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Martin Reincke[1$], Irina Bancos[2], Paolo Mulatero[3], Ute I Scholl[4], Michael Stowasser[5], Tracy Ann Williams[1,3]

[1] Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, München, Germany

[2] Division of Endocrinology, Metabolism and Nutrition, Department of Internal Medicine, Mayo Clinic, Rochester, MN, USA

[3] Division of Internal Medicine and Hypertension, Department of Medical Sciences, University of Turin, Italy

[4] Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Center of Functional Genomics, Charitéplatz 1, 10117 Berlin, Germany

[5] Endocrine Hypertension Research Centre, University of Queensland Diamantina Institute, Greenslopes and Princess Alexandra Hospitals, Brisbane, QLD, Australia

ORCID IDs:
MR:
IB: 0000-0001-9332-2524
PM: 0000-0002-5480-1116
UIS: 0000-0002-0309-8045
MS: 0000-0002-4317-8584
TAW: 0000-0002-2388-6444

[$]Corresponding author:

Prof. Martin Reincke, MD
Medizinische Klinik und Poliklinik IV
Klinikum der Universität München
Ziemssenstr. 1, 80336 Munich, Germany
Tel.: +49-89-4400-52100 | Fax: +49-89-4400-54428
E-Mail: martin.reincke@med.uni-muenchen.de

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Abstract

Primary aldosteronism is a common cause of secondary hypertension associated with excess cardiovascular morbidities. Primary aldosteronism is underdiagnosed because it does not have a specific, easily identifiable feature and clinicians are poorly aware of the disease. The diagnostic workup is a multistep process of screening, confirmatory testing, and subtype differentiation of unilateral from bilateral forms for therapeutic management. Adrenal venous sampling is key for reliable subtype identification but may be bypassed in patients with specific characteristics. For unilateral disease, surgery offers the possibility of cure with total laparoscopic unilateral adrenalectomy the treatment of choice. Bilateral forms are mainly treated with mineralocorticoid receptor antagonists. Prompt diagnosis of primary aldosteronism and implementation of targeted treatment strategies mitigate aldosterone-specific target organ damage, and outcomes are excellent with appropriate patient management. Advances in molecular histopathology challenge the traditional concept of primary aldosteronism as a binary disease, in which unilateral aldosterone-producing adenoma is juxtaposed with bilateral adrenal hyperplasia. Somatic mutations drive autonomous aldosterone production in most adenomas. Many of these same mutations have been identified in nodular lesions adjacent to an aldosterone-producing adenoma and in patients with bilateral disease. In addition, germ-line mutations cause rare familial forms of aldosteronism (familial hyperaldosteronism types I-IV). Genetic testing for inherited forms in suspect cases of familial hyperaldosteronism avoids the burdensome diagnostic workup in positive patients. In this review, we discuss recent advances and future management approaches in the diagnosis of primary aldosteronism.

Introduction including overview of the renin angiotensin aldosterone system

Arterial hypertension is a major cause of premature death affecting more than 1.4 billion adults worldwide.[1,2] Hypertension is responsible for 7.0% of the worldwide disability-adjusted life years.[3] The renin-angiotensin-aldosterone system (RAAS) is of paramount importance for homeostasis of plasma sodium concentrations, blood volume, and mean arterial blood pressure.[4] The RAAS is a hierarchical cascade that begins with the production of the aspartyl protease renin from the juxtaglomerular cells of the kidney (**Figure 1**). Renin release is stimulated by decreased renal perfusion, low sodium concentrations in the renal tubule, and central ß1 adrenergic receptor mediated sympathetic system activation.[4] Renin regulates the rate-limiting step of the RAAS through conversion of angiotensinogen to angiotensin I, which is activated to form the vasopressor octapeptide angiotensin II by angiotensin I-converting enzyme in the endothelium of the lung and kidney.[4] Angiotensin II triggers the release of aldosterone from the outer zone of the adrenal cortex- the zona glomerulosa- and acts on the nephron to increase sodium and water reabsorption. RAAS inhibition is a cornerstone of blood pressure management, with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and mineralocorticoid receptor antagonists blocking the RAAS at different levels of the RAAS cascade.[4,5] Primary aldosteronism (PA), first described by Jerome Conn in 1954,[6] results from excessive production of aldosterone independent of renin and angiotensin II. It leads to increased renal tubular resorption of sodium and volume expansion, resulting in hypertension and hypokalemia.[7] The increase in sodium chloride delivery to the juxtaglomerular apparatus and the rise in systemic blood pressure results in suppression of renin secretion. Consequently, the aldosterone-to-renin ratio (ARR) is increased. Aldosterone secretion in PA remains elevated, autonomous, and inappropriately high for blood volume and blood pressure.[7] Besides increased blood pressure, aldosterone excess is associated with excess cardiovascular morbidity and mortality.[7] PA represents the most frequent curable form of secondary hypertension and recommendations

for screening at-risk populations account for around 50% of the hypertensive population. Despite this, less than 1% of patients with PA are currently screened and treated during their lifetime.[8-12]

This review will give a timely overview on PA, describing its hallmarks including screening, confirmation, and subtyping. It will highlight the current standards of treatment and reflect controversial areas and fields of uncertainty.

Search strategy and selection criteria

We searched in the Cochrane Library (1993-2021) and in MEDLINE (1959-2021). We used the search terms “primary aldosteronism”, “Conn’s syndrome”, "hyperaldosteronism", “aldosterone to renin ratio”, "adrenal vein sampling", “genetics”. We selected mainly publications from the past 6 years without excluding relevant and highly referenced older publications.

Epidemiology of primary aldosteronism

Once considered rare, PA is currently thought to be the most common secondary endocrine form of hypertension. The introduction of the ARR as a screening test[13] and its application to a widening population of hypertensives has led to a marked increase in detection of PA, especially among normokalaemic patients. Most groups have reported PA prevalence rates between 5 and 15%, with the majority of patients being normokalaemic[14-16]. Variability in prevalence rates reported by different groups is explained by differences in diagnostic methods and cut-offs used in these studies and the variable degree of selectiveness of the cohorts that were examined (**Table 1**).

The prevalence of PA increases in line with the severity of hypertension among the cohorts studied.[17] In resistant hypertensive cohorts, prevalence rates have approximated 20% or even higher[18-21] (**Table 1**). The clinical need to continue antihypertensive medications that affect aldosterone and renin levels in those studies raises some doubt about the accuracy of the diagnosis in each case, with the potential for either over- or under-detection of PA. Nevertheless, mineralocorticoid antagonists have been reported to be particularly effective at lowering blood pressure in resistant hypertensive cohorts,[22] which argues in favour of a high prevalence of PA among them. High prevalence rates of PA have also been reported among hypertensive patients with atrial fibrillation[23] or diabetes mellitus[24,25] (**Table 1**). Among normotensive individuals in the Framingham cohort, both a rise in blood pressure and the incident development of hypertension over four years of follow up were positively correlated with aldosterone and the ARR and negatively correlated with renin, as would be expected if aldosterone secretion was autonomous.[26]

Table 1. Prevalence of primary aldosteronism in different cohorts

Patient Cohort	Prevalence of PA*	References
Hypertensives in a primary care setting	3.2-12.7% (median 5.9%)	Reviewed in Buffolo F, et al.[17]
Hypertensives referred to a referral centre	0.7-21.9% (median 7.2%)	Reviewed in Buffolo F, et al.[17]
Stage 1 hypertension	3.9% 6.6% 15.7%	Monticone S, et al.[27] Rossi GP, et al.[16] Brown JM, et al.[18]
Stage 2 hypertension	9.7%	Monticone S, et al.[27]

	15.5% 21.6%	Rossi GP, et al.[16] Brown JM, et al.[18]
Stage 3 hypertension	11.8% 19.0%	Monticone S, et al.[27] Rossi GP, et al.[16]
Resistant hypertension	20.5% 11.3% 29.1% 22.0%	Calhoun DA, et al.[19] Douma S, et al.[20] Parasiliti-Caprino M, et al.[21] Brown JM, et al.[18]
Hypertension and hypokalemia	28.1%	Burello J, et al.[28]
Adrenal incidentaloma	1.6% 4.3%	Mantero F, et al.[29] Li L, et al.[30]
Hypertension and atrial fibrillation	42.5%	Seccia TM, et al.[23]
Hypertension and diabetes mellitus	11.3% 19.1%	Murase K, et al.[24] Hu Y, et al.[25]

PA denotes primary aldosteronism
*Confirmed by suppression testing

Comorbidities in primary aldosteronism

Aldosterone excess in PA is associated with deleterious effects on the heart, vessels, brain and kidney, that are partly independent of elevated blood pressure determined by hyperaldosteronism.[31] These effects result in disproportionate levels of organ damage for blood pressure levels resulting in left ventricular hypertrophy, fibrosis and diastolic dysfunction[32-35] increased intima-media thickening, arterial stiffness, arterial wall inflammation and endothelial dysfunction[36-40] and renal hyperfiltration, albuminuria and glomerulosclerosis.[41,42] Aldosterone excess is also associated with reduced insulin sensitivity and secretion[43,44] and a higher rate of metabolic syndrome and type 2 diabetes mellitus.[35] The demonstration that adipocyte-derived factors can stimulate aldosterone production[45] provide the basis for a vicious circle between obesity, metabolic alterations, and hyperaldosteronism.[46] Obesity could also be the common pathophysiological link between PA and obstructive sleep apnea:[47] severity of sleep apnea may be worsened by aldosterone-mediated fluid retention and ameliorated by PA treatment.[48]

As a result, patients with PA display a significantly higher incidence of stroke, myocardial infarction, heart failure, atrial fibrillation, and deterioration of renal function[35,42,49,50] than patients with essential hypertension with similar blood pressure levels. The excess risk of cardio-, cerebrovascular and renal complications can be successfully reversed by specific treatment of unilateral PA by adrenalectomy and of bilateral forms of PA by therapy with mineralocorticoid receptor antagonists.[51-53] Of note, higher aldosterone levels are associated with all-cause mortality when renin is suppressed in a general population of adults.[54] Outside the cardiovascular system, aldosterone excess is also associated with lower serum calcium and higher urinary calcium excretion, with consequent increased production of parathyroid hormone[55] leading to an increased prevalence of osteoporosis and risk of bone fractures, which are reverted by PA treatment.[56]

These observations emphasize the importance of early and systematic detection of patients with PA and to implement efficient surgical or medical treatment to prevent or reverse the excess vascular events and mortality in this specific group of patients with secondary hypertension.[57]

Screening for primary aldosteronism
Who should be screened?

Patients with moderate to severe hypertension and those with hypertension and spontaneous or diuretic induced hypokalemia, adrenal incidentaloma, atrial fibrillation in the absence of structural heart disease, or a family history of early onset hypertension or cerebrovascular accident at a young age (<40 years), and all hypertensive first-degree relatives of patients with PA are candidates for screening.[58,59] Some investigators suggest screening of all hypertensives, given the high prevalence of this condition and the reported benefits from specific surgical or medical treatment.[60] Screening before commencing antihypertensive medications avoids potentially confounding effects of these agents on renin and aldosterone levels and permits earlier institution of specific treatment, which has been shown to be a major determinant of benefit achieved.[61]

How should patients be screened?

Measurement of the plasma ARR is currently the most popular method of screening for PA. False positive and false negative ARRs can result from a variety of pharmacological and physiological factors. B-adrenoceptor blockers, clonidine, α-methyldopa, and non-steroidal anti-inflammatory drugs are prone to cause false positives. Estrogen-containing oral contraceptive agents or hormone replacement therapy can also cause false positives when the direct renin concentration (but not plasma renin activity) is used to calculate the ARR. Diuretics (including potassium-sparing), angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and selective serotonin receptor inhibitors may cause false negatives.[62,63] In a patient suspected to have PA, screening can be performed without stopping any medications in selected cases, when hypertension is uncontrolled. Positive case detection testing in this case will decrease the need for sometimes unsafe periods of time needed for the washout. In milder cases, potentially interfering antihypertensives should be withdrawn (optimally for at least 4 weeks for diuretics and at least 2 weeks for other agents) prior to screening. Antihypertensives with lesser effects on the ARR that can be used to control hypertension during diagnostic workup of PA include verapamil SR, hydralazine (used in combination with verapamil SR to avoid reflex tachycardia), prazosin, doxazosin and moxonidine.[63]

ARR testing while patients are still on interfering medications can still be informative. For example, a raised ARR with suppressed renin during sole treatment with an angiotensin converting enzyme inhibitor, angiotensin receptor blocker or a diuretic including a mineralocorticoid receptor antagonist, would be highly suggestive of PA, whereas a normal ARR in a patient on a β-blocker would make PA unlikely.

False positives can be encountered during the luteal phase of the menstrual cycle in pre-menopausal women, advancing age, chronic kidney disease, a high dietary salt intake and the syndrome of familial hyperkalemic hypertension (Gordon syndrome). False negatives may occur during pregnancy, dietary salt restriction, vomiting or diarrhea, uncorrected hypokalemia and in patients with malignant hypertension or concomitant renovascular hypertension.[63] The ARR has greater sensitivity if measured in the morning in an upright (e.g., seated) posture.[64]

Controlling for the above factors or at least taking them into account enhances the usefulness of ARR testing in selecting patients for further diagnostic workup. The ARR also shows good within-patient reproducibility under such conditions.[65]

In the presence of extremely low renin levels, the ARR may be elevated even when plasma aldosterone is also very low and clearly not consistent with PA. While some investigators have suggested including a minimum plasma aldosterone concentration (for example, 415 pmol/L or 15 ng/dL) with an elevated ARR within the screening criteria to overcome this problem, others have found this to exclude patients who have subsequently been confirmed as having PA.[66] Normokalemic patients with aldosterone levels lower than the cut-off for suppression testing (for example 166 pmol/L or 6 ng/dL) almost certainly do not have PA. The ratio should be regarded as a screening test only, and should be measured more than once before deciding whether to go on to confirmatory testing for PA.

Confirmatory testing

Who to test?

Once PA is suspected from a positive screening test finding of an increased ARR, diagnosis of PA should be performed or excluded by one or more confirmatory tests.[58,59] This is required because of the relatively low specificity of the ARR as a screening test, even when performed under ideal conditions and especially when cut-offs are permissive to ensure high sensitivity as usually required for screening. Confirmatory testing is necessary to exclude patients with a falsely elevated ARR but without PA to undergo expensive, difficult, and invasive lateralizing procedures including AVS that should be dedicated to patients with a definitive diagnosis of PA.[58,59]

Since the ARR may be considered a quantitative and not only a qualitative test, the higher the ARR, the more likely a patient has PA. Thus, current guidelines suggest bypassing confirmatory tests in patients with a particularly florid phenotype, that is, patients with hypokalemia and undetectable renin/PRA levels and plasma aldosterone levels higher than 20 ng/dL (555 pmol/L). To further reduce the burden of confirmatory tests, scores have been developed to select patients for testing.[67]

How to test

Four tests are generally accepted to confirm or exclude PA, the fludrocortisone suppression test, the oral and the intravenous saline load test and the captopril challenge test (the furosemide-upright test is used only in Japan) (**Table 2**).[58,59,68] Confirmatory tests are based upon the assumption that complete suppression of renin production (for tests involving volume expansion) or the blockade of angiotensin II production (for the captopril test) should decrease aldosterone production if it is appropriately regulated. The fludrocortisone suppression test has been almost abandoned because it requires five days´ hospitalization.[58,59,69] The two most used tests are the oral and the intravenous saline load test.[58,59] In the oral saline load test, salt supplements are administered for three days to obtain a urinary sodium excretion rate higher than 200 mmoles/day, with potassium supplements to avoid hypokalemia. If urinary aldosterone levels remain above 12 μg/day (33 nmol/day), on the third day, PA is considered confirmed.[58,59,70] In the intravenous saline load test, 2 liters of 0.9% sodium chloride are infused intravenously. If aldosterone remains higher than a defined cut-off, PA is considered confirmed. Traditionally, the test was performed in the recumbent position. However, recent studies demonstrated a higher accuracy for detection of angiotensin II responsive forms of PA with the test performed in the sitting position,[64] which should therefore be the preferred approach.[58,59] The cut-off for PA diagnosis is commonly 5-6 ng/dL (171 pmol/L) when aldosterone is measured by mass spectrometry and 8 ng/dL (217 pmol/L) when measured by chemiluminescence.[69] If patients are at risk of volume expansion, the captopril test is the preferred confirmatory test. In this test, 25-50 mg of captopril are administered, and aldosterone and PRA (or renin) are measured after 1-2 hours. In patients without PA, plasma aldosterone concentrations are decreased by >30%, PRA is increased and

the ARR reduced. A recent study showed that the most accurate parameter in this test is the post-captopril plasma aldosterone concentration, with a cut-off of 11 ng/dL (305 pmol/L).[71]

Table 2: Comparison of Guideline recommendations for screening, confirmation, and subtyping of primary aldosteronism.

AII, angiotensin II; AC, aldosterone concentration; ACTH, adrenocorticotropic hormone; APA, aldosterone-producing adenoma; AVS, adrenal vein sampling; CT, computed tomography; DRC, direct renin concentration; LI, lateralization index; PA, primary aldosteronism; PRA, plasma renin activity; SI, selectivity index.

Guideline	Who to screen	How to screen	Who to confirm	How to confirm	Subtype diagnosis
Endocrine Society 2016[58]	Patients with blood pressure >150/100; resistant hypertension; hypokalemia (spontaneous or diuretic-induced); hypertension and adrenal incidentaloma; hypertension and sleep apnea; suspected familial form (family history of early onset hypertension/cerebrovascular accident at a young age; all hypertensive first-degree relatives of patients with PA)	Aldosterone to renin (or plasma renin activity) ratio	All patients with positive screening except those with: hypokalemia+ undetectable renin+ AC > 20 ng/dL	Seated intravenous or oral saline load; captopril test; fludrocortisone suppression test	CT scanning for confirmed patients and AVS for patients who are candidate for surgery (can skip AVS in patients with marked PA+age<35y+ unilateral lesion at CT>10mm)
European Society of Hypertension 2020[59]	Hypertension grade 2-3 or resistant; hypokalemia (spontaneous or diuretic-induced); hypertension and adrenal incidentaloma; hypertension and atrial fibrillation (without underlying cardiac structural cause); suspected familial forms	Aldosterone to renin (or plasma renin activity) ratio	All patients with positive screening except those with: hypokalemia+ undetectable renin (DRC<5 or PRA<0.2 ng/mL/h) + AC > 20 ng/dL	Seated intravenous saline load (captopril test if the former is considered risky)	CT scanning for confirmed patients and AVS for patients who are candidate for surgery (can skip AVS patients with marked PA+age<35y+ unilateral lesion at CT>10mm)
American Association of Clinical Endocrinologist 2006[72]	Hypertension and hypokalemia (spontaneous or diuretic-induced); resistant hypertension	Aldosterone to plasma renin activity ratio	All patients with positive screening test	Oral or intravenous saline load	CT scanning for confirmed patients and AVS with high probability of APA (can skip AVS patients with marked PA+age<40y+ unilateral lesion at CT>10mm)
Japan Society of Endocrinology 2009[68]	All patients with hypertension irrespective of severity	Aldosterone to plasma renin activity ratio	All patients with positive screening test using 2 confirmatory tests	Intravenous saline load; captopril test; upright-furosemide test	CT scanning for confirmed patients and AVS for patients who are candidate for surgery
French Society of Endocrinology 2016[73]	Hypertension grade 3; resistant hypertension, hypokalemia (spontaneous or diuretic-induced), hypertension and adrenal incidentaloma, organ damage or cardiovascular morbidity more severe than expected	Aldosterone to renin (or plasma renin activity) ratio	All patients with positive screening test except patients with AC > 20 ng/dL	Intravenous saline load (captopril test if the former is considered risky)	CT scanning for confirmed patients and AVS for patients who are candidate for surgery if age > 35y

Guideline	AVS performance	AVS criteria

Endocrine Society 2016[58]	Unstimulated (sequential or simultaneous) or continuous ACTH infusion (continuous or bolus)	SI threshold ≥2 (unstimulated); ≥5 (after ACTH), LI ≥2 (unstimulated); ≥4 (after ACTH)
European Society of Hypertension 2020[59]	Unstimulated or continuous ACTH infusion	SI threshold ≥2 (unstimulated); ≥5 (after ACTH), LI ≥4
American Association of Clinical Endocrinologist 2006[72]	Continuous ACTH infusion SI threshold ≥10, LI ≥3	SI threshold ≥10, LI ≥3
Japan Society of Endocrinology 2009[68]	Bolus ACTH	SI threshold ≥5, LI ≥2.6 (or AC >1400 ng/dL on one side)
French Society of Endocrinology 2016[73]	Simultaneous unstimulated cannulation	SI threshold ≥2, LI ≥4

Genetics and pathophysiology of primary aldosteronism

The last ten years have witnessed major progress in understanding the molecular and histopathologic basis of PA. Elucidation of the genetic basis of familial aldosteronism, single adenoma formation and the histology of adrenal hyperplasia has challenged the traditional model of PA as a binary disease of unilateral adenoma versus bilateral adrenal hyperplasia. The emerging concept implies an age-dependent remodeling of the adrenal cortex with gradual accumulation of nodular lesions with somatic mutations that contribute to autonomous aldosterone production.

Adrenal histopathology

Evaluation of morphology and CYP11B2 (aldosterone synthase) immunohistochemistry[74] of resected adrenals from patients with PA has helped determine the origin of aldosterone overproduction and unmask the complex associated histopathology.[75-78] This approach led to the identification of aldosterone-producing micronodules (formerly known as aldosterone-producing cell clusters) (**Figure 2**) that accumulate with age and may represent a source of age-related abnormal aldosterone physiology.[79,80] Aldosterone-producing micronodules drive excess aldosterone production in some bilateral forms of PA,[76,81] and, in a subset of cases, may transit into aldosterone-producing adenomas.[82,83] The international HISTALDO consensus established standardized nomenclature and criteria for the main histopathologic features observed in surgically removed adrenals from patients with PA and categorized adrenals into classical and nonclassical histopathologic forms of unilateral PA[84] (**Figure 2**). Classical forms are represented by a solitary aldosterone-producing adenoma or a dominant aldosterone-producing nodule. In contrast, nonclassical forms encompass specimens in which aldosterone overproduction is derived from multiple aldosterone-producing nodules or micronodules (previously referred to as nodular or micronodular hyperplasia), or, more seldomly, aldosterone-producing diffuse hyperplasia[85] (**Figure 2**). Assessment of a prospective cohort of adrenals from surgically treated patients for PA demonstrated that persistence of aldosteronism is more frequent in patients with the nonclassical histopathology.[85] Thus, the new classification takes both morphology and function into account, and emphasizes the value of CYP11B2 immunohistochemistry using specific monoclonal anti-CYP11B2 antibodies[74] for the routine diagnostic workup of PA. The HISTALDO consensus recommends,[84] in support of previous studies,[86,87] that the final histopathologic diagnosis of PA requires evaluation of both morphology and CYP11B2 immunohistochemistry to establish the functional correlation of autonomous aldosterone production.

Familial hyperaldosteronism

Familial hyperaldosteronism (FH) is a rare cause of PA. All known subforms (**Figure 3**) are autosomal dominant. Patients typically present with early-onset hypertension, but incomplete penetrance is common. FH-I (glucocorticoid-remediable hyperaldosteronism) was first described in 1966.[88] Family history is often positive, and early cerebral hemorrhage is an associated finding.[89] The historic diagnosis based on a decrease of aldosterone upon administration of dexamethasone has been largely replaced by genetic testing. FH-I is due to unequal crossing over between the genes *CYP11B1*, encoding 11ß-hydroxylase (involved in cortisol synthesis) and *CYP11B2*, encoding aldosterone synthase.[90] As a result, excess aldosterone and hybrid steroids 18-hydroxycortisol and 18-oxocortisol are produced under the control of adrenocorticotrophic hormone (ACTH) in the zona fasciculata. Therapy is mainly based on dexamethasone and mineralocorticoid receptor antagonists and amiloride. Screening of family members is advised. FH-II historically denoted cases with apparently familial PA

without known genetic cause. It now refers to patients with germline mutations in the *CLCN2* gene encoding the chloride channel ClC-2.[91,92] Here, increased chloride efflux from glomerulosa cells results in depolarization, calcium influx through voltage-gated calcium channels and increased aldosterone production. Patients present with moderate to severe hypertension and optional hypokalemia. FH-III encompasses patients with germline mutations in the *KCNJ5* gene, which codes for an inward rectifier potassium channel. Mutations cause abnormal sodium permeability of the channel and depolarization, again activating the calcium pathway. Different disease severities are associated with distinct mutations, with some showing massive bilateral hyperplasia and requiring bilateral adrenalectomy and others responding to therapy with mineralocorticoid receptor antagonists.[93-95] FH-IV is due to germline mutations in the *CACNA1H* gene encoding a T-type calcium channel;[96] mutations directly increase calcium signaling. Lastly, a complex syndrome of PA, seizures, and neurologic abnormalities (PASNA syndrome) has been associated with *de novo* germline gain-of-function mutations in the *CACNA1D* gene encoding an L-type calcium channel.[97] Associated symptoms may include epilepsy, autism, hypoglycemia, and heart defects.

Somatic mutations

Somatic mutations in genes that partially overlap with FH disease genes have been identified in aldosterone-producing adenomas and in aldosterone-producing micronodules. When using CYP11B2 immunohistochemistry and targeted next-generation sequencing for mutation detection, approximately 90% of aldosterone-producing adenomas carry somatic mutations in known disease genes. The most commonly mutated gene is *KCNJ5*,[94,98,99] followed by *CACNA1D*[97,100] (see above regarding the pathophysiology of both). Aldosterone-producing adenomas with *KCNJ5* mutations are associated with specific steroid profiles in plasma, which may in the future aid in the diagnosis of these tumors.[101] Somatic mutations not found in the germline include those in the *ATP1A1* gene (encoding a subunit of the Na^+/K^+-ATPase), the *ATP2B3* gene (encoding a calcium ATPase)[102] and in *CTNNB1* (encoding ß-catenin).[103] Mutations in ATPases confer a channel-like permeability to Na^+ or H^+ ions, causing depolarization and similar pathophysiology as *KCNJ5* mutations (**Figure 3**).[100,104] The pathophysiology of *CTNNB1* mutations may be related to a lack of cellular differentiation and subsequent hyperplasia.[105] More recently, rare mutations in the *CACNA1H* and *CLCN2* genes were identified in aldosterone-producing adenomas.[106,107] The relative proportions of mutations in different disease genes varies between ethnicities and sexes. For example, *KCNJ5* mutations, which account for approximately 40% of tumors, are more frequent in women[108] and possibly in patients of Asian ancestry,[109] whereas *CACNA1D* mutations appear to be more frequent in patients with recent African ancestry.[110] A matter of debate has been whether known somatic mutations explain only aldosterone production or also increased proliferation. Adrenal hyperplasia in FH-III suggests that *KCNJ5* mutations cause increased proliferation or a lack of differentiation.[94]

Somatic mutations in PA disease genes have also been identified in aldosterone-producing micronodules (**Figure 3**) in normal human adrenal glands[79] and, at increased size and density, in rare surgical specimens from patients with bilateral adrenal hyperplasia who had undergone unilateral adrenalectomy.[81] Mutations in *CACNA1D* are particularly common in aldosterone-producing micronodules. Yet, about 40% of aldosterone-producing micronodules remain genetically unexplained.[81]

Subtyping of primary aldosteronism

After confirming the diagnosis of PA, the next step is to determine whether PA is of unilateral or bilateral origin. Subtyping is driven by the intention to identify suitable candidates for

unilateral adrenalectomy who will be in biochemical remission postoperatively with a low risk of recurrence. The two subtypes of unilateral and bilateral PA, represent extremes of a spectrum of histomorphologic and biochemical phenotypes, which are not totally distinct. In other words, asymmetric bilateral PA with some lateralization is common, whereas true unilateral PA without any contralateral autonomous aldosterone secretion is rare. Certain demographic, clinical, biochemical, and imaging characteristics are associated with the PA subtypes.

Adrenal imaging by CT and MRI

Adrenal computed tomography is the first step for PA subtype evaluation and should be undertaken in every patient.[58,59] Patients with PA may present with normal adrenal imaging, unilateral or bilateral adenoma or micronodular hyperplasia, bilateral macronodular hyperplasia, or, rarely, as a unilateral adrenocortical carcinoma.[76,111] CT does not provide information about the secretory activity of a detected nodule.[112] Similarly, densitometry (Hounsfield units) and contrast wash-out do not distinguish secretory from non-secretory nodules.[58,113,114] As adrenal adenomas are more prevalent with age,[115] the accuracy of imaging in PA subtyping is low in older patients.[111,116,117] Overall, adrenal imaging is discordant with adrenal vein sampling (AVS) results in around 40% of patients with PA.[116,118,119] Conversely, as adenomas are uncommon in younger persons,[115] finding a unilateral small adenoma (usually <2 cm) in a patient with PA commonly indicates unilateral disease. The accuracy of imaging in establishing PA subtype is highest in patients younger than 35 years of age, but is not perfect.[116,120] Pre-AVS CT scanning also assists in localizing adrenal veins [3]. Magnetic resonance imaging is inferior to CT because its spatial resolution is lower and therefore, is a second-choice imaging technique.[58,121]

Several studies have proposed combinations of clinical, biochemical, and imaging variables in prediction scores for PA subtyping.[122-124] Incorporation of machine learning techniques produced a score for subtype diagnosis with a high performance, reproduced in an external validation cohort, thereby demonstrating its potential to guide surgical decision-making.[124]

Adrenal vein sampling

AVS is a technically challenging, expensive, and not well-standardized procedure with success rates varying widely between centers, depending on the expertise of the interventional radiologist. In experienced hands (usually in a high-volume PA center with more than 30 cases per year), the technical AVS success rate can be 90% or higher.[111,125-127]

It is a limitation of many studies supporting AVS use that they are based on low evidence, including retrospective design, lack of a comparator, and lack of a clinical endpoint (biochemical remission). Against these apparent weaknesses speaks that AVS is consistently used worldwide.[128] A single prospective randomized trial (SPARTACUS) compared AVS based treatment decision for adrenalectomy with CT based decision in 184 patients, finding no differences in post-surgery blood pressure outcomes.[129] However, this study has been intensely discussed and criticized for its overall study design and potential underpowering.[130,131] A multicentric international study using a non-randomized retrospective approach confirmed the biochemical success rates of the strategies in the SPARTACUS trial: CT-based treatment decision led to biochemical remission in 188 of 235 [80%] of patients, but in 491 of 526 [93%] of patients via AVS-based decision, (P<0.001).[132] AVS remains currently the gold standard for subtyping and is recommended by all guidelines and consensus statements.[58,59,68,72,73]

According to an international study, 46% of major centers use cosyntropin infusion during AVS.[128] Arguments in favour of cosyntropin use include decreasing stress-induced aldosterone secretion, increasing the technical success rate of AVS, and maximizing

aldosterone secretion from APAs. Arguments against its use include a reduction in the proportion of lateralized AVS results and therefore of surgically treatable patients.[133] A few studies have compared basal and post-cosyntropin AVS results, reporting discordant results in up to 25% of patients [132,133]. Detailed steroid profiling other than cortisol and aldosterone have been suggested as of potential value during AVS, especially when cosyntropin is not used.[134,135]

AVS is performed via a percutaneous femoral vein approach. Adrenal veins can be catheterized sequentially or simultaneously.[127] Blood is collected by slow aspiration from the left adrenal vein, which drains directly into the left renal vein, and from the right adrenal vein, which usually drains directly into the inferior vena cava and can be challenging to be located.[114] A sufficient gradient of cortisol from both adrenal veins to a peripheral blood sample validates technically successful AVS **(Table 3)**. Rapid cortisol measurements can be employed to improve technically success rates.[136-138] Complications of AVS include most commonly a groin hematoma, but also rarely adrenal hemorrhage or adrenal vein dissection, with an overall complication rate of around 2% in experienced AVS centers.[126,139-141]

AVS can be performed regardless of concomitant medications if the diagnosis was made correctly and the case detection testing is positive. For example, AVS can be performed in patients treated with suboptimal doses of mineralocorticoid receptor blocker, such as spironolactone or eplerenone, if renin plasma activity remains suppressed.[142,143] Concomitant cortisol hypersecretion is prevalent in patients with PA, in most cases mild.[144] When ACTH-independent hypercortisolism is diagnosed in a patient with concomitant PA, in general surgery targets therapy of cortisol excess, and AVS may not be needed. In mild autonomous cortisol secretion, interpretation of AVS does not seem to be significantly affected when performed under cosyntropin stimulation.[145] The diagnostic process of subtyping using AVS and the criteria for interpretation of AVS results are given in **Figure 4** and **Table 3**.

Table 3: Interpretation of AVS with and without ACTH stimulation. Several sequential steps are needed to correctly interpret AVS results. IVC, inferior vena cava; AV, adrenal vein

Required steps for AVS interpretation	***AVS with cosyntropin stimulation***	***AVS without cosyntropin stimulation (preferably simultaneous bilateral AVS)***
1. Documentation of successful adrenal vein catheterization (selectivity index)	Adrenal vein to IVC cortisol ratio should be at least 5:1, and is usually much higher than that (20-30:1)[121,126,140]	Adrenal vein to IVC cortisol ratio should be at least 2:1[121,127]
2. Correction for dilution	Dividing the AV aldosterone by respective AV cortisol concentrations (cortisol-corrected AV aldosterone ratio)	Dividing the AV aldosterone by respective AV cortisol concentrations (cortisol-corrected AV aldosterone ratio)
3. Determination of the aldosterone lateralization ratio (laterization index, LI)	Dividing the higher (ipsilateral, dominant) AV cortisol corrected aldosterone ratio by the lower (contralateral, nondominant) AV cortisol corrected aldosterone ratio. -LI≥4 indicates unilateral aldosterone excess[116,121,140] -LI<3 indicates bilateral aldosterone excess -LI 3-4 is indeterminate for bilateral versus unilateral aldosterone excess	Dividing the higher (ipsilateral, dominant) AV cortisol corrected aldosterone ratio by the lower (contralateral, nondominant) AV cortisol corrected aldosterone ratio. LI≥4 indicates unilateral aldosterone excess[114,121,146] LI<3 indicates bilateral aldosterone excess LI 3-4 is indeterminate for bilateral versus unilateral aldosterone excess
4. Calculation of the contralateral suppression index (CSI)	Dividing the lower (contralateral, nondominant) AV cortisol corrected aldosterone ratio by the IVC cortisol corrected aldosterone ratio (AV A/C : IVC A/C) -CSI<1 may predict surgical outcome in unilateral PA, helps establishing the diagnosis of unilateral PA when LI is between 3-4[140] but is not valuable in decision making when LI is >4 [147,148] -CSI<0.5 predicts unilateral contralateral PA if AVS was not successful bilaterally[149] -CSI<0.47 was associated with a higher risk for postoperative hyperkalemia[150]	Dividing the lower (contralateral, nondominant) AV cortisol corrected aldosterone ratio by the IVC cortisol corrected aldosterone ratio (AV A/C : IVC A/C) -CSI<1 may predict surgical outcome in unilateral PA

Steroid profiling

Several studies demonstrated a variable diagnostic accuracy of 18-oxocortisol and 18-hydroxycortisol when analyzed in the AVS samples in attempt to distinguish unilateral from bilateral PA.[151-154] Analysis of the peripheral samples with a LC-MS based 15 steroid assay correctly identified the subtype of 80% patients with PA.[154] A 7 steroid fingerprint correctly classified 92% of 79 patients with PA according to their genotype.[155] However, the performance of steroid profiling was lower when tested on a larger cohort of patients. In a large multicenter study of patients with PA and patients with hypertension, steroid profiling combined with machine learning algorithms diagnosed PA with a sensitivity of 69% and specificity of 94%, and identified PA associated with KCNJ5 with a sensitivity of 85% and specificity 97%.[101]

Functional imaging

Several functional imaging techniques have been recently explored for PA subtyping. ^{11}C-metomidate positron emission tomography (PET) has been used in several small studies demonstrating reasonable accuracy for PA diagnosis.[156-158] The procedure presents several challenges: the need for pretreatment with dexamethasone due to low selectivity of ^{11}C-metomidate for CYP11B2 (aldosterone synthase) over CYP11B1 (11β-hydroxylase), limited number of centers capable of performing the test due to the short life of ^{11}C. One study showed only a 51% concordance of ^{11}C-metomidate PET imaging with AVS results, although pretreatment with dexamethasone was not used in all patients.[159]

Recently, a high expression of CXCR4 (CXC chemokine receptor type 4) was reported in aldosterone-producing adenomas that correlated with expression of CYP11B2. CXCR4 ligand ^{68}Ga-pentixafor positron tomography successfully detected PA in an initial small study of 9 patients.[160] In another study of 36 patients with a reference standard based on clinical follow up and immunohistochemistry, ^{68}Ga-pentixafor PET diagnosed PA with 88% sensitivity and 100% specificity.[161] Further larger studies with optimal reference standard are needed to validate these initial findings.

Surgical management

Candidates for surgery

Adrenalectomy should be offered in principle to all patients with predominant unilateral aldosterone secretion at AVS.[58,121] When AVS is only unilaterally successful, machine learning algorithms may provide reliable information to guide surgical decision-making in patients with contralateral suppression of aldosterone secretion.[162] In centers where AVS is unavailable, diagnostic modelling techniques can be used for subtype diagnosis to select patients for surgery.[163] Surgical treatment for PA normalizes blood pressure in less than half of patients[164] and predictive scores have been developed to differentiate those with post-surgical clinical remission from those who will require close follow-up due to persistent hypertension.[165,166]

Glucocorticoid co-secretion

In some cases, APAs may also produce detectable amounts of cortisol.[144] Cortisol co-secretion from APAs may influence subtype diagnosis in PA[114,121] and post-surgical management[167] and may be associated with an increased incidence of metabolic alterations[43] and organ damage.[168] Therefore, cortisol co-secretion should always be investigated by an overnight 1 mg dexamethasone suppression test,[104,121] especially in patients with an adrenal nodule at CT scanning larger than 10 mm diameter. After adrenalectomy, these patients should be closely followed up to identify and treat potential adrenal insufficiency.[167]

Outcome assessment

The international PASO consensus established criteria to assess the clinical and biochemical response to adrenalectomy and timeline for follow-up evaluation.[164] The consensus recommended a first post-surgical outcome assessment within 3 months, and a final outcome assessment at 6-12 months after adrenalectomy to evaluate clinical and biochemical outcomes categorized as complete, partial or absent success.[164] The criteria allow comparison of reports from different international case series and, in some centres, form part of routine clinical care of surgically treated patients. Persistence of hypertension (partial or absent clinical success) may be determined by factors that are independent of aldosteronism such as long duration of hypertension (associated with organ damage, which in turn maintains high blood pressure) or concomitant primary hypertension. Persistence of aldosteronism (partial or absent biochemical success) indicates presurgical bilateral aldosteronism and thus biochemical outcomes provide a quality measure of patient diagnosis with complete biochemical success defining the correct diagnosis and appropriate treatment.[164]

Interventional approaches

Complete unilateral adrenalectomy is the treatment of choice for patients diagnosed with unilateral PA.[58,121] Laparoscopic adrenalectomy, performed by an expert adrenal surgeon, is considered the technique of choice because it is associated with a shorter duration of hospitalisation and a lower rate of clinical complications compared with laparotomy.[121,169] Correction of hypokalemia and normalisation of blood pressure levels should be obtained preoperatively to minimize peri-procedural complications.[121] To avoid post-surgical hyperkalaemia, mineralocorticoid receptor antagonists and potassium supplementation should both be stopped. Except for specific cases identified by super-selective AVS, partial adrenalectomy (adrenal sparing nodulectomy) should be avoided because AVS can only determine the side of aldosterone hyper-secretion and the largest nodule is not always the culprit lesion responsible for hyperaldosteronism.

Medical therapy

Patients with bilateral adrenal hyperplasia should receive mineralocorticoid receptor blockade using one of the agents mentioned below and depicted in **Table 4**. Patients with unilateral hyperaldosteronism may prefer medical therapy over surgery and can be effectively treated accordingly. Retrospective cohort studies demonstrate that surgery appears to be more effective than mineralocorticoid receptor blockade in controlling blood pressure and reducing the number of hypertensive drugs.[170] In addition, surgery reverses left ventricular hypertrophy more effectively,[171] reduces the risk for atrial fibrillation[172] and chronic kidney failure[52] and normalizes quality of life more efficiently.[173] Also, long-term mortality appears to be lower following adrenalectomy.[174,175]

Spironolactone

Spironolactone is a competitive antagonist of the mineralocorticoid receptor belonging to the group of potassium-sparing diuretics. Due to spironolactone-mediated inhibition of aldosterone binding the mineralocorticoid receptor in the collecting ducts of the kidneys, the epithelial sodium channels in the principal cells are inhibited.[7] This reduces sodium reabsorption and water flux into the cortical collecting ducts and the vascular system, thus lowering blood pressure. Oral spironolactone is converted after absorption into long-acting metabolites including canrenoate, which are responsible for the main pharmacological effects.[176] Due to off-target effects at the androgen and progesterone receptor, spironolactone has dose-dependent antiandrogenic side effects, including gynecomastia and impotency in males and menstrual irregularities in females.[177] In the RALES study at a dose of 25 mg

spironolactone, gynecomastia was observed in 10% of males.[178] Therefore, the starting dose is 12.5 to 25 mg per day, and long-term management is effective at doses of spironolactone of 25 to 50 mg/day.[121] In one study, this dose reduced the need for antihypertensive drugs by 0.5 drugs, and systolic and diastolic blood pressure by 15 mm Hg and 8 mm Hg, respectively. Half of the patients were able to be managed with spironolactone monotherapy and had a blood pressure <140/90 mm Hg.[179] If these doses are not sufficient, combination therapy using additional hypertension drugs is effective. Severe and life-threatening hyperkalaemia is a frequent side effect in heart failure,[180] but rarely seen in PA because of the underlying mineralocorticoid excess increasing tubular potassium losses. The onset of action on blood pressure is slow, requiring weeks to months, reaching 20-25 mm mercury in systolic blood pressure.[181]

Eplerenone

Eplerenone is an option in patients who cannot tolerate spironolactone because of side effects. It is a selective steroidal mineralocorticoid receptor antagonist with a short half-life of 3 to 6 hours, and its main indication is left ventricular dysfunction after myocardial infarction.[182] Eplerenone is not approved for hypertension treatment or treatment of PA in every country. In clinical practice, higher doses of 100-300 mg eplerenone have to be used to achieve a similar blood pressure effect as spironolactone,[183] and it is administered in this indication twice daily.

Amiloride and other hypertensives

Mineralocortioid antagonist treatment can be substituted by or complemented with agents like amiloride, which directly block the epithelial sodium channel. Amiloride is less effective in terms of blood pressure control, therefore preferably used in milder forms of PA. The relative potency of amiloride versus spironolactone in correcting hypokalaemia was 3 to 1, and doses of 5 to 20 mg twice daily are required for optimal effect.[184] Thiazide diuretics and calcium channel blockers can be synergistically combined with mineralocorticoid receptor antagonist treatment or amiloride. For theoretical consideration angiotensin converting enzyme inhibitors and angiotensin receptor blockers are less good combination partners, as renin and angiotensin levels should be suppressed in PA. However, in clinical practice they act often synergistically. One explanation might be that the effective blockade of the mineralocorticoid receptor will antagonise suppressed renin levels, thus, re-expose the RAAS to pharmacologic inhibition.[7] The detection of agonistic angiotensin receptor antibodies in the plasma of patients with PA[185] bestow a pathophysiologic basis for the use of angiotensin receptor blockers. Most patients with bilateral PA require 1 to 3 additional drugs to sufficiently control blood pressure levels. Patients with PA consume at average a high salt diet of more than 10 g sodium chloride per day.[186] One of the reasons may be hyperaldosteronism-induced impaired sensory salt tasting.[187] Unilateral adrenalectomy can restore the salt testing threshold and is associated with spontaneous reduction of dietary sodium chloride intake.[186] Since medically treated patients with PA do retain spontaneously a high salt diet during mineralocorticoid receptor blockade, dietary sodium restriction should be implemented because the deleterious consequences of hyperaldosteronism are dependent on salt loading.[181]

Outcome of medical treatment

Medical management of PA aims at normalizing systolic and diastolic blood pressure, in accordance with current guidelines.[188] Also, serum potassium concentrations should be normalized. Long-term follow-up should be provided to assess and treat cardiovascular and metabolic comorbidities. PA must be considered as a relevant and independent risk factor for adverse long-term-outcome of hypertension. Effective blockade of the mineralocorticoid receptor appears to be a cornerstone of optimal treatment for bilateral PA. In a large cohort

study of patients treated with mineralocorticoid antagonists, patients with consistently suppressed plasma renin activity (< 1 ng/mL/h) had an adverse cardiovascular, renal, and metabolic outcome and increased mortality, compared to patients with primary hypertension. In contrast, patients with unsuppressed renin levels and patients after unilateral adrenalectomy had no significant excess risk,[51-53] data confirmed by other recent studies.[189,190]

Table 4: Specific medical therapies in primary aldosteronism.
FH-I: familial hyperaldosteronism type I.

Compound	Starting dose per day	Maximum dose	Application schedule	Typical side effects
Spironolactone	12.5-25 mg	Males: 50 mg Females: 100 mg	Once daily	Males: gynecomastia, impotency Females: menstrual irregularities Both sexes: hyperkalaemia
Eplerenone	50 mg	300 mg	Twice daily	Hyperkalaemia
Amiloride	5 mg	20-40 mg		Hyperkalaemia Nausea, stomach pain, loss of appetite
Dexamethasone (in FH-I)	0.125 mg	0.25 mg	At bedtime	At higher doses: weight gain, osteoporosis, diabetes, Cushingoid phenotype, adrenal insufficiency, growth failure

Perspectives

PA is characterized by the paradox of being a common cause and augmenter of hypertension whilst its high incidence and burden of disease is widely ignored in most if not all health care systems. In a society with a 20% prevalence of hypertension, every 83rd individual will have PA, and one in every 250 will have a surgically curable form of unilateral PA.[27] Affected patients will often develop resistant hypertension associated with a low quality of life, and adverse cardiovascular and cerebrovascular outcomes. Disappointingly, an astonishingly low proportion, around 1% of patients with PA, are ever evaluated and treated for the condition. This screening paradox is only partially attributed to the 'monosymptomatic' phenotype of hypertension or the challenging diagnostic cascade. In the opinion of the authors, it is the misconception of PA as a rare, distinct disease that must be treated by specialists, that underlies the current 'ignorance' of the disorder. Clinical recognition of its long-term impact on hypertension outcomes by extended screening would be cost-saving[191] and result in gains in quality-adjusted life years.[192] Contrary to the conception of PA as a distinct disease, it should be regarded as a cardiovascular risk factor, like other classical risk factors, such as diabetes mellitus, hypercholesterolemia, or smoking, which aggravate and potentiate adverse outcomes of hypertension. Therefore, instead of screening selected at-risk populations, general screening of subjects with hypertension should be the future strategy to reduce the disease-burden in a cost-effective way.[193]

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Figure 1: **Physiology and pathophysiology of the renin angiotensin aldosterone system**. a.) physiologic regulation; b.) regulation in PA. Abbreviations: ACE, angiotensin converting enzyme.

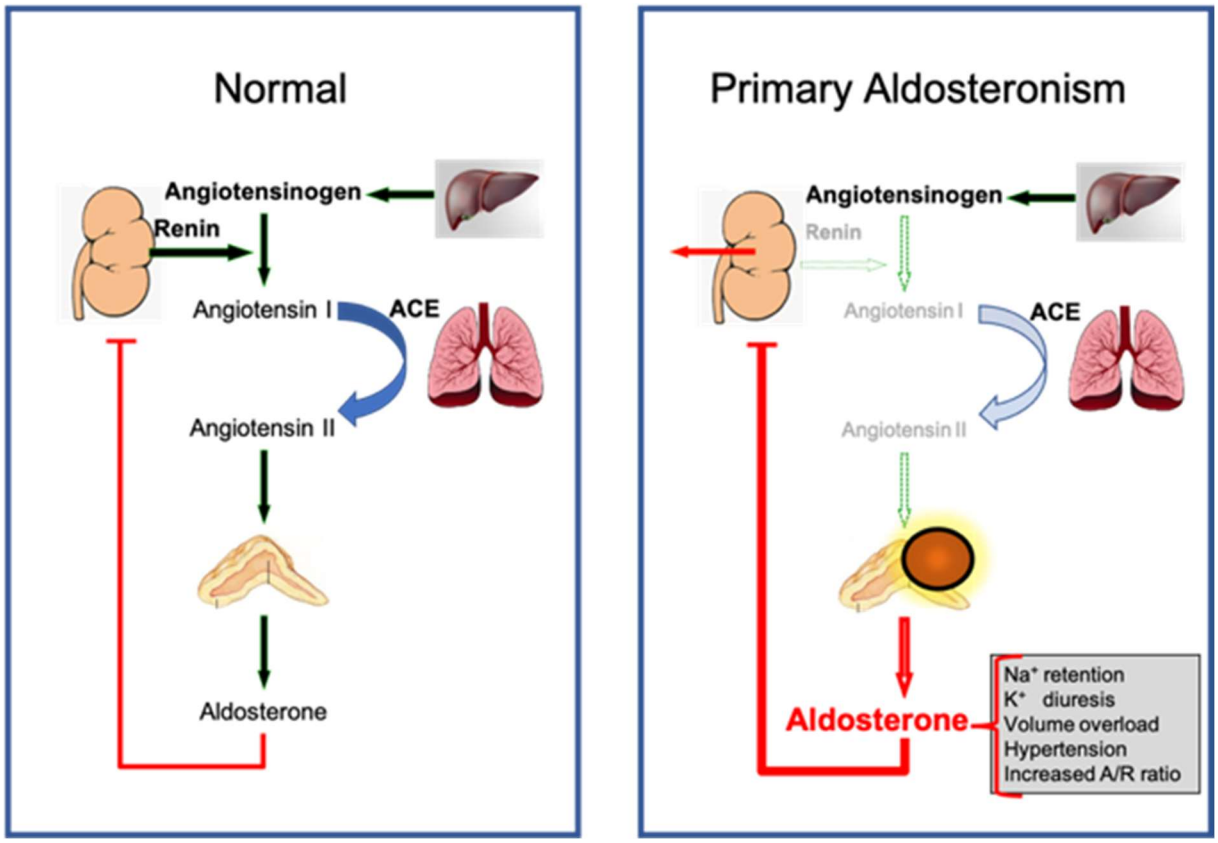

Figure 2: Histopathologic classification of primary aldosteronism
CYP11B2 (aldosterone synthase) immunohistochemistry showing resected adrenals with a well circumscribed solitary aldosterone-producing adenoma (≥ 10 mm diameter) with homogeneous (**A**) or heterogeneous (**B**) immunostaining; a 5 mm diameter aldosterone-producing nodule which is morphologically distinguishable by haematoxylin-eosin staining (**C**); multiple aldosterone-producing micronodules, which are not morphologically distinguishable from adjacent cells by haematoxylin-eosin staining (**D**); aldosterone-producing diffuse hyperplasia (**E**). Scale bars indicate 2 mm (main images), 200 μm (insets).

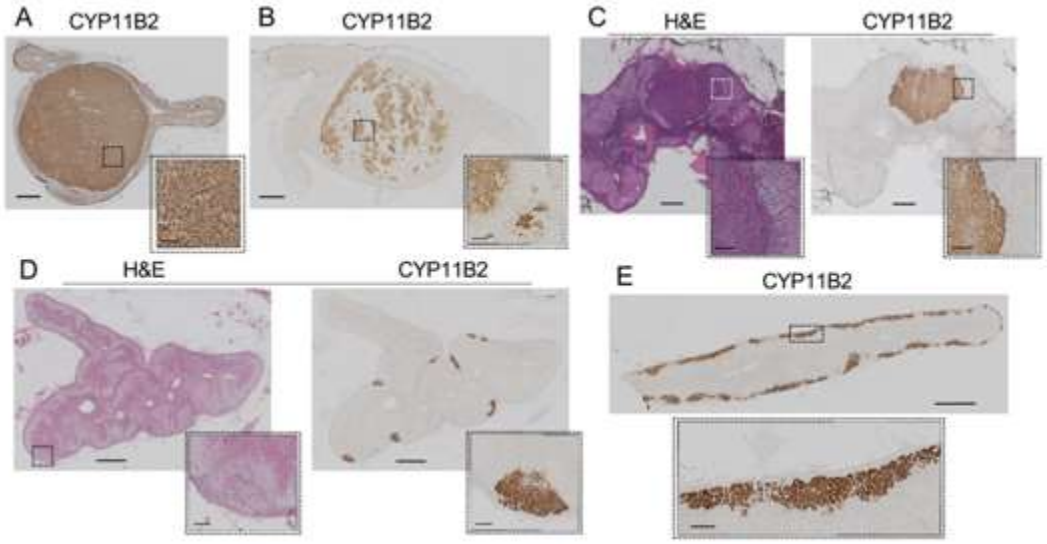

Figure 3: **Pathophysiology of somatic and germline mutations in primary aldosteronism**. In FH-I, transcription of a hybrid *CYP11B1*/*CYP11B2* gene is activated by ACTH. Channel-like permeability of mutant ATPases *ATP1A1* and *ATP2B3* for Na^+ or H^+ ions, leads to depolarization, activation of voltage-gated calcium channels, calcium influx and aldosterone production. Similarly, abnormal Na^+ permeability of mutant KCNJ5 channels causes depolarization in FH-III and in adenomas, as does enhanced chloride efflux via mutant *CLCN2* in FH-II and rare cases of adenomas. Gain-of-function mutations in calcium channel genes *CACNA1D* (PASNA syndrome, adenomas, aldosterone-producing micronodules) and *CACNA1H* (FH-IV, rare cases of adenomas) directly increase Ca^{2+} influx.

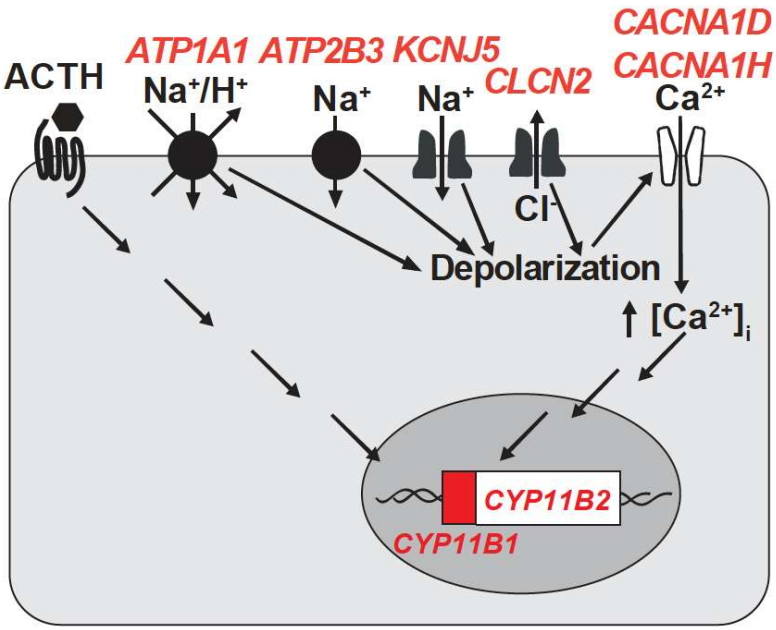

Figure 4: Flow diagram of subtyping in primary aldosteronism. * Selectivity indices according to Table 3; ** Lateralization indices according to Table 3; *** Contralateral suppression indices according to Table 3.