Hypertension

REVIEW

Histopathology and Molecular Profiling of Primary Aldosteronism

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ABSTRACT: Primary aldosteronism is the most common surgically treatable form of hypertension. Surgically amenable forms are characterized by lateralized aldosterone overproduction, which may occur along a spectrum ranging from true unilateral hypersecretion to asymmetrical bilateral production. Historically attributed to solitary aldosterone-producing adenomas, lateralized primary aldosteronism is now recognized to encompass a range of histopathologic lesions, including aldosterone-producing micronodules, reflecting a continuum from subclinical autonomous secretion to overt primary aldosteronism. This review synthesizes recent evidence demonstrating how histopathology, genetics, and cellular mapping can collectively explain some of the heterogeneity in surgical outcomes. The Histopathology of Primary Aldosteronism consensus classifies lateralized primary aldosteronism into classical (aldosterone-producing adenoma-predominant) and nonclassical (aldosterone-producing micronodule-predominant) forms, with the former linked to biochemical remission and the latter associated with persistent or recurrent aldosteronism. Somatic driver mutations further correlate with histology and outcomes, while single-cell and spatial omics data reveal distinct pathogenic trajectories and metabolic profiles underlying tumor progression. Together, these advances offer a more precise, pathophysiology-based framework to improve diagnosis, subtyping, and outcome prediction, advancing tailored management for this disease.

Key Words: adenoma ■ adrenal cortex ■ aldosterone ■ consensus ■ hyperaldosteronism ■ hypertension ■ mutation

Primary aldosteronism (PA) represents the most common form of endocrine and surgically curable hypertension.[1,2] The resultant aldosterone excess leads to sodium retention, potassium excretion and a disproportionate increase in cardiovascular and renal risk that is independent of blood pressure elevation.[3,4] Biochemically, PA is characterized by inappropriately elevated aldosterone concentrations leading to renal sodium retention, volume expansion, and suppression of renin. Despite its clinical significance, PA remains greatly underdiagnosed, with persistently low screening rates even among high-risk populations.[5,6] Prevalence estimates vary considerably by clinical setting, ranging from ≈5% in primary care populations to up to 30% in patients with resistant hypertension or those with hypertension and hypokalemia, and exceeding 40% in patients with hypertension and comorbid atrial fibrillation.[7–10] This diagnostic shortfall is concerning given that PA is a treatable condition with targeted, disease-specific therapies available.[2,11]

PA manifests in both sporadic and familial forms, with sporadic disease accounting for the vast majority of cases. The most common sporadic subtypes are unilateral aldosterone-producing adenomas (APAs) and bilateral aldosterone-producing lesions, an important distinction that determines treatment strategy because while lateralized disease is amenable to surgical cure, bilateral disease typically requires lifelong medical management.[12,13] Somatic mutations in ion channels and transporters are now known to underlie most APAs, while germline mutations account for several familial syndromes.[14,15] This review will focus on lateralized forms of PA.

The development of highly specific monoclonal antibodies against CYP11B2 (aldosterone synthase) has transformed our understanding of APA pathophysiology.[16,17] This tool has enabled precise identification and classification of aldosterone-producing lesions, guided targeted sampling for somatic mutation analysis and aided integration with tissue-omics approaches to refine the cellular and molecular profile of adrenal lesions in PA.[17–19] Consequently, the systematic analysis of resected adrenal glands from patients with lateralized

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Nonstandard Abbreviations and Acronyms

APA	aldosterone-producing adenoma
APM	aldosterone-producing micronodule
AVS	adrenal vein sampling
CYP11B2	aldosterone synthase
HISTALDO	Histopathology of Primary Aldosteronism
mTOR	mechanistic target of rapamycin
PA	primary aldosteronism
ZG	zona glomerulosa

PA, guided by adrenal vein sampling (AVS) and combined with next-generation sequencing and multi-omics platforms, has provided unprecedented insight into disease pathophysiology.

In this review, we discuss how specific types of aldosterone-producing lesions and their associated somatic driver mutations correlate with postsurgical outcomes. Furthermore, we explore how emerging single-cell and spatial omics technologies offer the potential to dissect cellular heterogeneity within resected tissue and map molecular profiles onto tissue architecture. The integration of these approaches may provide deeper mechanistic insight into disease pathogenesis and elucidate the biological determinants underlying variable postoperative outcomes.

ADRENAL HISTOPATHOLOGY IN PA: SPECTRUM, CLASSIFICATION, AND CLINICAL CORRELATIONS

AVS is the standard method for selecting patients with PA for unilateral adrenalectomy, based on the lateralization index (the ratio of cortisol-corrected aldosterone from the dominant to nondominant adrenal vein). High lateralization indices (typically >4:1) usually guide the decision for surgery.[2] The combination of AVS-guided adrenalectomy and subsequent morphology and CYP11B2 immunohistochemistry[16] has revealed that lateralized PA originates from a spectrum of histologically distinct lesions.[17,20] This pathological diversity underlies the heterogeneous clinical and biochemical features of PA.[21–23] The prototypical lesion is an APA, which is frequently associated with a more florid biochemical phenotype, including more pronounced hypokalemia, elevated aldosterone-to-renin ratios, and resistant hypertension.[8,21,24–26]

Following adrenalectomy for AVS-directed lateralized PA, a minority of patients fail to achieve complete biochemical success.[12] Analysis of resected adrenal glands from these patients with persistent biochemical disease (ie, those with absent or partial biochemical success) revealed significant pathological differences compared with age- and sex-matched patients who achieved remission, including a higher prevalence of adrenal hyperplasia (49% versus 21%) and a lower prevalence of APA (44% versus 79%).[27] They also exhibited lower lateralization indices and higher contralateral ratios on presurgical AVS. Together, these findings suggest that AVS lateralization may not indicate purely unilateral disease but rather asymmetrical bilateral aldosterone production, where 1 gland is dominant despite underlying bilateral pathology. Supporting this, a multicenter study of 283 patients who underwent adrenalectomy based on strong AVS lateralization found that 16% lacked biochemical remission following surgery.[28] Within this group with persistent PA, 73% had multiple CYP11B2-positive adenomas in the resected adrenal, compared with only 23% in those who achieved biochemical remission. Thus, this histological evidence directly links adrenal pathology to biochemical outcomes and confirms that AVS cannot definitively exclude contralateral aldosteronism. Beyond these biological factors, interpretation of AVS results may also be influenced by methodological differences, including between-center variability in sampling protocols, the use of ACTH stimulation, and differences in lateralization index thresholds.

To standardize the classification of this variable adrenal morphology, the international Histopathology of Primary Aldosteronism (HISTALDO) consensus was established[29] and later endorsed by the WHO Classification of Adrenal Cortical Tumors[30] (Table 1). This consensus recommends using CYP11B2 immunohistochemistry to identify aldosterone-producing regions and categorize lesions into APAs, aldosterone-producing nodules, aldosterone-producing micronodules (APMs), or aldosterone-producing diffuse hyperplasia (Figure 1). The HISTALDO system distinguishes 2 broad categories of classical and nonclassical lateralized PA. Classical forms feature a solitary APA or dominant aldosterone-producing nodule and a mixed phenotype where the dominant lesion coexists with other aldosterone-producing lesions. Nonclassical forms comprise multiple aldosterone-producing nodules, APMs, or aldosterone-producing diffuse hyperplasia, without a single dominant nodule.[21–23]

Application of the HISTALDO criteria in studies of consecutively resected adrenals from patients with AVS-confirmed lateralized PA has demonstrated classical histology in 75% to 88% of cases and nonclassical histology in 12% to 25% (cohort sizes: 60–240 patients). Among classical cases, 41% to 58% displayed the mixed phenotype.[21–23] Critically, this histopathologic classification correlates with preoperative disease severity and postoperative outcomes. The classical histology is associated with a more severe biochemical presentation, including higher baseline plasma aldosterone concentrations (262 versus 155 pg/mL), higher aldosterone-to-renin ratios (81 versus 42 [pg/mL]/[mU/L]), lower serum potassium, and greater contralateral adrenal suppression on AVS.[21] The mixed phenotype demonstrates

Table 1. HISTALDO Morphology Definitions

Histopathologic entity	Definition
Aldosterone-producing adenoma (APA)	A CYP11B2-positive, solitary, and well-circumscribed neoplasm (≥10 mm diameter) composed of clear or compact eosinophilic cells or both cell types
Aldosterone-producing nodule (APN)	A CYP11B2-positive lesion visible on standard H&E staining with a diameter <10 mm
Aldosterone-producing micronodule (APM)	A subcapsular CYP11B2-positive lesion with a diameter <10 mm composed of zona glomerulosa cells that are morphologically similar to adjacent cells on H&E stain. Often shows a gradient of CYP11B2 immunostaining decreasing in intensity from the outer to the inner part of the lesion
Aldosterone-producing diffuse hyperplasia (APDH)	A CYP11B2-positive lesion characterized by a broad and uninterrupted zone of glomerulosa cells, with positive immunostaining present in more than half of this cellular population

Standardized histopathologic classification of aldosterone-producing lesions adapted from the HISTALDO consensus.[29] Definitions are based on lesion size, morphological characteristics on hematoxylin and eosin (H&E) staining, and CYP11B2 (aldosterone synthase) immunohistochemistry using a monoclonal CYP11B2 antibody.[16] HISTALDO indicates Histopathology of Primary Aldosteronism.

intermediate biochemical severity between the classical and nonclassical groups.[22]

Regarding postoperative outcomes, a significantly higher proportion of patients with classical histology achieve complete biochemical success (97% to 98% versus 67% to 69%, $P<0.001$) or complete clinical success (34% versus 11%, $P<0.001$) compared with those with nonclassical forms.[21,23] Patients with classical and mixed histology also require fewer postoperative antihypertensive medications.[22] Multivariable analysis confirmed classical histology as an independent predictor of both biochemical and clinical remission.[23] The inferior biochemical outcomes in the nonclassical group correlate with increased aldosterone production from the contralateral adrenal, providing a pathophysiological basis for persistent disease.[21]

The clinical impact of nonclassical histology extends beyond short-term outcome assessment. Long-term follow-up demonstrates a risk of biochemical recurrence after initial remission. While 93% of patients achieved biochemical success at 12 months, 23% developed recurrent PA at a median follow-up of 89 months. Recurrence was far more common in patients with nonclassical histology (60% versus 14% in classical cases), suggesting progressive disease in the contralateral gland.[31] Although recurrence may be more common in lateralized PA without a discrete APA, adrenalectomy frequently still provides meaningful clinical improvement in blood pressure and medication burden. Table 2 summarizes studies linking histopathology to postsurgical outcomes based on international consensus criteria.[12,29]

One study proposed refining the HISTALDO system by incorporating the B2 ratio (the size ratio of the largest to second-largest CYP11B2-positive nodule) to improve diagnostic accuracy.[40] Using the original HISTALDO criteria, this study classified 73% of lateralized PA cases (55 of 75) as nonclassical- a proportion notably higher than the 12% to 25% reported in other cohorts.[21–23] Applying the B2 ratio reclassified 29 nonclassical cases as classical, yielding a revised distribution of 65% classical and 35% nonclassical. The authors suggest such quantitative metrics may reduce overestimation of nonclassical cases. However, the substantial initial discrepancy likely reflects interobserver variability or cohort-specific differences, including differences in diagnostic criteria for surgical candidates, underscoring the need for replication in multicenter cohorts.

GENETIC BASIS OF ALDOSTERONE-PRODUCING LESIONS: DRIVER MUTATIONS AND CLINICAL IMPLICATIONS

The histopathologic spectrum of APAs is underpinned by a diverse but a generally mechanistically convergent genetic landscape. Somatic driver mutations can be identified in over 90% of APAs, with most leading to sustained intracellular calcium ($Ca2^{+}$) signaling, which constitutively induces aldosterone synthase gene (*CYP11B2*) expression. The somatic drivers, their mechanisms, and some clinicopathological correlates are summarized in Table 3.

The most common drivers are mutations in genes encoding ion channels and pumps that disrupt cellular Ca^{2+} homeostasis through pathological membrane depolarization, directly increased Ca^{2+} entry, or impaired Ca^{2+} export.[41,51,62] *KCNJ5* mutations are the most prevalent and are typified by larger adenomas comprised mainly of clear (zona fasciculata-like) cells and more pronounced hyperaldosteronism.[50,68] In contrast, mutations in *CACNA1D* and *ATP1A1* are more frequently linked to smaller adenomas composed mostly of eosinophilic (ZG-like) cells.[52,69] *ATP2B3*-mutated APAs are also smaller but display an overlapping clear cell morphology with *KCNJ5*-mutated tumors.[70] This mutational landscape has direct implications for clinical outcomes (Table 2). *KCNJ5* mutations are reported to predict higher rates of postsurgical clinical remission,[31,34–39] whereas *CACNA1D* mutations are linked to lower remission rates.[31,38] This difference may reflect the bilateral disease continuum, with additional aldosterone-producing lesions often present in the contralateral adrenal. Consistent with this continuum, *CACNA1D* mutations are preferentially associated with APMs,[20,71] which frequently occur as bilateral lesions.[72] Consequently, aldosterone production may persist after unilateral adrenalectomy. In contrast, *KCNJ5* mutations are predominantly associated with APAs,[51] which are generally linked to more favorable surgical outcomes.[21,31,34–39] These genotype-histopathology relationships may also reflect distinct developmental pathways of aldosterone-producing lesions, as discussed in later sections.

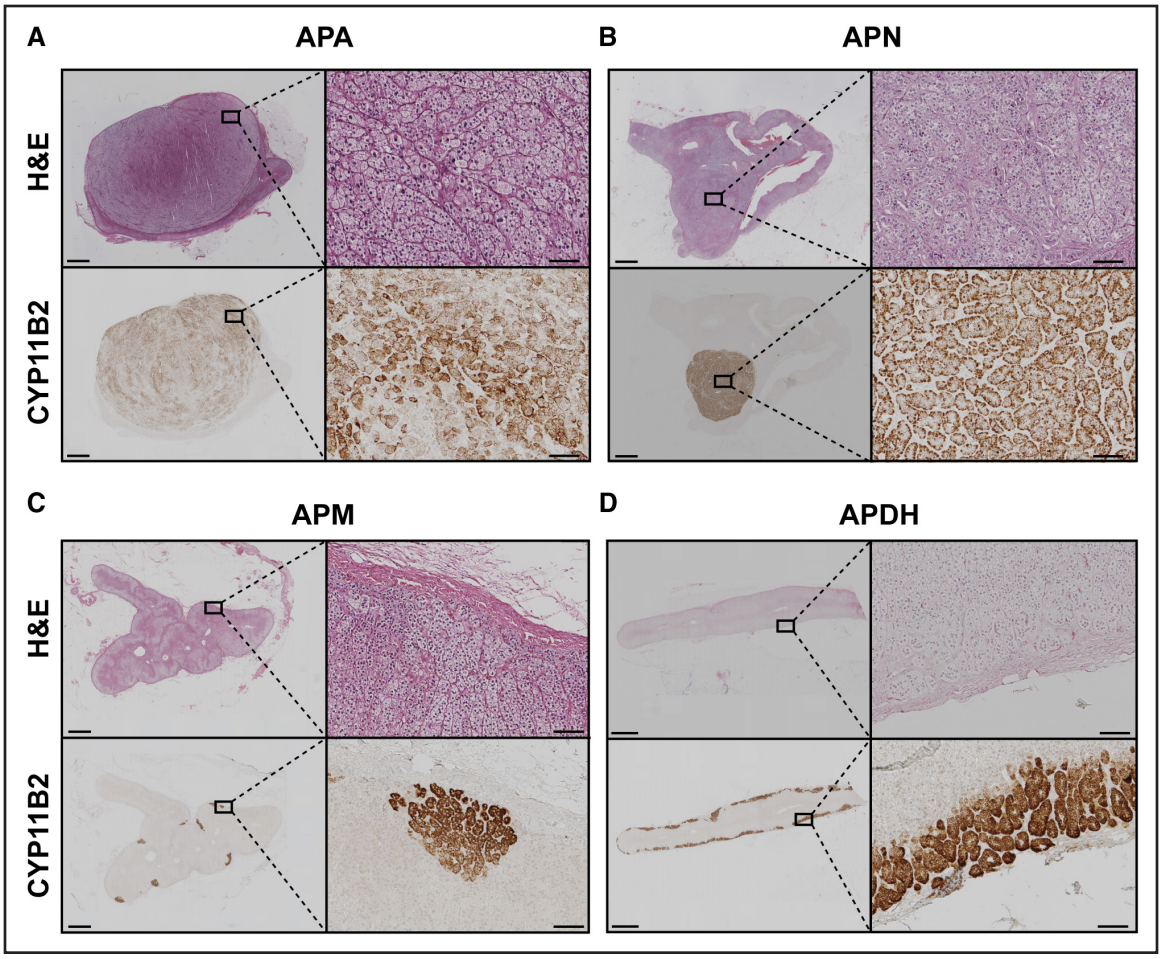

Figure 1. Histopathologic spectrum of aldosterone-producing lesions in primary aldosteronism (PA).
Representative adrenal tissue sections from patients operated for lateralized PA, illustrating the morphological diversity of aldosterone-producing lesions according to Histopathology of Primary Aldosteronism (HISTALDO) criteria.[29] Each lesion type is shown with paired hematoxylin and eosin (H&E) staining and CYP11B2 (aldosterone synthase) immunohistochemistry. **A**, Aldosterone-producing adenoma (APA). **B**, Aldosterone-producing nodule (APN). **C**, Aldosterone-producing micronodules (APMs). **D**, Aldosterone-producing diffuse hyperplasia (APDH). The classical histopathology of lateralized PA is represented in **A** and **B**, whereas **C** and **D** demonstrate the nonclassical pattern. Refer to Table 1 for morphology definitions. High-magnification insets are also shown. Scale bars, 2 mm (lower left), 100 µm (lower right).

A growing list of rarer variants in other genes, such as *CACNA1H*, *CLCN2*, *MCOLN3*, and *SLC30A1*,[56,59,61,64] further reinforces dysregulated ion handling and sustained intracellular Ca^{2+} increase as the dominant final common pathway for aldosterone hypersecretion. However, not all PA driver mutations function through altered ion conductance. Somatic APA mutations in *CTNNB1* activate Wnt/β-catenin signaling and likely drive adrenocortical proliferation.[73,74] Of interest, *GNA11/GNAQ* mutations seem clinically silent in isolation but can synergistically increase aldosterone secretion when they cooccur with mutations in *CTNNB1*.[65] Rare mutations in *CADM1* propose a novel paradigm of disrupted intercellular communication within the adrenal cortex, attenuating the physiological suppression of *CYP11B2*.[67]

The spectrum of aldosteronism extends beyond overt clinical PA, suggesting a population-wide continuum. Approximately 14% of normotensive individuals with low renin demonstrate autonomous aldosterone secretion fulfilling diagnostic criteria for PA, indicating a background of renin-independent aldosteronism.[75] This phenomenon is age-dependent, with aging associated with progressive accumulation of APMs, a decline in CYP11B2 expression in the zona glomerulosa (ZG), and a pattern of autonomous aldosteronism characterized by inappropriately elevated aldosterone-to-renin ratios despite sodium loading and blunted aldosterone responses to sodium restriction.[76]

APMs seem to represent an important histological intermediate within this continuum. They are widespread in normal adrenal glands, and around one-third harbor somatic mutations in known aldosterone-driver genes, usually in *CACNA1D*.[20,71] Metabolic profiling of PA adrenal sections without an APA but with multiple APMs (nonclassical forms) revealed distinct APM subgroups, one of which, minor in number, displayed a metabolic phenotype converging with that of APAs,

Table 2. Histopathology and Genotype Predictors of Biochemical or Clinical Outcomes After Adrenalectomy

Predictors	Surgical outcome	Correlation			References
Histopathology	Biochemical outcome	Total cohort	Classical histopathology	Nonclassical histopathology	
	Complete success (biochemical remission)	90.8% (118/130)	97.0% (98/101)	69.0% (20/29)	23
		79.1% (72/91)	86.3% (63/73)	50.0% (9/18)	32
		90.7% (49/54)	97.6% (41/42)	66.7% (8/12)	21
		64.7% (22/34)	81.8% (18/22)	33.3% (4/12)	29
	Clinical outcome	Total cohort	Classical histopathology	Nonclassical histopathology	
	Complete success (hypertension remission)	29.1% (37/127)	34.3% (34/99)	10.7% (3/28)	23
		55.0% (55/100)*	62.1% (41/66)	41.2% (14/34)	33
KCNJ5 mutation	Clinical outcome	Total cohort	*KCNJ5* mutation	Nonmutated *KCNJ5*	
	Complete success (hypertension remission)	55.3% (198/358)*	67.0% (126/188)	42.4% (72/170)	34
		56.0% (187/334)	65.5% (150/229)	35.2% (37/105)	35
		62.9% (73/116)	65.1% (69/106)	40.0% (4/10)	36
		34.2% (26/76)	57.6% (19/33)	16.3% (7/43)	37
		37.9% (22/58)	77.8% (14/18)	20.0% (8/40)	38
		33.3% (15/45)	55.6% (10/18)	18.5% (5/27)	31
		35.7% (10/28)	80.0% (8/10)	11.1% (2/18)	39
CACNA1D mutation	Clinical outcome	Total cohort	*CACNA1D* mutation	Nonmutated *CACNA1D*	
	Partial + absent success (persistent hypertension)	62.1% (36/58)	84.2% (16/19)	51.3% (20/39)	38
		66.7% (30/45)	100% (7/7)	60.5% (23/38)	31

Summary of studies reporting significant associations between adrenal histopathology or APA-*KCNJ5* or *CACNA1D*-mutation status and outcomes after adrenalectomy for lateralized PA. Outcomes were assessed in accordance with the PASO (Primary Aldosteronism Surgical Outcome) consensus[12] and are shown as proportions of patients (%) with absolute numbers in parenthesis. Complete biochemical success is defined as normalization of aldosterone and renin parameters and correction of hypokalemia (if present presurgery); complete clinical success is defined as normalization of blood pressure without antihypertensive therapy. Classical and nonclassical histopathology were assessed using HISTALDO criteria (refer to Table 1 and Figure 1).[29]

*Outcome assessment at > 12 mo.

supporting a model of progression from microscopic lesions to macroscopic adenomas.[77] Further, the evolution from an APM background to bilateral disease is suggested by findings from unilateral adrenalectomy in bilateral PA, in which APMs in the resected gland are significantly more numerous and larger than in normotensive controls.[72] Furthermore, most of these lesions carried *CACNA1D* aldosterone-driver mutations (58%), suggesting that bilateral disease may arise from multiple mutated APMs. The genetic overlap between APMs and APAs underlines the continuum concept. This is further reinforced by a genome-wide association study, which identified common susceptibility loci shared between lateralized and bilateral forms of PA, underscoring a shared polygenic basis.[78]

Collectively, these findings support a spectrum model wherein PA results from varying degrees of clonal expansion of adrenocortical cells harboring somatic mutations in PA driver genes. The nodular remodeling from prevalent APMs to clinical APA, illustrated in Figure 2, is thus a manifestation of a broader continuum. This continuum ranges from age-related autonomous aldosteronism in normotensive individuals to overt lateralized or bilateral PA. These genotype-driven clonal expansions give rise to distinct cellular trajectories and molecular profiles, which can now be dissected using advanced single-cell and spatial omics technologies.

CELLULAR AND MOLECULAR LANDSCAPE OF ALDOSTERONE-PRODUCING LESIONS

The histopathologic and genetic diversity of PA stems from cellular reprogramming and remodeling within the adrenal cortex. Single-cell combined with spatial omics approaches help delineate this complexity by linking molecular profiles directly to tissue morphology. Cortical homeostasis relies on the centripetal migration of cells from the outer cortex inward, accompanied by differentiation and lineage conversion.[79,80] A recent study has established a high-resolution molecular reference map of normal adrenal homeostasis.[81] This details the signaling basis of the centripetal model, tracing cellular progression from the outer capsule through the ZG, fasciculata and reticularis at single-cell resolution and defining the conserved signaling pathways, including Wnt/β-catenin, sonic hedgehog, and fibroblast growth factor, that drive this zonation.

Single-cell transcriptomic studies indicate that APMs originate from ZG cells, a process potentially initiated by stress-response factors like NR4A2 (nuclear receptor subfamily 4A2).[82,83] APMs harbor somatic driver mutations (primarily in *CACNA1D*) also found in APAs, providing a direct molecular link between these lesions.[71] A key genetic divergence, however, is the notable rarity of *KCNJ5* mutations in APMs,[20,71] despite *KCNJ5* being the

Table 3. Somatic Mutations in Aldosterone-Producing Adenomas

Target gene	Protein abbreviation	Protein full name	Function	Mechanism/effect of mutation	Prevalence	Comments
Ion channels						
KCNJ5	GIRK4 (or Kir3.4)	Potassium inwardly rectifying channel subfamily J member 5	Maintains resting membrane potential through selective potassium efflux and G-protein-mediated signaling regulation	Impairs potassium selectivity, allowing aberrant Na^+ influx, causing membrane depolarization and subsequent voltage-gated Ca^{2+} channel activation[41]	*34–73%[31,42–46]	More frequent in women, in younger individuals, and in larger tumors[47–50]
CACNA1D	$Ca_v1.3$	Calcium channel, voltage-dependent, L type, alpha 1D subunit	Mediates voltage-dependent Ca^{2+} influx in response to membrane depolarization, regulating cellular excitability and hormone secretion	Increases Ca^{2+} influx through enhanced channel activity, sustaining membrane depolarization and triggering autonomous aldosterone production[51,52]	*8–42%[31,42–46]	More frequent in men and in smaller tumors[49,52]
CACNA1H	$Ca_v3.2$	Calcium voltage-gated channel subunit alpha-1 H	Regulates low-voltage-activated (T-type) Ca^{2+} currents controlling membrane potential and cellular excitability	(1) loss of normal membrane potential regulation, (2) increased basal Ca^{2+} influx, and (3) altered sensitivity to extracellular K^+ and Na^+ concentrations[53–55]	3 of 75 APAs[56]	2 men, 1 woman
CLCN2	ClC-2	Chloride voltage-gated channel 2	Maintains cellular chloride homeostasis and regulates membrane potential through voltage-dependent Cl^- conductance	Promotes membrane depolarization through increased Cl^- efflux, disrupting the normal hyperpolarized state[57,58]	1 of 80[59] and 2 of 115 APAs[60]	1 man, 1 woman[60]
MCOLN3	TRPML3	Mucolipin transient receptor potential cation channel 3	Regulates endolysosomal Ca^{2+} release and intracellular cation homeostasis	Produces constitutively active Ca^{2+}- and Na^+-permeable channels, causing dysregulated calcium signaling through direct influx or indirect mechanisms[61]	4 of 408 APAs[61]	4 men, 0 women
Ion transporters						
ATP1A1	Na^+/K^+-ATPase $\alpha1$	Sodium/potassium-transporting ATPase subunit alpha-1	Maintains electrochemical gradients by actively transporting 3 Na^+ out and 2 K^+ into cells, establishing resting membrane potential	Reduces Na^+ and K^+ binding affinity, impairing pump function and causing membrane depolarization due to disrupted ion gradients[52,62]	*5–27%[31,42–46]	More frequent in men and in smaller tumors[52,62]
ATP2B3	PMCA3	Plasma membrane calcium-transporting ATPase 3	Actively exports Ca^{2+} from cytoplasm to maintain low intracellular Ca^{2+} concentrations and cellular calcium homeostasis	(1) reduced Ca^{2+} export due to impaired pump function, and (2) increased Ca^{2+} influx via depolarization-activated channels and potential Ca^{2+} leak through the mutated pump[62,63]	*4–11%[31,42–46]	More frequent in men, in older individuals and in smaller tumors[62]
SLC30A1	ZnT1	Zinc transporter 1 (solute carrier family 30 member 1)	Exports zinc from cytoplasm to extracellular space, maintaining intracellular zinc homeostasis and protecting cells from zinc toxicity	Generates pathological Na^+ influx through aberrant Na^+ current, causing depolarization of resting membrane potential and subsequent activation of voltage-gated Ca^{2+} channels[64]	1.0–1.9% (5 cases identified)[64]	5 men, 0 women
G-protein signaling						
GNAQ/GNA11	$G\alpha q/G\alpha11$	Guanine nucleotide-binding protein G(q) subunit alpha/G (11) subunit alpha	Mediates signal transduction from G-protein-coupled receptors to activate phospholipase C, leading to intracellular Ca^{2+} mobilization and regulation of cellular responses	Solitary *GNA11* mutations are clinically silent; pathogenic aldosterone hypersecretion requires co-occurring *CTNNB1* mutation. Double *CTNNB1*+*GNA11* mutations synergistically increase aldosterone secretion beyond either mutation alone[65]	Coexists with 16 of 27 *CTNNB1*-mutated APAs[65]	1 prepubescent man, 15 women
Cell adhesion and proliferation						
CTNNB1	β-catenin	Catenin beta 1	Functions as structural component of adherens junctions and key transcriptional co-activator in Wnt signaling pathway, regulating cell proliferation and differentiation	Constitutive activation of Wnt/β-catenin signaling, primarily driving adrenocortical cell proliferation and tumor formation[66]	5.1 % (10 of 198 APAs)[66]	4 men, 6 women
CADM1	SYNCAM/IGSF4A	Cell adhesion molecule 1	Mediates cell-cell adhesion, synaptic organization, and tumor suppression through homophilic and heterophilic interactions at intercellular gap junctions	Inhibition of gap junction communication between zona glomerulosa cells, thereby disrupting the physiological suppression of *CYP11B2* gene expression and causing aldosterone hypersecretion[67]	0.5–1.0% (6 cases identified)[67]	4 men, 2 women

Somatic mutations identified in aldosterone-producing adenomas (APAs), grouped by functional category. Sex distribution and tumor size associations are noted where reported. Rare mutations in *CACNA1H*, *CLCN2*, *MCOLN3*, *SLC30A1*, and *CADM1* have been identified in small numbers of cases as indicated. *CTNNB1* mutations frequently cooccur with *GNA11* mutations, which are not pathogenic in isolation but synergistically enhance aldosterone secretion when coexisting with *CTNNB1* pathogenic variants.

*Indicates prevalence ranges derived from multiple studies that used CYP11B2-guided next-generation sequencing.

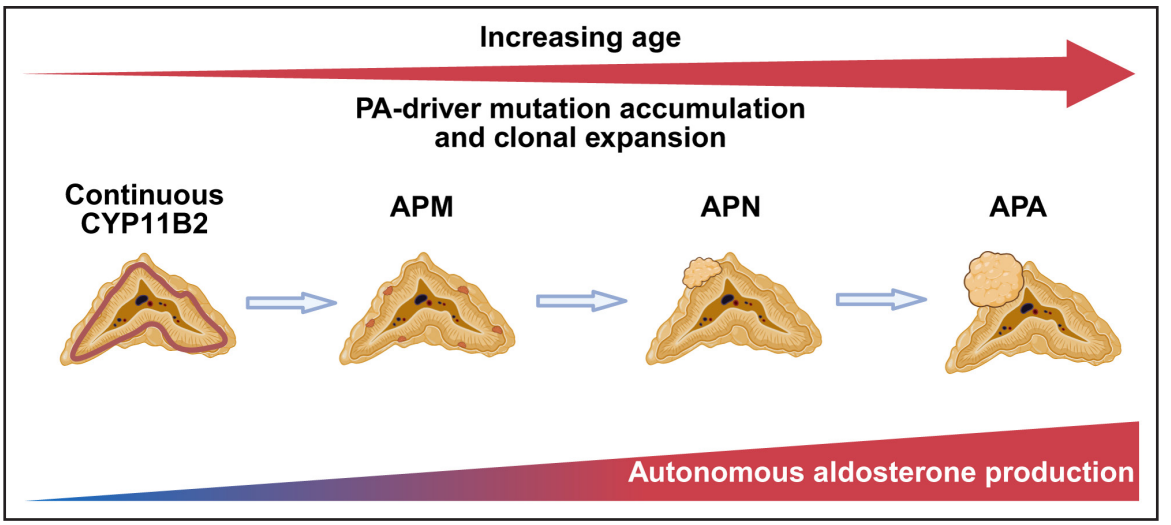

Figure 2. Model of the histopathologic evolution from physiological zona glomerulosa (ZG) to aldosterone-producing adenoma.

This schematic integrates morphological, molecular and functional observations to illustrate how aldosterone-producing adenomas (APAs) may represent the end stage of a multistep pathogenic process. The leftmost stage represents the physiological ZG in young adrenal glands, where CYP11B2 (aldosterone synthase) expression is continuous and aldosterone production remains under renin-angiotensin-aldosterone system control. With aging, CYP11B2 expression becomes discontinuous, giving rise to small foci of CYP11B2-positive cells termed aldosterone-producing micronodules (APMs), many of which do not harbor known aldosterone-driver mutations. Somatic mutations in aldosterone-driver genes may arise in individual ZG cells, and clonal expansion of mutated cells may contribute to the growth of some APMs and increasing autonomy of aldosterone production. In bilateral primary aldosteronism (PA), APMs are typically more numerous and larger than in normal adrenals. Further expansion may lead to the formation of larger aldosterone-producing nodules (APNs) and, in some cases, to an APA. Multiple APMs and APNs may coexist within the same gland without forming a dominant nodule, reflecting the nonclassical histopathologic patterns observed in some patients with PA. Created in BioRender. Pang, Y. (2026) https://app.biorender.com/illustrations/69733e2408e00e7ba2255df5.

most frequently mutated gene in APAs.[50] These observations suggest that *KCNJ5*-mutant APAs arise through a distinct pathogenic pathway, potentially from a different cellular lineage or precursor. Convergent histopathologic, genetic, and spatial-omics observations support a model in which APAs arise through distinct developmental trajectories. Recent integrated single-cell and spatial transcriptomic analyses further suggest 2 biologically feasible trajectories of APA development that correlate with *KCNJ5* genotype status.[84] One trajectory involves a direct progression from ZG cells to APA, observed exclusively in *KCNJ5*-mutant tumors. The other is a stepwise progression from ZG cells to APM and then to APA, which is present in APAs with or without a *KCNJ5* mutation.

These alternative trajectories help reconcile the mutation spectrum observed across adrenal lesions. *CACNA1D* mutations are frequently detected in APMs, whereas *KCNJ5* mutations are rarely observed in these lesions, suggesting that *KCNJ5*-mutant APAs predominantly arise through the direct ZG-to-APA pathway and bypass the APM stage. By contrast, *KCNJ5*–wild-type tumors seem more dependent on the APM precursor pool.[84]

An explanation for this divergence is that the APM stage may represent a metabolic bottleneck during APA progression. In the stepwise pathway, clonal expansion of mutated ZG cells leads to APM formation, where cells may experience metabolic and oxidative stress associated with sustained steroidogenic activity. *KCNJ5*-mutated cells seem better adapted to this stress. Spatial metabolomic analyses demonstrate enrichment of antioxidant metabolites and a shift from glycolysis toward the pentose phosphate pathway, generating NADPH required for redox buffering, while immunohistochemistry for malondialdehyde (a marker of oxidative lipid damage) shows lower levels in *KCNJ5*-mutant APAs compared with *KCNJ5*–wild-type tumors.[19,85] These observations suggest that *KCNJ5*-mutant cells may more readily bypass the APM stage, whereas *KCNJ5* wild-type APAs seem to develop predominantly through progression from APM intermediates (Figure 3).

Within APAs, cellular reprogramming generates spatially segregated functional states, including aldosterone-producing, cortisol-producing and proliferative stromal-like cells.[86] Transcriptional profiling further identifies 2 major *CYP11B2*-positive transcriptional signatures: a ZG-like profile and a fasciculata/reticularis-like profile, the latter enriched in *KCNJ5*-mutant APAs.[19] The tumor microenvironment is also actively remodeled, featuring an immunosuppressive landscape with specific immune infiltration, angiogenic activation and fibroblast-driven stromal reorganization mediated in part by Hedgehog and Wnt signaling.[84,87] Clinically, intratumoral cortisol synthesis in *KCNJ5*-mutant APAs correlates with systemic hypercortisolemia and an increased

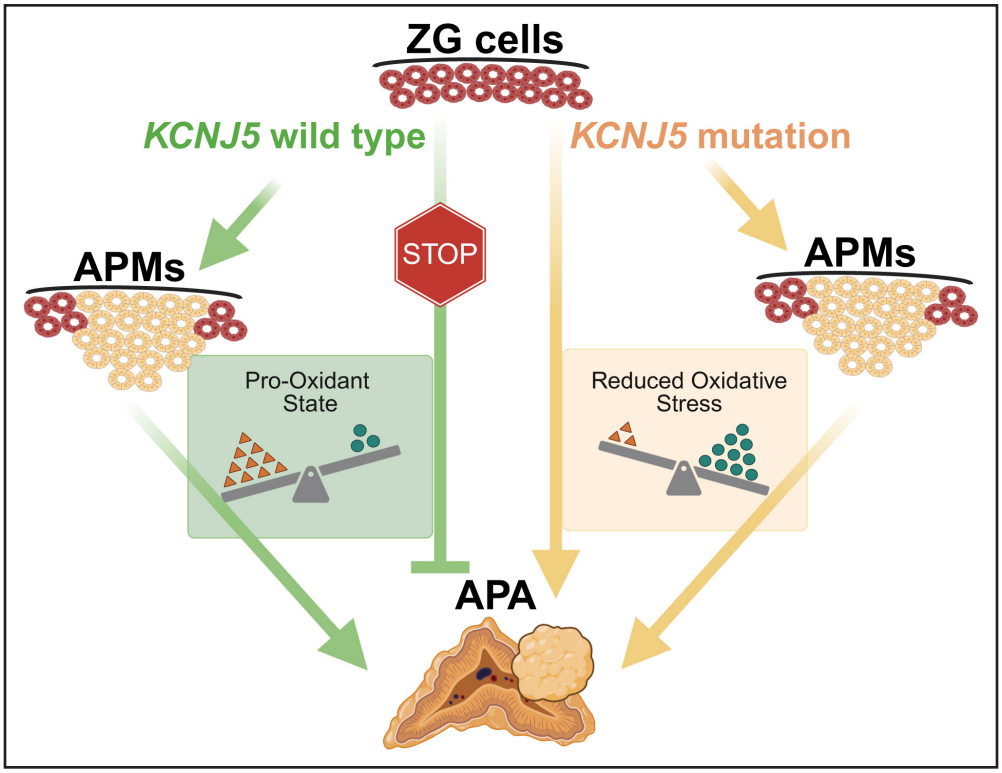

Figure 3. Distinct developmental trajectories and redox environments of *KCNJ5*-wild-type and *KCNJ5*-mutated aldosterone-producing adenomas.

Schematic model of genotype-specific developmental trajectories of aldosterone-producing adenomas (APAs) according to *KCNJ5* genotype status. Spatial transcriptomic and spatial metabolomic profiling identified distinct redox environments in APAs according to *KCNJ5* genotype. *KCNJ5*-wild-type APAs are associated with a pro-oxidant state, whereas *KCNJ5*-mutant tumors exhibit reduced oxidative stress. Pseudotime analysis of integrated scRNA-seq and spatial transcriptomics data suggests that this reduced oxidative environment is progressively established during the development of *KCNJ5*-mutant tumors. Trajectory inference further indicates that *KCNJ5*–wild-type APAs follow a single developmental route requiring progression from zona glomerulosa (ZG) cells through an aldosterone-producing micronodule (APM) intermediate (green arrows). In this model, direct ZG-to-APA transition is not observed. Conversely, *KCNJ5*-mutant APAs may arise either through a direct ZG→APA pathway or via the stepwise ZG→APM→APA route (yellow arrows). These differences may reflect distinct cellular responses to oxidative stress, whereby the APM stage represents a metabolic bottleneck in APA development that *KCNJ5*-mutant cells may more readily bypass. Consistent with this model, *CACNA1D* mutations are frequently detected in APMs, whereas *KCNJ5* mutations are rarely observed in these lesions. Created in BioRender. Sun, Z. (2026) https://app.biorender.com/illustrations/697f3d177e6ee3749c1b94e5.

risk of vertebral fractures.[86] Furthermore, the cortex adjacent to APAs harbors *CYP11B2* expression-negative, APA-like regions, indicating the presence of precursor states, with molecular changes in this peritumoral region potentially mediated by factors such as the AP-1 transcription factors FOS (fos proto-oncogene, AP-1 transcription factor subunit) and JUN (jun proto-oncogene, AP-1 transcription factor subunit).[19,88]

These genotype-driven programs manifest as divergent molecular and metabolic profiles. *KCNJ5*-mutant APAs exhibit a metabolic state that favors growth and steroidogenic capacity, characterized by upregulated lipogenesis, oxidative phosphorylation, and mTOR (mechanistic target of rapamycin) signaling.[19,82,87] Furthermore, histopathologic subtypes of PA also correlate with distinct metabolic profiles, including specific fatty acid alterations.[89] Conversely, non-*KCNJ5*–mutated APAs accumulate metabolites associated with oxidative stress and cell death, aligning with their mandatory progression through an APM precursor state.[19,84] This metabolic divergence is spatially resolved; high-resolution mass spectrometry imaging confirms distinct tumor metabolomes, with *KCNJ5*-mutant APAs displaying unique signatures in purine and steroidogenic pathways.[90]

The integration of these multi-omics data sets is now translating molecular maps into functional insights and practical tools. Computational analyses have identified novel regulatory nodes within APA tumors. For instance, *ATP2A3* is a positive regulator of *CYP11B2* expression and aldosterone secretion, while *EGR1* functions within an oxidative stress–steroidogenesis axis, suppressing both processes.[85,91] These analyses have also identified specific markers with diagnostic utility, such as ABCC3 (ATP-binding cassette C3), a novel marker of CYP11B2-negative ZG cells, providing a histopathologic tool to distinguish normal adrenal zonation from aldosterone-producing lesions.[92]

In conclusion, spatial and single-cell omics define PA as a disease of divergent cellular trajectories. The dichotomy between *KCNJ5*-mutant and APM-driven pathways

can help explain the clinical and molecular heterogeneity of aldosterone-producing lesions. This detailed map of adrenal reprogramming is an important step toward a precise, molecularly informed understanding of the disease.

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Disclosures

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REFERENCES

1. Young WF. Primary aldosteronism: renaissance of a syndrome. *Clin Endocrinol (Oxf)*. 2007;66:607–618. doi: 10.1111/j.1365-2265.2007.02775.x
2. Adler GK, Stowasser M, Correa RR, Khan N, Kline G, McGowan MJ, Mulatero P, Murad MH, Touyz RM, Vaidya A, et al. Primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2025;110:2453–2495. doi: 10.1210/clinem/dgaf284
3. Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, Mulatero P. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol*. 2018;6:41–50. doi: 10.1016/S2213-8587(17)30319-4
4. Monticone S, Sconfienza E, D'Ascenzo F, Buffolo F, Satoh F, Sechi LA, Veglio F, Mulatero P. Renal damage in primary aldosteronism: a systematic review and meta-analysis. *J Hypertens*. 2020;38:3–12. doi: 10.1097/HJH.0000000000002216
5. Cohen JB, Cohen DL, Herman DS, Leppert JT, Byrd JB, Bhalla V. Testing for primary aldosteronism and mineralocorticoid receptor antagonist use among U.S. veterans: a retrospective cohort study. *Ann Intern Med*. 2021;174:289–297. doi: 10.7326/M20-4873
6. Hundemer GL, Imsirovic H, Vaidya A, Yozamp N, Goupil R, Madore F, Agharazii M, Knoll G, Sood MM. Screening rates for primary aldosteronism among individuals with hypertension plus hypokalemia: a population-based retrospective cohort study. *Hypertension*. 2022;79:178–186. doi: 10.1161/HYPERTENSIONAHA.121.18118
7. Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, Gabetti L, Mengozzi G, Williams TA, Rabbia F, et al. Prevalence and clinical manifestations of primary aldosteronism encountered in primary care practice. *J Am Coll Cardiol*. 2017;69:1811–1820. doi: 10.1016/j.jacc.2017.01.052
8. Parasiliti-Caprino M, Lopez C, Prencipe N, Lucatello B, Settanni F, Giraudo G, Rossato D, Mengozzi G, Ghigo E, Benso A, et al. Prevalence of primary aldosteronism and association with cardiovascular complications in patients with resistant and refractory hypertension. *J Hypertens*. 2020;38:1841–1848. doi: 10.1097/HJH.0000000000002441
9. Seccia TM, Letizia C, Muiesan ML, Lerco S, Cesari M, Bisogni V, Petramala L, Maiolino G, Volpin R, Rossi GP. Atrial fibrillation as presenting sign of primary aldosteronism: results of the Prospective Appraisal on the Prevalence of Primary Aldosteronism in Hypertensive (PAPPHY) Study. *J Hypertens*. 2020;38:332–339. doi: 10.1097/HJH.0000000000002250
10. Burrello J, Monticone S, Losano I, Cavaglià G, Buffolo F, Tetti M, Covella M, Rabbia F, Veglio F, Pasini B, et al. Prevalence of hypokalemia and primary aldosteronism in 5100 patients referred to a tertiary hypertension unit. *Hypertension*. 2020;75:1025–1033. doi: 10.1161/HYPERTENSIONAHA.119.14063
11. Reincke M, Bancos I, Mulatero P, Scholl UI, Stowasser M, Williams TA. Diagnosis and treatment of primary aldosteronism. *Lancet Diabetes Endocrinol*. 2021;9:876–892. doi: 10.1016/S2213-8587(21)00210-2
12. Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, Satoh F, Amar L, Quinkler M, Deinum J, et al; Primary Aldosteronism Surgery Outcome (PASO) investigators. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol*. 2017;5:689–699. doi: 10.1016/S2213-8587(17)30135-3
13. Yang J, Burrello J, Goi J, Reincke M, Adolf C, Asbach E, Brűdgam D, Li Q, Song Y, Hu J, et al. Outcomes after medical treatment for primary aldosteronism: an international consensus and analysis of treatment response in an international cohort. *Lancet Diabetes Endocrinol*. 2025;13:119–133. doi: 10.1016/S2213-8587(24)00308-5
14. Mulatero P, Scholl UI, Fardella CE, Charmandari E, Januszewicz A, Reincke M, Gomez-Sanchez CE, Stowasser M, Dekkers OM. Familial hyperaldosteronism: an European Reference Network on Rare Endocrine Conditions clinical practice guideline. *Eur J Endocrinol*. 2024;190:G1–G14. doi: 10.1093/ejendo/lvae041
15. Stölting G, Tran Vo KN, Haus J, Scholl UI. The genetics of hypertension. *Nat Rev Nephrol*. 2026;22:137–151. doi: 10.1038/s41581-025-01020-6
16. Gomez-Sanchez CE, Qi X, Velarde-Miranda C, Plonczynski MW, Parker CR, Rainey W, Satoh F, Maekawa T, Nakamura Y, Sasano H, et al. Development of monoclonal antibodies against human CYP11B1 and CYP11B2. *Mol Cell Endocrinol*. 2014;383:111–117. doi: 10.1016/j.mce.2013.11.022
17. Nakamura Y, Maekawa T, Felizola SJ, Satoh F, Qi X, Velarde-Miranda C, Plonczynski MW, Ise K, Kikuchi K, Rainey WE, et al. Adrenal CYP11B1/2 expression in primary aldosteronism: immunohistochemical analysis using novel monoclonal antibodies. *Mol Cell Endocrinol*. 2014;392:73–79. doi: 10.1016/j.mce.2014.05.002
18. Nanba K, Rainey WE, Udager AM. Approaches to gene mutation analysis using formalin-fixed paraffin-embedded adrenal tumor tissue from patients with primary aldosteronism. *Front Endocrinol (Lausanne)*. 2021;12:683588. doi: 10.3389/fendo.2021.683588
19. Gong S, Sun N, Meyer LS, Tetti M, Koupourtidou C, Krebs S, Masserdotti G, Blum H, Rainey WE, Reincke M, et al. Primary aldosteronism: spatial multiomics mapping of genotype-dependent heterogeneity and tumor expansion of aldosterone-producing adenomas. *Hypertension*. 2023;80:1555–1567. doi: 10.1161/HYPERTENSIONAHA.123.20921
20. Omata K, Anand SK, Hovelson DH, Liu CJ, Yamazaki Y, Nakamura Y, Ito S, Satoh F, Sasano H, Rainey WE, et al. Aldosterone-producing cell clusters frequently harbor somatic mutations and accumulate with age in normal adrenals. *J Endocr Soc*. 2017;1:787–799. doi: 10.1210/js.2017-00134
21. Meyer LS, Handgriff L, Lim JS, Udager AM, Kinker IS, Ladurner R, Wildgruber M, Knösel T, Bidlingmaier M, Rainey WE, et al. Single-center prospective cohort study on the histopathology, genotype, and postsurgical outcomes of patients with primary aldosteronism. *Hypertension*. 2021;78:738–746. doi: 10.1161/HYPERTENSIONAHA.121.17348
22. Ahn CH, Lee YB, Kim JH, Oh YL, Kim JH, Jung KC. Correlation of histopathologic subtypes of primary aldosteronism with clinical phenotypes and postsurgical outcomes. *J Clin Endocrinol Metab*. 2024;109:e1582–e1592. doi: 10.1210/clinem/dgad747
23. Goldbaum TS, Ledesma FL, Guimaraes AG, Okubo J, Kawahara EZ, Calsavara VF, Bortolotto LA, Chambo JL, Fragoso M, Pereira MAA, et al. Histopathological evaluation based on CYP11B2 staining predicts outcomes in unilateral primary aldosteronism. *Eur J Endocrinol*. 2025;192:763–775. doi: 10.1093/ejendo/lvaf118
24. Wang H, Wang F, Zhang Y, Wen J, Dong D, Chang X, Sun H, Ma X, Cui Y, Chen S, et al. Surgical outcomes of aldosterone-producing adenoma on the basis of the histopathological findings. *Front Endocrinol (Lausanne)*. 2021;12:663096. doi: 10.3389/fendo.2021.663096
25. Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, Ganzaroli C, Giacchetti G, Letizia C, Maccario M, et al; PAPY Study Investigators. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *J Am Coll Cardiol*. 2006;48:2293–2300. doi: 10.1016/j.jacc.2006.07.059
26. Rossi GP, Rossitto G, Amar L, Azizi M, Riester A, Reincke M, Degenhart C, Widimsky J, Naruse M, Deinum J, et al. Drug-resistant hypertension in primary aldosteronism patients undergoing adrenal vein sampling: the AVIS-2-RH study. *Eur J Prev Cardiol*. 2022;29:e85–e93. doi: 10.1093/eurjpc/zwaa108
27. Meyer LS, Wang X, Sušnik E, Burrello J, Burrello A, Castellano I, Eisenhofer G, Fallo F, Kline GA, Knösel T, et al. Immunohistopathology and steroid profiles associated with biochemical outcomes after adrenalectomy for unilateral primary aldosteronism. *Hypertension*. 2018;72:650–657. doi: 10.1161/HYPERTENSIONAHA.118.11465
28. Turcu AF, Tezuka Y, Lim JS, Salman Z, Sehgal K, Liu H, Larose S, Parksook WW, Williams TA, Cohen DL, et al. Multifocal, asymmetric bilateral primary aldosteronism cannot be excluded by strong adrenal vein sampling

lateralization: an international retrospective cohort study. *Hypertension*. 2024;81:604–613. doi: 10.1161/HYPERTENSIONAHA.123.21910
29. Williams TA, Gomez-Sanchez CE, Rainey WE, Giordano TJ, Lam AK, Marker A, Mete O, Yamazaki Y, Zerbini MCN, Beuschlein F, et al. International histopathology consensus for unilateral primary aldosteronism. *J Clin Endocrinol Metab*. 2021;106:42–54. doi: 10.1210/clinem/dgaa484
30. Mete O, Erickson LA, Juhlin CC, de Krijger RR, Sasano H, Volante M, Papotti MG. Overview of the 2022 WHO classification of adrenal cortical tumors. *Endocr Pathol*. 2022;33:155–196. doi: 10.1007/s12022-022-09710-8
31. Tetti M, Brüdgam D, Burrello J, Udager AM, Riester A, Knösel T, Beuschlein F, Rainey WE, Reincke M, Williams TA. Unilateral primary aldosteronism: long-term disease recurrence after adrenalectomy. *Hypertension*. 2024;81:936–945. doi: 10.1161/HYPERTENSIONAHA.123.22281
32. Bruedgam D, Adolf C, Schneider H, Schwarzlmueller P, Mueller L, Handgriff L, Bidlingmaier M, Kunz S, Zimmermann P, Deniz S, et al. Postoperative ACTH-stimulated aldosterone predicts biochemical outcome in primary aldosteronism. *Eur J Endocrinol*. 2023;189:611–618. doi: 10.1093/ejendo/lvad159
33. Fang CW, Hsieh HL, Wang SM, Huang KH, Peng KY, Lin YH, Wu VC, Chueh JS. A comparative study of postadrenalectomy hyperuricemia and renal impairment in patients with unilateral primary aldosteronism: Does histopathology subtype matter? *BMC Nephrol*. 2024;25:347. doi: 10.1186/s12882-024-03750-4
34. Chan CK, Yang WS, Lin YH, Chang CC, Wu VC, Chueh JS. KCNJ5 somatic mutations are associated with better long-term outcomes in patients with unilateral primary aldosteronism. *J Clin Endocrinol Metab*. 2025;110:e2918–e2928. doi: 10.1210/clinem/dgaf007
35. Zhang C, Wu L, Jiang L, Su T, Zhou W, Zhong X, Xie J, Sun F, Zhu Y, Jiang Y, et al. KCNJ5 mutation contributes to complete clinical success in aldosterone-producing adenoma: a study from a single center. *Endocr Pract*. 2021;27:736–742. doi: 10.1016/j.eprac.2021.01.007
36. Kitamoto T, Omura M, Suematsu S, Saito J, Nishikawa T. KCNJ5 mutation as a predictor for resolution of hypertension after surgical treatment of aldosterone-producing adenoma. *J Hypertens*. 2018;36:619–627. doi: 10.1097/HJH.0000000000001578
37. Vilela LAP, Rassi-Cruz M, Guimaraes AG, Moises CCS, Freitas TC, Alencar NP, Petenuci J, Goldbaum TS, Maciel AAW, Pereira MAA, et al. KCNJ5 somatic mutation is a predictor of hypertension remission after adrenalectomy for unilateral primary aldosteronism. *J Clin Endocrinol Metab*. 2019;104:4695–4702. doi: 10.1210/jc.2019-00531
38. Wu X, Senanayake R, Goodchild E, Bashari WA, Salsbury J, Cabrera CP, Argentesi G, O'Toole SM, Matson M, Koo B, et al. [11C]metomidate PET-CT versus adrenal vein sampling for diagnosing surgically curable primary aldosteronism: a prospective, within-patient trial. *Nat Med*. 2023;29:190–202. doi: 10.1038/s41591-022-02114-5
39. Arnesen T, Glomnes N, Strømsøy S, Knappskog S, Heie A, Akslen LA, Grytaas M, Varhaug JE, Gimm O, Brauckhoff M. Outcome after surgery for primary hyperaldosteronism may depend on KCNJ5 tumor mutation status: a population-based study from Western Norway. *Langenbecks Arch Surg*. 2013;398:869–874. doi: 10.1007/s00423-013-1093-2
40. Solhusløkk Höse K, Stenman A, Falhammar H, Volpe C, Larsson C, Zedenius J, Juhlin CC. Improving diagnosis in primary aldosteronism using HISTALDO and nodule size metrics. *Eur J Endocrinol*. 2025;193:278–288. doi: 10.1093/ejendo/lvaf157
41. Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Patel A, Men CJ, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science*. 2011;331:768–772. doi: 10.1126/science.1198785
42. Nanba K, Omata K, Else T, Beck PCC, Nanba AT, Turcu AF, Miller BS, Giordano TJ, Tomlins SA, Rainey WE. Targeted molecular characterization of aldosterone-producing adenomas in White Americans. *J Clin Endocrinol Metab*. 2018;103:3869–3876. doi: 10.1210/jc.2018-01004
43. Nanba K, Omata K, Gomez-Sanchez CE, Stratakis CA, Demidowich AP, Suzuki M, Thompson LDR, Cohen DL, Luther JM, Gellert L, et al. Genetic characteristics of aldosterone-producing adenomas in blacks. *Hypertension*. 2019;73:885–892. doi: 10.1161/HYPERTENSIONAHA.118.12070
44. De Sousa K, Boulkroun S, Baron S, Nanba K, Wack M, Rainey WE, Rocha A, Giscos-Douriez I, Meatchi T, Amar L, et al. Genetic, cellular, and molecular heterogeneity in adrenals with aldosterone-producing adenoma. *Hypertension*. 2020;75:1034–1044. doi: 10.1161/HYPERTENSIONAHA.119.14177
45. Guo Z, Nanba K, Udager A, McWhinney BC, Ungerer JPJ, Wolley M, Thuzar M, Gordon RD, Rainey WE, Stowasser M. Biochemical, histopathological, and genetic characterization of posture-responsive and unresponsive APAs. *J Clin Endocrinol Metab*. 2020;105:e3224–e3235. doi: 10.1210/clinem/dgaa367
46. Guimaraes AG, Goldbaum TS, Ledesma FL, Freitas-Castro F, Santana LS, Sobrinho J, Rossetti LB, Okubo J, Kawahara EZ, Bortolotto LA, et al. Somatic drivers in aldosterone-producing adenomas and nodules: Insights from a Brazilian cohort of primary aldosteronism. *J Clin Endocrinol Metab*. 2026:dgag002. doi: 10.1210/clinem/dgag002
47. Boulkroun S, Beuschlein F, Rossi GP, Golib-Dzib JF, Fischer E, Amar L, Mulatero P, Samson-Couterie B, Hahner S, Quinkler M, et al. Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism. *Hypertension*. 2012;59:592–598. doi: 10.1161/HYPERTENSIONAHA.111.186478
48. Åkerström T, Crona J, Delgado Verdugo A, Starker LF, Cupisti K, Willenberg HS, Knoefel WT, Saeger W, Feller A, Ip J, et al. Comprehensive re-sequencing of adrenal aldosterone producing lesions reveal three somatic mutations near the KCNJ5 potassium channel selectivity filter. *PLoS One*. 2012;7:e41926. doi: 10.1371/journal.pone.0041926
49. Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, Strom TM, Monticone S, Amar L, Meatchi T, et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension*. 2014;64:354–361. doi: 10.1161/HYPERTENSIONAHA.114.03419
50. Lenzini L, Rossitto G, Maiolino G, Letizia C, Funder JW, Rossi GP. A meta-analysis of somatic KCNJ5 K(+) channel mutations in 1636 patients with an aldosterone-producing adenoma. *J Clin Endocrinol Metab*. 2015;100:E1089–E1095. doi: 10.1210/jc.2015-2149
51. Scholl UI, Goh G, Stölting G, de Oliveira RC, Choi M, Overton JD, Fonseca AL, Korah R, Starker LF, Kunstman JW, et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet*. 2013;45:1050–1054. doi: 10.1038/ng.2695
52. Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, Maniero C, Garg S, Bochukova EG, Zhao W, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet*. 2013;45:1055–1060. doi: 10.1038/ng.2716
53. Reimer EN, Walenda G, Seidel E, Scholl UI. CACNA1H(M1549V) mutant calcium channel causes autonomous aldosterone production in HAC15 cells and is inhibited by mibefradil. *Endocrinology*. 2016;157:3016–3022. doi: 10.1210/en.2016-1170
54. Daniil G, Fernandes-Rosa FL, Chemin J, Blesneac I, Beltrand J, Polak M, Jeunemaitre X, Boulkroun S, Amar L, Strom TM, et al. CACNA1H mutations are associated with different forms of primary aldosteronism. *EBioMedicine*. 2016;13:225–236. doi: 10.1016/j.ebiom.2016.10.002
55. Gürtler F, Jordan K, Tegtmeier I, Herold J, Stindl J, Warth R, Bandulik S. Cellular pathophysiology of mutant voltage-dependent Ca2+ channel CACNA1H in primary aldosteronism. *Endocrinology*. 2020;161:bqaa135. doi: 10.1210/endocr/bqaa135
56. Nanba K, Blinder AR, Rege J, Hattangady NG, Else T, Liu CJ, Tomlins SA, Vats P, Kumar-Sinha C, Giordano TJ, et al. Somatic CACNA1H mutation as a cause of aldosterone-producing adenoma. *Hypertension*. 2020;75:645–649. doi: 10.1161/HYPERTENSIONAHA.119.14349
57. Scholl UI, Stölting G, Schewe J, Thiel A, Tan H, Nelson-Williams C, Vichot AA, Jin SC, Loring E, Untiet V, et al. CLCN2 chloride channel mutations in familial hyperaldosteronism type II. *Nat Genet*. 2018;50:349–354. doi: 10.1038/s41588-018-0048-5
58. Fernandes-Rosa FL, Daniil G, Orozco IJ, Göppner C, El Zein R, Jain V, Boulkroun S, Jeunemaitre X, Amar L, Lefebvre H, et al. A gain-of-function mutation in the CLCN2 chloride channel gene causes primary aldosteronism. *Nat Genet*. 2018;50:355–361. doi: 10.1038/s41588-018-0053-8
59. Dutta RK, Arnesen T, Heie A, Walz M, Alesina P, Söderkvist P, Gimm O. A somatic mutation in CLCN2 identified in a sporadic aldosterone-producing adenoma. *Eur J Endocrinol*. 2019;181:K37–K41. doi: 10.1530/EJE-19-0377
60. Rege J, Nanba K, Blinder AR, Plaska S, Udager AM, Vats P, Kumar-Sinha C, Giordano TJ, Rainey WE, Else T. Identification of somatic mutations in CLCN2 in aldosterone-producing adenomas. *J Endocr Soc*. 2020;4:bvaa123. doi: 10.1210/jendso/bvaa123
61. van Rooyen D, Bandulik S, Coon GA, Laukemper M, Kumar-Sinha C, Udager AM, Lerario AM, Lee C, Wachtel H, Cohen DL, et al. Somatic mutations in MCOLN3 are associated with aldosterone-producing adenomas. *Hypertension*. 2025;82:1778–1788. doi: 10.1161/HYPERTENSIONAHA.125.24909
62. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, Penton D, Schack VR, Amar L, Fischer E, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet*. 2013;45:440–4, 444e1. doi: 10.1038/ng.2550
63. Tauber P, Aichinger B, Christ C, Stindl J, Rhayem Y, Beuschlein F, Warth R, Bandulik S. Cellular pathophysiology of an adrenal adenoma-associated mutant of the plasma membrane Ca(2+)-ATPase ATP2B3. *Endocrinology*. 2016;157:2489–2499. doi: 10.1210/en.2015-2029

64. Rege J, Bandulik S, Nanba K, Kosmann C, Blinder AR, Plain A, Vats P, Kumar-Sinha C, Lerario AM, Else T, et al. Somatic SLC30A1 mutations altering zinc transporter ZnT1 cause aldosterone-producing adenomas and primary aldosteronism. *Nat Genet*. 2023;55:1623–1631. doi: 10.1038/s41588-023-01498-5
65. Zhou J, Azizan EAB, Cabrera CP, Fernandes-Rosa FL, Boulkroun S, Argentesi G, Cottrell E, Amar L, Wu X, O'Toole S, et al. Somatic mutations of GNA11 and GNAQ in CTNNB1-mutant aldosterone-producing adenomas presenting in puberty, pregnancy or menopause. *Nat Genet*. 2021;53:1360–1372. doi: 10.1038/s41588-021-00906-y
66. Åkerström T, Maharjan R, Sven Willenberg H, Cupisti K, Ip J, Moser A, Stålberg P, Robinson B, Alexander Iwen K, Dralle H, et al. Activating mutations in CTNNB1 in aldosterone producing adenomas. *Sci Rep*. 2016;6:19546. doi: 10.1038/srep19546
67. Wu X, Azizan EAB, Goodchild E, Garg S, Hagiyama M, Cabrera CP, Fernandes-Rosa FL, Boulkroun S, Kuan JL, Tiang Z, et al. Somatic mutations of CADM1 in aldosterone-producing adenomas and gap junction-dependent regulation of aldosterone production. *Nat Genet*. 2023;55:1009–1021. doi: 10.1038/s41588-023-01403-0
68. Azizan EA, Lam BY, Newhouse SJ, Zhou J, Kuc RE, Clarke J, Happerfield L, Marker A, Hoffman GJ, Brown MJ. Microarray, qPCR, and KCNJ5 sequencing of aldosterone-producing adenomas reveal differences in genotype and phenotype between zona glomerulosa- and zona fasciculata-like tumors. *J Clin Endocrinol Metab*. 2012;97:E819–E829. doi: 10.1210/jc.2011-2965
69. Monticone S, Castellano I, Versace K, Lucatello B, Veglio F, Gomez-Sanchez CE, Williams TA, Mulatero P. Immunohistochemical, genetic and clinical characterization of sporadic aldosterone-producing adenomas. *Mol Cell Endocrinol*. 2015;411:146–154. doi: 10.1016/j.mce.2015.04.022
70. Gao X, Yamazaki Y, Ono Y, Satoh F, Li F, Zhou H, Sasano H. Histopathological features of aldosterone-producing lesions according to their different somatic genetic mutations. *Vitam Horm*. 2025;129:125–141. doi: 10.1016/bs.vh.2024.09.001
71. Nishimoto K, Tomlins SA, Kuick R, Cani AK, Giordano TJ, Hovelson DH, Liu CJ, Sanjanwala AR, Edwards MA, Gomez-Sanchez CE, et al. Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands. *Proc Natl Acad Sci U S A*. 2015;112:E4591–E4599. doi: 10.1073/pnas.1505529112
72. Omata K, Satoh F, Morimoto R, Ito S, Yamazaki Y, Nakamura Y, Anand SK, Guo Z, Stowasser M, Sasano H, et al. Cellular and genetic causes of idiopathic hyperaldosteronism. *Hypertension*. 2018;72:874–880. doi: 10.1161/HYPERTENSIONAHA.118.11086
73. Backman S, Åkerström T, Maharjan R, Cupisti K, Willenberg HS, Hellman P, Björklund P. RNA sequencing provides novel insights into the transcriptome of aldosterone producing adenomas. *Sci Rep*. 2019;9:6269. doi: 10.1038/s41598-019-41525-2
74. Gao Y, Wang Y, Zhou Y, Ding J, Chang X, Qiu L, Huo L, Wen J, Zhang Y, Tong A. Two-hit model for the development of aldosterone-producing adenoma: supporting from two new cases. *J Hypertens*. 2025;43:544–548. doi: 10.1097/HJH.0000000000003888
75. Baudrand R, Guarda FJ, Fardella C, Hundemer G, Brown J, Williams G, Vaidya A. Continuum of renin-independent aldosteronism in normotension. *Hypertension*. 2017;69:950–956. doi: 10.1161/HYPERTENSIONAHA.116.08952
76. Nanba K, Vaidya A, Williams GH, Zheng I, Else T, Rainey WE. Age-related autonomous aldosteronism. *Circulation*. 2017;136:347–355. doi: 10.1161/CIRCULATIONAHA.117.028201
77. Sun N, Meyer LS, Feuchtinger A, Kunzke T, Knösel T, Reincke M, Walch A, Williams TA. Mass spectrometry imaging establishes 2 distinct metabolic phenotypes of aldosterone-producing cell clusters in primary aldosteronism. *Hypertension*. 2020;75:634–644. doi: 10.1161/HYPERTENSIONAHA.119.14041
78. Le Floch E, Cosentino T, Larsen CK, Beuschlein F, Reincke M, Amar L, Rossi GP, De Sousa K, Baron S, Chantalat S, et al. Identification of risk loci for primary aldosteronism in genome-wide association studies. *Nat Commun*. 2022;13:5198. doi: 10.1038/s41467-022-32896-8
79. Pignatti E, Leng S, Carlone DL, Breault DT. Regulation of zonation and homeostasis in the adrenal cortex. *Mol Cell Endocrinol*. 2017;441:146–155. doi: 10.1016/j.mce.2016.09.003
80. Lyraki R, Schedl A. Adrenal cortex renewal in health and disease. *Nat Rev Endocrinol*. 2021;17:421–434. doi: 10.1038/s41574-021-00491-4
81. Altieri B, Secener AK, Sai S, Fischer C, Sbiera S, Arampatzi P, Kircher S, Herterich S, Landwehr LS, Vitcetz SN, et al. Single-nucleus and spatial transcriptome reveal adrenal homeostasis in normal and tumoural adrenal glands. *Clin Transl Med*. 2024;14:e1798. doi: 10.1002/ctm2.1798
82. Iwahashi N, Umakoshi H, Kitamura Y, Kaneko H, Fujita M, Ogasawara T, Fukumoto T, Sakamoto R, Seki M, Matsumoto T, et al. Role of stress-responsive NR4A2 in aldosterone-producing cell cluster formation. *Hypertension*. 2025. doi: 10.1161/HYPERTENSIONAHA.125.24825
83. Iwahashi N, Umakoshi H, Seki T, Gomez-Sanchez CE, Mukai K, Suematsu M, Umezawa Y, Oya M, Kosaka T, Seki M, et al. Characterization of Aldosterone-producing Cell Cluster (APCC) at single-cell resolution. *J Clin Endocrinol Metab*. 2022;107:2439–2448. doi: 10.1210/clinem/dgac394
84. Sun Z, Jing C, Tetti M, Gong S, Wei J, Pang Y, Reincke M, Williams TA. Integrated transcriptomics reveals evolutionary trajectories and cell density-dependent mechanisms in aldosterone-producing adenomas. *Adv Sci (Weinh)*. 2026;e05410. doi: 10.1002/advs.202505410
85. Pang Y, Gong S, Tetti M, Sun Z, Mir-Bashiri S, Bidlingmaier M, Knösel T, Wolf E, Reincke M, Kemter E, et al. EGR1 regulates oxidative stress and aldosterone production in adrenal cells and aldosterone-producing adenomas. *Redox Biol*. 2025;80:103498. doi: 10.1016/j.redox.2025.103498
86. Yokomoto-Umakoshi M, Fujita M, Umakoshi H, Ogasawara T, Iwahashi N, Nakatani K, Kaneko H, Fukumoto T, Nakao H, Haji S, et al. Multiomics analysis unveils the cellular ecosystem with clinical relevance in aldosterone-producing adenomas with KCNJ5 mutations. *Proc Natl Acad Sci U S A*. 2025;122:e2421489122. doi: 10.1073/pnas.2421489122
87. Huang J, Qin F, Li J. Single-cell transcriptomic analysis reveals metabolic reprogramming and tumor microenvironment remodeling in aldosterone-producing adenoma. *Genomics*. 2026;118:111164. doi: 10.1016/j.ygeno.2025.111164
88. Wei J, Duregon E, Papotti MG, Knösel T, Bidlingmaier M, Sbiera S, Reincke M, Williams TA. FOS and JUN regulate oxidative stress and steroidogenesis in human aldosterone-producing adenomas. *Redox Biol*. 2026;89:103982. doi: 10.1016/j.redox.2025.103982
89. Yang Y, Liu Y, Williams TA, Gao M, Yan Y, Bao M, Tao J, Ma G, Wang M, Xia Z, et al. Metabolic phenotypes and fatty acid profiles associated with histopathology of primary aldosteronism. *Hypertens Res*. 2025;48:1363–1378. doi: 10.1038/s41440-025-02143-w
90. Murakami M, Rhayem Y, Kunzke T, Sun N, Feuchtinger A, Ludwig P, Strom TM, Gomez-Sanchez C, Knösel T, Kirchner T, et al. In situ metabolomics of aldosterone-producing adenomas. *JCI Insight*. 2019;4:e130356. doi: 10.1172/jci.insight.130356
91. Sun Z, Kemter E, Pang Y, Bidlingmaier M, Wolf E, Reincke M, Williams TA. ATP2A3 in primary aldosteronism: machine learning-based discovery and functional validation. *Hypertension*. 2025;82:319–332. doi: 10.1161/HYPERTENSIONAHA.124.23817
92. Pang Y, Mir-Bashiri S, Sun Z, Yang Y, Castellano I, Knösel T, Reincke M, Williams TA. ABCC3 is a differential marker of CYP11B2-negative zona glomerulosa cells in human adrenal cortex. *Endocr Pathol*. 2025;36:17. doi: 10.1007/s12022-025-09862-3