [38], which, coupled with next-generation sequencing approaches, has led researchers to determine a high combined prevalence of somatic variants in APAs that often exceeds 90% [32, 33, 39-42].

Herein, we review the latest advances on the pathophysiology of PA due aldosterone-driver gene variants, and the histopathology of surgically treated forms including the relationship between histopathology and postsurgical outcomes.

Gene Variants and Cellular Pathophysiology

In 1992, Lifton et al., established the genetic basis of FH-I (or GRA, glucocorticoid remediable aldosteronism) [22, 43]. Since this breakthrough, major advances in sequencing technologies have aided the identification of germline and somatic pathogenic variants that drive dysregulated aldosterone production in familial and sporadic forms of PA [44, 45] (Table 1).

Germline Variants

FH is caused by rare germline pathogenic variants with an autosomal dominant pattern of inheritance. FH-I is derived from an unequal crossover recombination between *CYP11B1* (encoding 11β-hydroxylase that produces cortisol in the zona fasciculata) and *CYP11B2* (encoding aldosterone synthase that produces aldosterone in the zona glomerulosa). The resultant chimeric gene comprises the ACTH-responsive promoter region of *CYP11B1* and the proceeding exons of *CYP11B2*. Thus, the chimeric gene is expressed in the zona fasciculata with aldosterone production regulated by ACTH instead of angiotensin II [22, 43].

FH-II is due to pathogenic germline variants in the *CLCN2* gene that encodes the CIC-2 voltage-gated chloride channel [21, 24]. Pathogenic *CLCN2* variants in FH-II display strong CIC-2 currents and abolish voltage-dependent gating that depolarizes the zona glomerulosa cell membrane [21, 24, 46, 47]. In turn, the opening of voltage-gated calcium channels and activation of intracellular calcium signaling stimulates *CYP11B2* gene expression and autonomous aldosterone production. *Knock-in* mice with constitutively open CIC-2 channels with similar electrophysiologic properties as PA-associated *CLCN2* variants have hypertension, hypokalemia, high serum aldosterone and low renin levels [46].

Pathogenic *KCNJ5* germline variants cause FH-III [19]. In normal aldosterone physiology, KCNJ5- a G-protein activated inward rectifying potassium channel- functions in membrane depolarization in response to angiotensin II [48]. Pathogenic *KCNJ5* variants associated with PA cause a loss of channel selectivity for potassium and abnormal sodium conductance that results in depolarization of zona glomerulosa cells, the opening of voltage-gated calcium channels, and activation of calcium signaling and dysregulated aldosterone production [49].

Increased Ca^{2+} signaling elicited by *KCNJ5* variants was proposed to account for enhanced cell proliferation in addition to excess aldosterone production [19]. Evidence in favor of this is derived from a study by Scholl and co-workers relating the clinical phenotype of four kindreds with FH-III with the underlying *KCNJ5* variant and the associated cellular physiology [50]. Two kindreds with a KCNJ5 p.G151R substitution displayed severe aldosteronism with massive adrenocortical hyperplasia. In contrast, hypertension was easily controlled in the other two kindreds with a KCNJ5 p.G151E substitution without detectable adrenal hyperplasia [50]. The greater Na^+ currents elicited by KCNJ5 p.G151E compared with p.G151R were associated with higher Na^+-dependent cell lethality (likely due to greater osmotic shock) that may impede the development of an adrenocortical mass and severity of the clinical phenotype [50]. Following this, inducible expression of *KCNJ5* variants in adrenocortical cells indicated that expression level of the mutated *KCNJ5* can modulate cell growth through effects on cell death and proliferation [51].

Cases of genetic mosaicism for a *KCNJ5* pathogenic variant (encoding p.KCNJ5-G151R) in patients with bilateral aldosterone-producing nodules have been described [52, 53]. Maria et al. identified the *KCNJ5* variant in 11 aldosterone-producing nodules from a patient with early onset-PA using Sanger sequencing [52]. Low-level mosaicism for the *KCNJ5* variant was uncovered by whole exome deep sequencing. The variant allele frequency in

aldosterone-producing nodules with the *KCNJ5* variant reached 39% compared with 0.1%-0.23% in germline cells (peri-adrenal fat, peripheral blood leukocytes) [52].

Germline pathogenic variants in the *CACNA1H* gene (encoding the T-type calcium channel Cav3.2) lead to FH-IV [20, 23]. These variants directly result in increased calcium signaling mainly through a defect in channel inactivation and consequently, increased *CYP11B2* gene transcription and aldosterone production [23, 54]. A mouse model replicating an FH-IV *CACNA1H* variant (equivalent to the human p.M1549V) confirmed its effect on enhancing intracellular Ca^{2+} concentrations and the relative autonomy of aldosterone production [55].

De novo germline variants in *CACNA1D* (encoding the L-type calcium channel Cav1.3) can cause PA, via a similar mechanism of activation of intracellular calcium signaling described above, in the context of complex neurologic disorders referred to as PASNA (PA, seizures, and neurologic abnormalities) [28]. PASNA is not usually referred to as a familial form of PA because no families with germline pathogenic *CACNA1D* variants have been reported.

Germline pathogenic variants in *ARMC5* (encoding the armadillo repeat-containing protein 5) are frequent in cortisol-producing primary bilateral macronodular adrenal hyperplasia [56-60]. Of note is the presence of *ARMC5* germline variants in bilateral adrenocortical nodules in African American patients with PA, but not in Caucasians [61, 62]. As for PASNA, Mendelian inheritance of *ARMC5* variants in PA has not been demonstrated and thus, PA associated with *ARMC5* variants is not designated a familial form of the disease.

Somatic Variants

Several germline pathogenic variants in PA are also found as somatic variants in APAs (Box 1). Accordingly, APAs may carry variants in *CLCN2*, *KCNJ5*, *CACNA1H* and *CACNA1D* [19, 26, 28-30] that also cause FH-II, FH-III, FH-IV, and PASNA, respectively when they

occur in the germline [19-21, 23, 24] (Table 1). In addition, recurrent somatic pathogenic variants exist in *ATP1A1* (encoding Na^+/K^+-ATPase subunit 1), *ATP2B3* (encoding a Ca^{2+}-ATPase), and *CTNNB1* (encoding ß-catenin) [25-27].

Using CYP11B2 immunohistochemistry to localize the probable origin of aldosterone production in tissue sections combined with targeted next generation sequencing panels determines a higher combined prevalence of somatic APA variants (85-96% of APAs) than conventional approaches [32, 33, 39-41] (Table 1). APA genotype data from the same group (LMU, Munich, Germany) illustrates a prominent increased detection of *KCNJ5* pathogenic variants using the former method (Figure 1).

In most reported cohorts, APAs with *KCNJ5* variants predominate, particularly in East Asians but *CACNA1D* variants prevail in African Americans [64] (Table 1). Further, differences in sex distribution are also evident, with a higher incidence of *KCNJ5* variants in women and *CACNA1D* variants in men [64]. The reasons underlying the relative prevalence according to race and sex are unclear.

ATP1A1 variants impair pump function and K^+ binding [27, 65] and lead to adrenocortical cell depolarization and dysregulated aldosterone production via a mechanism not fully explained by increased intracellular Ca^{2+} signaling but rather, related to cell acidification [66]. In contrast, *ATP2B3* variants cause increased Ca^{2+} influx and decreased Ca^{2+} export which underscores the associated amplification of *CYP11B2* gene expression and aldosterone production in adrenocortical cells [67].

Somatic variants in *CTNNB1* (encoding β-catenin) are frequent in adrenocortical tumors including nonfunctioning adenomas, cortisol-producing adenomas, and adrenocortical carcinomas in addition to APAs [68]. These variants constitutively activate β-catenin signaling leading to β-catenin bypassing ubiquitin-proteosome degradation and thus

accumulating in the cytoplasm and nucleus [68]. A defined role for β-catenin in adrenocortical hyperplasia was demonstrated by generation of mice with stabilized β-catenin specifically expressed in zona glomerulosa cells. The mice displayed progressive hyperplastic expansion of the zona glomerulosa layer that resulted not from increased proliferation but rather, from a block in the transdifferation of zona glomerulosa to zona fasciculata cells [69].

Despite a prevalence of *CTNNB1* APA variants of around 5% [25], β-catenin accumulation is evident in a high proportion of APAs (70% of 47 APAs) suggesting a broad function in APA pathophysiology [70]. Berthon et al., showed that a constitutively active form of β-catenin was associated with increased expression of the NURR1 (NR4A2) and NUR77 (NR4A1) transcription factors that activate *CYP11B2* gene expression [70]. Further, transgenic mouse models with activated β-catenin signaling display mild aldosteronism [69, 71].

Overall, the above observations suggest a pathophysiological role for *CTNNB1* variants in PA in aldosterone production in addition to tumorigenesis. In a recent development, 59% of 27 APAs with a *CTNNB1* variant had a concurrent co-driver somatic variant in *GNA11* or in *GNAQ* (encoding homologous guanine nucleotide-binding proteins) [72]. The co-existing G protein variants result in a missense substitution at p.Q209 and show synergy with *CTNNB1* variants for aldosterone production [72].

Adrenal Histopathology

The generation of highly specific monoclonal antibodies against human adrenal steroidogenic enzymes [31, 35] has been key to precisely localize apparent production sites of steroids in immunohistochemistry and immunofluorescence studies of resected adrenals. Notable among these are monoclonals against CYP11B2 (aldosterone synthase, to identify the source of aldosterone) [31], CYP11B1 (11β hydroxylase, for cortisol) [31], and against CYP17A1

(17α-hydroxylase/17,20-lyase [37], to identify cells that may produce the hybrid steroids 18-hydroxycortisol and 18-oxocortisol when used in combination with CYP11B2 or with both CYP11B2 and CYP11B1 in double or triple immunostaining studies) [37, 73-75].

The use of these antibodies in immunohistochemistry has had an enormous impact on PA research. These advances include characterization of the diverse and complex histopathologic landscape of PA adrenals [32-35, 74, 76, 77], identification of novel aldosterone-producing lesions [78, 79] that may function in the pathophysiology of some unilateral and bilateral forms of PA [78, 80, 81] (Figure 2), incorporation into CYP11B2-guided sequencing to detect aldosterone-driver variants, and for the diagnosis of different histopathologic subforms of PA [36, 82].

Adrenal Morphology and Steroidogenesis

APAs comprise predominantly clear, lipid-rich cells ("zona fasciculata-like cells") or compact eosinophilic cells ("zona glomerulosa-like"). Decades prior to the discovery of somatic APA pathogenic variants, Tunny et al. [83] reported subclasses of APAs with distinct cellular compositions. Specifically, a class of APAs with predominantly compact cells, produced aldosterone in response to upright posture (that activates the renin-angiotensin-aldosterone system) or angiotensin II infusion. A second subgroup of APAs, comprising mostly clear cells that were unresponsive to these stimuli, were associated with elevated production of the hybrid steroids 18-oxocortisol and 18-hydroxycortisol and this morphologic difference was proposed to have an underlying genetic basis [83, 84].

A link between *KCNJ5* variants and the predominant clear cell composition of APAs was reported by Azizan et al. [85] and thereafter by others [77, 86] and more recently, Guo et al confirmed elevated hybrid steroid production in posture-unresponsive APAs [42]. APAs with *KCNJ5* variants show higher *CYP17A1* gene expression than those without a detected *KCNJ5*

variant [85] and more intense CYP11B1 immunostaining [86]. Expression of CYP17A1 permits 11-deoxycortisol and cortisol production for subsequent synthesis of hybrid steroids when CYP11B2 is expressed in the same cell. The distinct cellular composition of these tumors therefore, likely underlies their characteristic steroidogenic activity [42, 75, 87] and account for the success of plasma 18-oxocorticol alone to differentiate unilateral from bilateral forms of PA in a cohort from Japan [88]- a population with a particularly high prevalence of APA *KCNJ5* pathogenic variants [41] (Table 1). In contrast, in other cohorts, LC-MS/MS (liquid chromatography with tandem mass spectrometry) measurement of multiple steroids in peripheral blood are required to differentiate unilateral from bilateral forms of PA [89-92] and, when combined with machine learning technologies, may be useful in a clinical setting to predict pathogenic *KCNJ5* variant status to identify a subset of patients with an APA [89]. A further development on the clinical exploitation of *KCNJ5* variants is the potential application of macrolide antibiotics that selectively inhibit forms of KCNJ5 with a p.G151R or p.L168R variant [93]. Such compounds could potentially be used in the diagnosis and treatment of APAs harboring these pathogenic variants [93, 94].

The broad adrenal histopathology in PA prompted the international HISTALDO consensus to establish a standardized approach for the evaluation, classification and nomenclature of the main aldosterone-producing adrenocortical lesions observed in surgically treated PA for the final histopathologic diagnosis (Figure 3) [36]. In HISTALDO, the role of immunohistochemistry with the specific CYP11B2 monoclonal antibody [31] was underlined, as in other studies [35, 76, 78, 82] to identify aldosterone-producing lesions with analysis of the multiple pieces of adrenal often resected by laparoscopic adrenalectomy, in addition to the routine morphologic assessment with hematoxylin-eosin staining. Thus, functional lesions in PA are documented that encompass APAs, aldosterone-producing

nodules, aldosterone-producing micronodules (APMs, previously called aldosterone-producing cell clusters) and aldosterone-producing diffuse hyperplasia [36].

Histopathologic Classification

APAs and aldosterone-producing nodules are composed of clear and compact eosinophilic cells visualized by hematoxylin-eosin staining. APAs are larger than aldosterone-producing nodules (lesion diameter ≥ 10 mm *versus* < 10 mm) and are more likely to be visualized by adrenal imaging methods (computed tomography scanning and magnetic resonance imaging) [36]. Aldosterone-producing nodules cannot be differentiated by their size from APMs and both may show polarized CYP11B2 immunostaining that decreases in intensity from the outer to inner part of the lesion. However, APMs comprise only zona glomerulosa cells and their morphology is indistinguishable from that of adjacent cells. Thus, evaluation of both morphology (hematoxylin-eosin) and function (CYP11B2 immunohistochemistry) is required to differentiate an APM from an aldosterone-producing nodule [36]. Aldosterone-producing diffuse hyperplasia is defined by a broad layer of zona glomerulosa cells with > 50% CYP11B2 positive immunostaining, irrespective of the presence of APMs.

HISTALDO also categorized adrenals from surgically treated patients into classical and nonclassical histopathology of unilateral PA [36]. The classical cases include a solitary APA or a dominant aldosterone-producing nodule; nonclassical cases comprise multiple aldosterone-producing nodules, APMs, or aldosterone-producing diffuse hyperplasia (Figure 3). Histopathologic assessment of adrenals using the HISTALDO criteria from 60 consecutively operated patients with PA from a single center showed 75% with the classical histopathology and 25% with the nonclassical type [33].

Aldosterone-Producing Micronodules

APMs are distinct histopathologic lesions first identified as subcapsular nests of zona glomerulosa cells with positive CYP11B2-immunostaining [79]. APMs are present in adrenals from patients with PA, and in other adrenals, including normal adrenals obtained from kidney donors [79, 95]. Aldosterone-driver variants in *CACNA1D*, *ATP1A1*, and *ATP2B3*, but rarely in *KCNJ5*, are present in 35% of APMs in normal adrenals but may reach a higher incidence in PA adrenals [76, 80, 95].

APMs develop with age. In young persons (<12 years), immunohistochemistry of adrenals from kidney donors revealed continuous CYP11B2 expression throughout the outer zona glomerulosa layer. This progressively changes with age to a discontinuous pattern of CYP11B2 immunostaining and an accumulation of APMs [4, 78] (Figure 2). The aldosterone-to-renin ratio, that highlights inappropriate aldosterone production if elevated, was positively associated with age in a cohort of individuals without PA suggesting a link between APMs and age-related abnormal aldosterone physiology [4].

CYP11B2 immunohistochemistry of 15 adrenals of patients with bilateral idiopathic hyperaldosteronism (and without detectable abnormalities on adrenal imaging) showed that aldosterone-producing diffuse hyperplasia was present in 4 cases, in contrast, APMs were identified in all 15 adrenals. An increase in number and size of APMs was also observed in the bilateral idiopathic hyperaldosteronism cases compared with normal adrenals and adrenals from patients with unilateral PA [80]. A higher incidence of somatic variants in aldosterone-driver genes was also determined in adrenals from idiopathic hyperaldosteronism than in normal adrenals (57% *versus* 35%). Overall, Omata et al. highlighted a conceivable role for APMs in the pathogenesis and pathophysiology of a form of bilateral PA [80].

APMs may progress to APAs. This hypothesis is supported by observations of hybrid aldosterone-producing lesions with features of APMs and APAs with outer zona glomerulosa cells and inner zona fasciculata cells, and in some cases, different aldosterone-driver variants in the outer and the inner portions of the lesion [78]. Additional evidence derives from in situ MALDI (matrix assisted laser desorption/ionization)-mass spectrometry imaging of adrenals from unilateral PA [81]. Two highly distinct APM metabolic phenotypes (APM subgroups 1 and 2) were identified from 27 APMs. Subgroup 2 (7 of 27 APMs) and APAs displayed similar metabolic profiles. In contrast, the majority of APMs in subgroup 1 (20 of 27) showed a clearly distinct pattern of metabolites [81]. These differences appeared independent of aldosterone-driver variants. The metabolic phenotype of APM subgroup 2 showed enrichment of biological pathways related to cell proliferation. Thus, some observations from molecular pathology favor the concept of a minority of APMs progressing to APAs (Figure 2).

Postsurgical Outcome Assessment

The international PASO consensus established criteria to assess the clinical and biochemical response to total unilateral adrenalectomy to treat patients diagnosed with unilateral PA by adrenal venous sampling [96]. A key characteristic of the PASO criteria is the separation of clinical and biochemical outcomes, each categorized as complete, partial, and absent success of surgical treatment (Figure 4).

Biochemical Outcomes

Complete biochemical success defines cure of aldosteronism, partial or absent biochemical success characterize different levels of persistence of aldosteronism. In PASO, 94% of 699 patients (83-100%) achieved complete biochemical success highlighting the rewarding

outcome of adrenalectomy for patients with unilateral disease and subsequent reduction in the future risk of aldosterone-mediated cardiovascular toxicities [97]. The remaining 6% of 699 patients, who likely had presurgical bilateral aldosteronism, indicates a limitation of adrenal venous sampling in a small proportion of patients with asymmetrical bilateral aldosterone overproduction [96].

Clinical Outcomes

Complete clinical success is defined by resolution of hypertension without anti-hypertensive medication. Persistence of hypertension is classified as partial or absent clinical success which may be influenced by factors such as sex, and age [96], long duration of hypertension and organ damage [98, 99] or indicate background essential hypertension. However, partial clinical success denotes improvement with substantial patient benefit and reduced cardiovascular morbidity. In the PASO study, 37% of 705 patients (between-center range, 17-62%) achieved complete clinical success, 47% (35-66%) partial clinical success, demonstrating the significant clinical benefit of unilateral adrenalectomy (clinical + partial clinical success) [14, 97].

The PASO criteria allow cohort analysis of individual patient data between different studies and centers [100-103] and have been used (i) to evaluate approaches for diagnosis and treatment [89, 103-106]; (ii) develop prediction scores for clinical outcomes [98, 107]; and (iii) establish the link between adrenal histopathology and outcomes [33, 34, 82].

Pathophysiology and Postsurgical Outcomes

The presence of aldosterone-producing nodules or micronodules, or aldosterone producing diffuse hyperplasia in the dominant resected adrenal suggests their existence in the unresected contralateral adrenal and may reflect persistence of milder hypertension [104].

Patients with resected adrenal lesions classified with a classical histology display a more florid aldosteronism at baseline compared with the classical phenotype [33] and more favorable postsurgical biochemical outcomes [33, 36]. Consistently, steroidogenic activity in unresected (contralateral) adrenals is associated with postsurgical outcomes [33, 104, 106]. Hence, adrenals with a nonclassical histopathology are associated with higher production of aldosterone from the unresected gland compared with the classical group [33].

Comparing data from patients categorized by postsurgical outcomes demonstrated differences in contralateral adrenal steroidogenesis between patients with biochemical or clinical persistence than those who are cured. Thus, using patient data from segmental adrenal vein sampling procedures (to identify the adrenal segment originating aldosterone overproduction for partial adrenalectomies to spare unaffected segments), Nakai et al [106] established higher baseline aldosterone production in the tributary and central veins of the unresected adrenal in patients with postsurgical incomplete (absent + partial success) biochemical success.

A few studies reported better clinical outcomes after surgery in patients with an APA carrying a *KCNJ5* variant [89, 108, 109]. However, these studies did not include CYP11B2 immunohistochemistry to identify those cases with histopathologic confirmation of an APA and thus may have included non-functioning adenomas or multiple nodules. In a prospective study, that used HISTALDO and PASO criteria for histopathology and outcome assessment, and CYP11B2-guided genotype analysis of APAs demonstrated similar clinical and biochemical outcomes in groups with or without a *KCNJ5* variant with 36 of 37 patients showing postsurgical complete biochemical success [33].

Concluding Remarks

Somatic variants in genes that control intracellular homeostasis account for aldosterone excess in most APAs. A dual role for these variants in autonomous aldosterone production and the promotion of tumorigenesis is unclear. Currently, APA variant status is not employed in diagnosis and therapy but accurate prediction of the presence of a *KCNJ5* pathogenic variant (by steroid profiling or hormone response to macrolide antibiotics) may find a future application in refining patient selection for treatment (see Outstanding Questions). Consensus criteria to evaluate outcomes and histopathology are useful tools to study a wide array of applications, including a better understanding of the pathophysiology of PA.

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Text Box 1. Somatic and Germline Pathogenic Variants in Primary Aldosteronism

Sporadic forms of PA include unilateral disease, usually due to an APA. APAs are overwhelmingly caused by pathogenic somatic variants in genes encoding an ion channel (KCNJ5 potassium channel, CACNA1D and CACNA1H calcium channels, CLCN2 chloride channel), either of two ion pumps (ATP1A1, ATP2B3), or the CTNNB1 cell signaling/cell-cell adhesion molecule. CTNNB1 variants may co-exist with variants in the G proteins, GNA11 or GNAQ (Figure I). Genes encoding somatic APA variants in ion channels are also targets of germline pathogenic variants causing familial forms of PA (FH II-IV) or PASNA (PA with seizures and neurologic abnormalities). PASNA is caused by de novo germline *CACNA1D* variants and familial transmission has not been demonstrated. FH-I is due to a hybrid *CYP11B1-CYP11B2* gene (Figure I). Somatic *CACNA1D* variants predominate in APMs over variants in *ATP1A1* and *ATP2B3*. APMs have been implicated in the pathophysiology of a form of bilateral PA (IHA) and in nonclassical unilateral forms of PA.

Gene	Protein	White Americans (*N*=75)	Black Americans (*N*=73)	Brisbane (Australia) (*N*=40)	Sendai (Japan) (*N*=106)	Paris (France) (*N*=48)	Munich (Germany) (*N*=41)	PA with germline variants
KCNJ5	GIRK4	43%	34%	35%	73%	44%	56%	FH-III
CACNA1D	Cav1.3	21%	42%	33%	14%	27%	10%	PASNA
CACNA1H	Cav3.2	4%	-	-	1%	0%	0%	FH-IV
CLCN2	CIC-2	3%	-	-	-	0%	2%	FH-II
ATP1A1	Na^+/K^+ ATPase1	17%	8%	20%	5%	13%	12%	-
ATP2B3	Ca^{2+}-ATPase	4%	4%	5%	4%	10%	5%	-
CTNNB1	β-catenin	3%	0%	0%	0%	0%	0%	-
Total prevalence		**95%**	**89%**	**93%**	**97%**	**94%**	**85%**	-

Table 1. Genes with Common Somatic Variants in Primary Aldosteronism

Relative prevalence of variants in each gene is reported from studies using CYP11B2-guided sequencing incorporating NGS panels [30, 32, 33, 39-42, 110]. The indicated genes function in intracellular anion homeostasis (*KCNJ5*, *CACNA1D*, *CACNA1H*, *ATP1A1*, and *ATP2B3*), intracellular cation homeostasis (*CLCN2*), or cell signaling and cell-cell adhesion (*CTNNB1*).

APAs, aldosterone-producing adenomas; Ca^{2+}-ATPase, plasma membrane calcium-ATPase; Cav1.3, calcium channel, voltage-dependent, L type, alpha 1D subunit; Cav3.2, calcium channel, voltage-dependent, T type, alpha 1H subunit; CIC-2, chloride voltage-gated channel-2; FH, familial hyperaldosteronism; GIRK4, G protein-activated inward rectifier potassium channel 4; Na^+/K^+ ATPase 1, sodium/potassium-transporting alpha 1 subunit; PA, primary aldosteronism; PASNA, primary aldosteronism with seizures, and neurologic abnormalities. *N* indicates the total number of APAs in each cohort. FH-I is caused by a hybrid *CYP11B1-CYP11B2* gene (see text for details).

Figure Legends

Text Box Figure I. Somatic and Germline Pathogenic Variants Cause Unilateral and Bilateral Forms of Primary Aldosteronism.

APA, aldosterone-producing adenoma; APM, aldosterone-producing micronodule (formerly known as aldosterone-producing cell cluster); IHA, idiopathic hyperaldosteronism; PA, primary aldosteronism; PASNA, PA with seizures and neurologic abnormalities; FH, familial hyperaldosteronism. Figure created using BioRender (https://biorender.com/).

Figure 1. Prevalence of APA Variants in Aldosterone-Driver Genes: Conventional versus CYP11B2-Guided Sequence Analysis.

The figure shows the reported prevalence of variants in aldosterone driver genes in a single center (LMU Munich, Germany) using the traditional approach (employed in 2014) without CYP11B2 immunohistochemistry to guide tissue sampling for sequencing [111], compared with CYP11B2-guided sequencing and incorporation of next generation sequencing panels in 2021 [33].

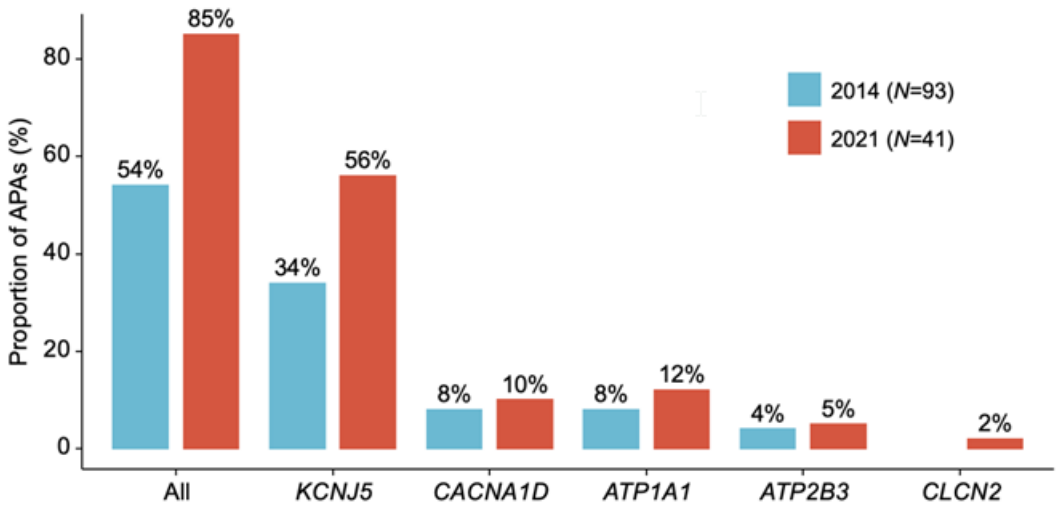


Figure 2. Aldosterone-Producing Micronodules and Aldosterone Pathophysiology.

APMs are distinct histopathologic lesions beneath the adrenal capsule (see Box 1 for details). APMs accumulate with age. Investigation of a form of idiopathic hyperaldosteronism in bilateral PA (without detectable abnormalities on computer tomography scanning) implicates a role for APMs in PA pathophysiology. In some cases, an APM may evolve into an APA. Inappropriate aldosterone production for renin levels may increase with age to a subclinical form of PA and further develop to idiopathic hyperaldosteronism. Dominant aldosterone overproduction from a single gland is usually higher than bilateral forms. Figure created using BioRender (https://biorender.com/)

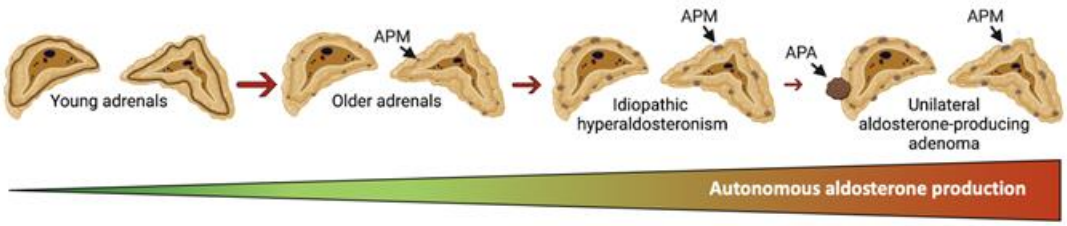


Figure 3. HISTALDO Evaluation of Adrenals from Patients with Primary Aldosteronism.

The HISTALDO (histopathology of primary aldosteronism) consensus established standardized nomenclature for the identifiable histopathologic features (see text for details). Adrenals are categorized into those exhibiting a classical or nonclassical histopathology of unilateral PA. A higher incidence of complete biochemical success (according to the PASO criteria) is observed in patients after surgical resection of classical forms compared with removal of nonclassical adrenal lesions.

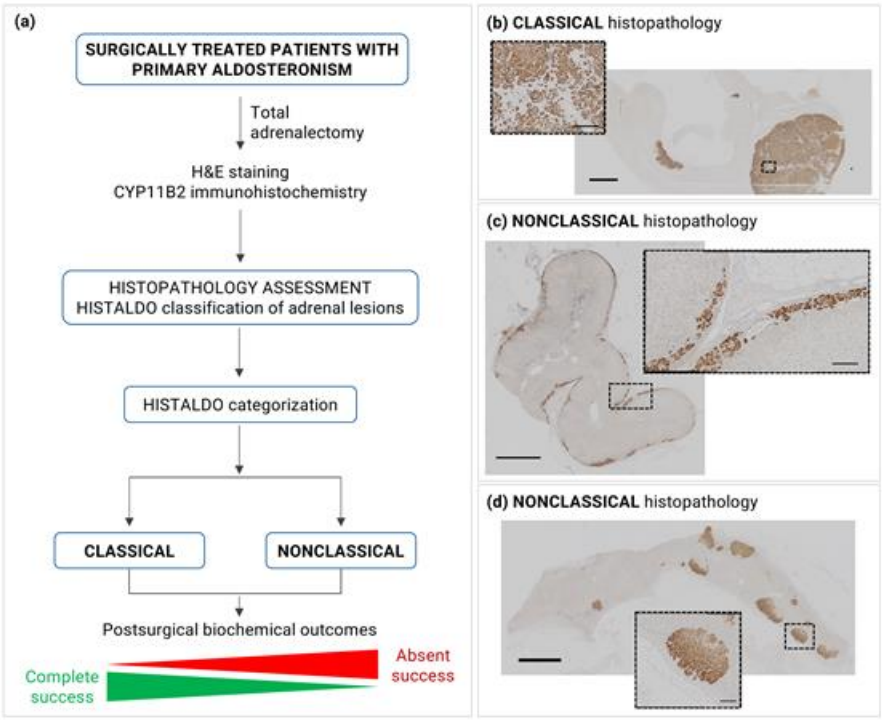
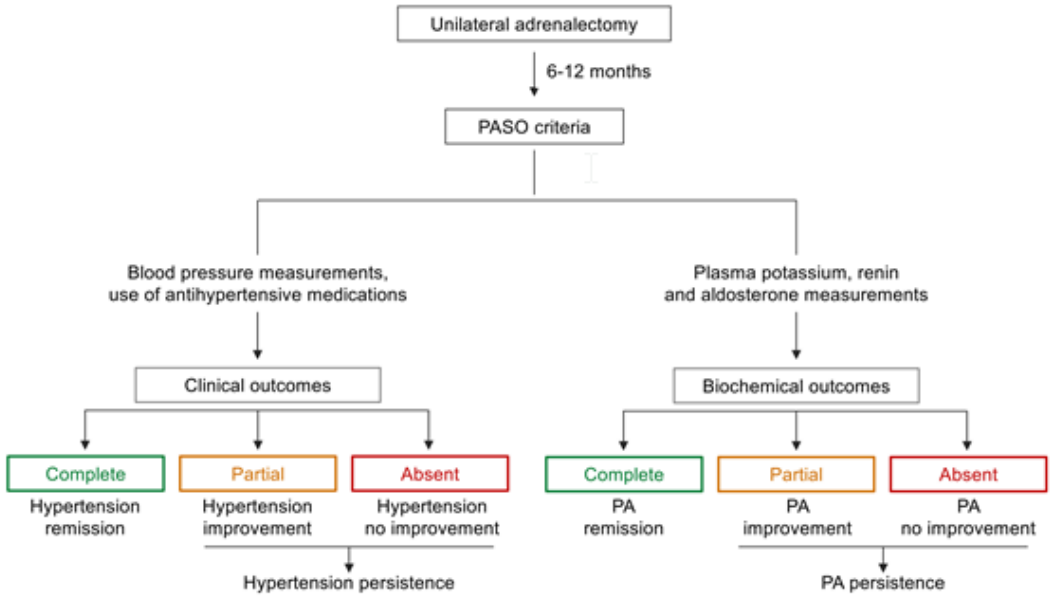
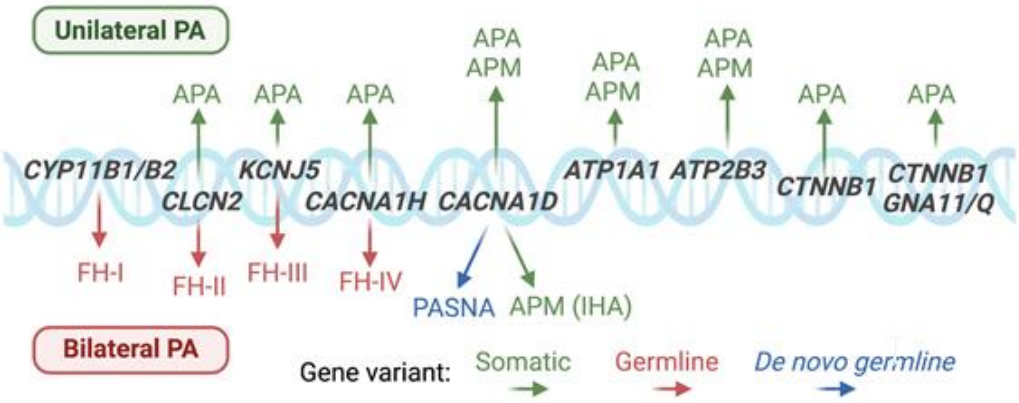


Figure 4. Primary Aldosteronism Surgical Outcome (PASO) Criteria.

The PASO (primary aldosteronism surgical outcome) criteria allow the standardized assessment of postsurgical outcomes. Clinical and biochemical outcomes after adrenalectomy are evaluated in accordance with defined criteria that stratify outcomes into complete, partial, or absent clinical or biochemical success. Partial and absent success indicate persistence of hypertension (clinical outcomes) or aldosteronism (biochemical outcomes).


Unilateral adrenalectomy
6-12 months
PASO criteria
Blood pressure measurements, use of antihypertensive medications
Plasma potassium, renin and aldosterone measurements
Clinical outcomes
Biochemical outcomes
Complete
Partial
Absent
Complete
Partial
Absent
Hypertension remission
Hypertension improvement
Hypertension no improvement
PA remission
PA improvement
PA no improvement
Hypertension persistence
PA persistence


Unilateral PA
APA
APM
CYP11B1/B2
CLCN2
KCNJ5
CACNA1H
CACNA1D
ATP1A1
ATP2B3
CTNNB1
CTNNB1
GNA11/Q
FH-I
FH-II
FH-III
FH-IV
PASNA
APM (IHA)
Bilateral PA
Gene variant:
Somatic
Germline
De novo germline

Highlights

- Primary aldosteronism is a frequent form of endocrine hypertension caused by aldosterone overproduction principally from one adrenal gland or relatively equivalently from both (unilateral or bilateral disease)
- Unilateral forms are usually caused by an aldosterone-producing adenoma in which somatic mutations alter the properties of ion channels and ion pumps to disturb intracellular ion homeostasis leading to increased *CYP11B2* (aldosterone synthase) transcription
- Adrenal CYP11B2 immunohistochemistry aids the final histopathologic diagnosis of primary aldosteronism and is central to a refined approach for the identification of aldosterone-driver variants
- Adrenal histopathology is linked to postsurgical outcomes and abnormal aldosterone production from the unresected contralateral adrenal gland
- Some bilateral forms of primary aldosteronism may be caused by distinct histopathologic lesions called aldosterone-producing micronodules that in some cases, may evolve into aldosterone-producing adenomas

Outstanding Questions

- Do mutations in ion channels and ion pumps also stimulate dysregulated cell growth in aldosterone-producing adenomas or are there other requisite modulators?
- Can the predicted presence of KCNJ5 pathogenic variants in aldosterone-producing adenomas be exploited in personalized medicine for streamlined patient management?
- Do bilateral forms of sporadic primary aldosteronism exhibit a broad histopathologic spectrum as in unilateral forms?
- What is the molecular mechanism that triggers aldosterone-producing diffuse hyperplasia?
- Do aldosterone-producing lesions in the adjacent cortex to an aldosterone-producing adenoma function in disease etiology, for example, in asymmetrical bilateral primary aldosteronism?
- Are there subcategories of aldosterone-producing micronodules with distinct roles in pathogenesis?