Hypertension

REVIEW

Genetics of Primary Aldosteronism

Ute I. Scholl

ABSTRACT: Primary aldosteronism is considered the commonest cause of secondary hypertension. In affected individuals, aldosterone is produced in an at least partially autonomous fashion in adrenal lesions (adenomas, [micro]nodules or diffuse hyperplasia). Over the past decade, next-generation sequencing studies have led to the insight that primary aldosteronism is largely a genetic disorder. Sporadic cases are due to somatic mutations, mostly in ion channels and pumps, and rare cases of familial hyperaldosteronism are caused by germline mutations in an overlapping set of genes. More than 90% of aldosterone-producing adenomas carry somatic mutations in K^+ channel Kir3.4 (*KCNJ5*), Ca^{2+} channel $Ca_V1.3$ (*CACNA1D*), alpha-1 subunit of the Na^+/K^+ ATPase (*ATP1A1*), plasma membrane Ca^{2+} transporting ATPase 3 (*ATP2B3*), Ca^{2+} channel $Ca_V3.2$ (*CACNA1H*), Cl^- channel ClC-2 (*CLCN2*), β-catenin (*CTNNB1*), and/or G-protein subunits alpha q/11 (*GNAQ/11*). Mutations in some of these genes have also been identified in aldosterone-producing (micro)nodules, suggesting a disease continuum from a single cell, acquiring a somatic mutation, via a nodule to adenoma formation, and from a healthy state to subclinical to overt primary aldosteronism. Individual glands can have multiple such lesions, and they can occur on both glands in bilateral disease. Familial hyperaldosteronism, typically with early onset, is caused by germline mutations in steroid 11-beta hydroxylase/ aldosterone synthase (*CYP11B1/2*), *CLCN2*, *KCNJ5*, *CACNA1H*, and *CACNA1D*.

Key Words: adenoma ■ aldosterone ■ hyperaldosteronism ■ mutation

Aldosterone, the main mineralocorticoid hormone, is physiologically produced in the zona glomerulosa of the adrenal cortex. By binding to the mineralocorticoid receptor, it activates signaling cascades leading to increased renal salt and water reabsorption, as well as increased potassium and proton secretion. The production of aldosterone is normally tightly regulated. Angiotensin II (the main product of the renin-angiotensin system) and elevated serum potassium levels are the main stimuli of aldosterone production; adrenocorticotropic hormone can also temporarily increase aldosterone levels.[1] In primary aldosteronism (PA), levels of aldosterone are inappropriate for salt, volume, and/or potassium status. This excess production causes variable degrees of hypertension, possible hypokalemia, and disproportionately high levels of cardiovascular disease.[2] PA is considered the most important cause of secondary hypertension. An Italian study of 1672 primary care patients with hypertension, following the Endocrine Society guidelines for diagnosis, reported an overall prevalence of 5.9%, ranging from 3.9 in stage 1 hypertension to 11.8% in stage 3 hypertenson.[3] Recently, using urinary aldosterone for diagnosis, even higher prevalence estimates for biochemically overt PA were reported, from 11.3% in normotension to 22.0% in stage 3 hypertension.[4]

Traditionally, several subforms of PA were distinguished: bilateral adrenal hyperplasia, also known as idiopathic hyperaldosteronism (about 60% of cases), aldosterone-producing adenomas (APAs; about 30% of cases), unilateral hyperplasia (less common), malignancy, and familial hyperaldosteronism (FH; both very rare).[5] Recent histological and genetic studies have challenged this concept and will be discussed in this review. The diagnosis of PA is complicated, based upon the aldosterone/renin ratio as screening parameter and subsequent confirmatory testing. Of clinical importance is the distinction between unilateral and bilateral forms because unilateral forms are amenable to potentially curative surgery, whereas bilateral forms are treated with mineralocorticoid receptor antagonists.[5]

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Nonstandard Abbreviations and Acronyms	
APA	aldosterone-producing adenoma
APCC	aldosterone-producing cell cluster
AT1R	angiotensin 1 receptor
FH	familial hyperaldosteronism
KCNJ5	K^+ channel Kir3.4
PA	primary aldosteronism
TCF/LEF	T-cell factor/lymphoid enhancer factor

Until 2011, the molecular mechanisms underlying autonomous aldosterone production in PA were largely unknown. Discoveries made over the last decade, their potential future impact on clinical PA management and open questions in the field of PA genetics will be covered in this review.

SOMATIC MUTATIONS IN APAS

Because APAs are typically treated by unilateral adrenalectomy, tumor tissue is available for genetic studies. Advances in sequencing technology enabled the discovery of somatic (tumor specific) mutations in APAs. The initial study sequenced the exomes of 4 APAs and corresponding normal tissue. Two tumors carried heterozygous mutations in the *KCNJ5* gene, which encodes the inward rectifier potassium channel Kir3.4. One mutation, G151R, was located within the selectivity filter of the channel, which enables K^+ to pass through the channel and blocks the passage of Na^+, and the second mutation, L168R, was close by. Both mutations led to abnormal sodium permeability and depolarization of the cell,[6] which results in opening of voltage-gated calcium channels, calcium influx and increased aldosterone production.[7] Sanger sequencing identified 6 additional somatic *KCNJ5* mutations, all G151R and L168R, in 18 other APAs.[6] Follow-up studies showed that G151R and L168R are recurrently mutated in APAs and account for >40% of APAs (Table 1). Other *KCNJ5* mutations are rare.[9,13] Among rare mutations, T158A has been functionally studied, demonstrating activation of transcriptional regulators *NURR1* and *ATF2* downstream of calcium signaling and upstream of *CYP11B2*.[14,15] Further exome sequencing studies of KCNJ5 (K^+ channel Kir3.4)-negative tumors revealed heterozygous mutations in *CACNA1D*, which encodes the L-type calcium channel $Ca_V1.3$.[16,17] *CACNA1D* is large, and mutations cluster much less than in *KCNJ5*, which is why early Sanger sequencing may have missed mutations; recent studies (albeit with rather small case numbers) show frequencies between 14 and 42% (Table 1).[9,11,18] Another frequently mutated gene with heterozygous somatic mutations is *ATP1A1*,[17,19] which encodes the α-1 subunit of the Na^+/K^+-ATPase. Somatic mutations cause abnormal permeability to Na^+ or H^+ and loss of pump function, leading to depolarization, aldosterone production and proliferation, likely via increased intracellular calcium and cellular acidification.[17,18,20,21] Less frequently, plasma membrane Ca^{2+} ATPase (*ATP2B3*) mutations cause abnormal Na^+ and potentially Ca^{2+} permeability, along with reduced Ca^{2+} transport capacity, all resulting in elevated intracellular Ca^{2+} levels.[19,22] Heterozygous somatic gain-of-function mutations in *CACNA1H*, encoding the T-type calcium channel $Ca_V3.2$, are also infrequent.[10] These mutations confer gain-of-function, with increased calcium influx, like *CACNA1D* mutations. Similarly rare are mutations in the gene *CLCN2*, encoding the ClC-2 chloride channel.[23,24]

Whereas the mutations discussed above affect ion channels or pumps and either directly or indirectly cause increased intracellular calcium levels (Figure 1), somatic mutations in *CTNNB1*, encoding β-catenin, point to a different mechanism. β-Catenin is part of the Wnt signaling pathway. Somatic *CTNNB1* mutations are present in several tumor types,[25] including benign and malignant, hormone-producing and nonproducing tumors of the adrenal gland, among them APAs.[26–28] One study suggested an association of *CTNNB1* mutations in APAs with pregnancy,[29] which, however, was doubted[30]; such mutations were also found in males.[31,32] Interestingly, unlike the mutations described above, *CTNNB1* mutations have been described to frequently cooccur with other aldosterone-driver mutations, such as a *CACNA1D* mutation[11] or mutations in the *GNA11* and *GNAQ* genes, encoding G-protein α subunits G11 and Gq, respectively,[33] downstream of the AT1R (angiotensin 1 receptor). Mutations in *GNA11* and *GNAQ* have only been found in conjunction with *CTNNB1* mutations and are likely not sufficient for APA formation alone. Binding of angiotensin II to the AT1R results in exchange of guanosine diphophate for guanosine triphosphate, liberation of Gβγ and activation of downstream phospholipase Cβ, which then cleaves phosphatidylinositol 4,5-bisphosphate into diacyl glycerol and inositol trisphosphate. Inositol trisphosphate causes Ca^{2+} release from intracellular stores (Figure 1).[1] Somatic mutations prevent the hydrolysis of GTP that usually terminates signaling, causing constitutive activity and aldosterone production. Concurrent mutations in *CTNNB1* and *GNA11/Q* were associated with increased expression of luteinizing hormone/choriogonadotropin receptor (*LHCGR*), potentially explaining an association with pregnancy.[33] In mice, expression of a *Ctnnb1* gain-of-function allele to the zona glomerulosa leads to a block of transdifferentiation of zona glomerulosa cells into zona fasciculata cells, resulting in progressive hyperplastic expansion of the zona glomerulosa and increased aldosterone levels.[34] This

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Table 1. Somatic Mutation Frequencies in APAs From Different Ethnicities and Sexes[8]

% Mutations	White Americans (N=75)[8–10]			Black Americans (N=73)[11]			Japanese (N=106)[12]		
Sex	Male	Female	Total	Male	Female	Total	Male	Female	Total
KCNJ5	24	70	41	13	57	34	60	95	73
CACNA1D	33	3	21	55	29	42	21	3	14
ATP1A1	22	10	17	13	3	8	7	0	5
ATP2B3	7	0	4	5	3	4	6	0	4
CTNNB1	0	7	3	3*	0	1	0	0	0
CACNA1H	4	3	4	NA	NA	NA	1	0	1
CLCN2	2	3	3	NA	NA	NA	0	0	0
Negative	7	3	5	13	9	11	4	3	4

APAs indicates aldosterone-producing adenomas; and NA, not assessed.
*Concomitant *CACNA1D* mutation.

observation suggests that *CTNNB1* mutations may act primarily by increasing the number of aldosterone-producing cells, which may be sufficient to cause PA once tumors are large enough. Additional somatic mutations may cause increased aldosterone production on the individual cell level, aggravating the phenotype. *GNA11* mutations may precede *CTNNB1* mutations based on the finding of *GNA11* single mutations in hyperplastic areas outside of double-mutant APAs.[33] Last, *PRKACA* mutations, common in cortisol-producing adenomas, have been described in 2 PA cases, however, their role in causing PA is doubtful.[35]

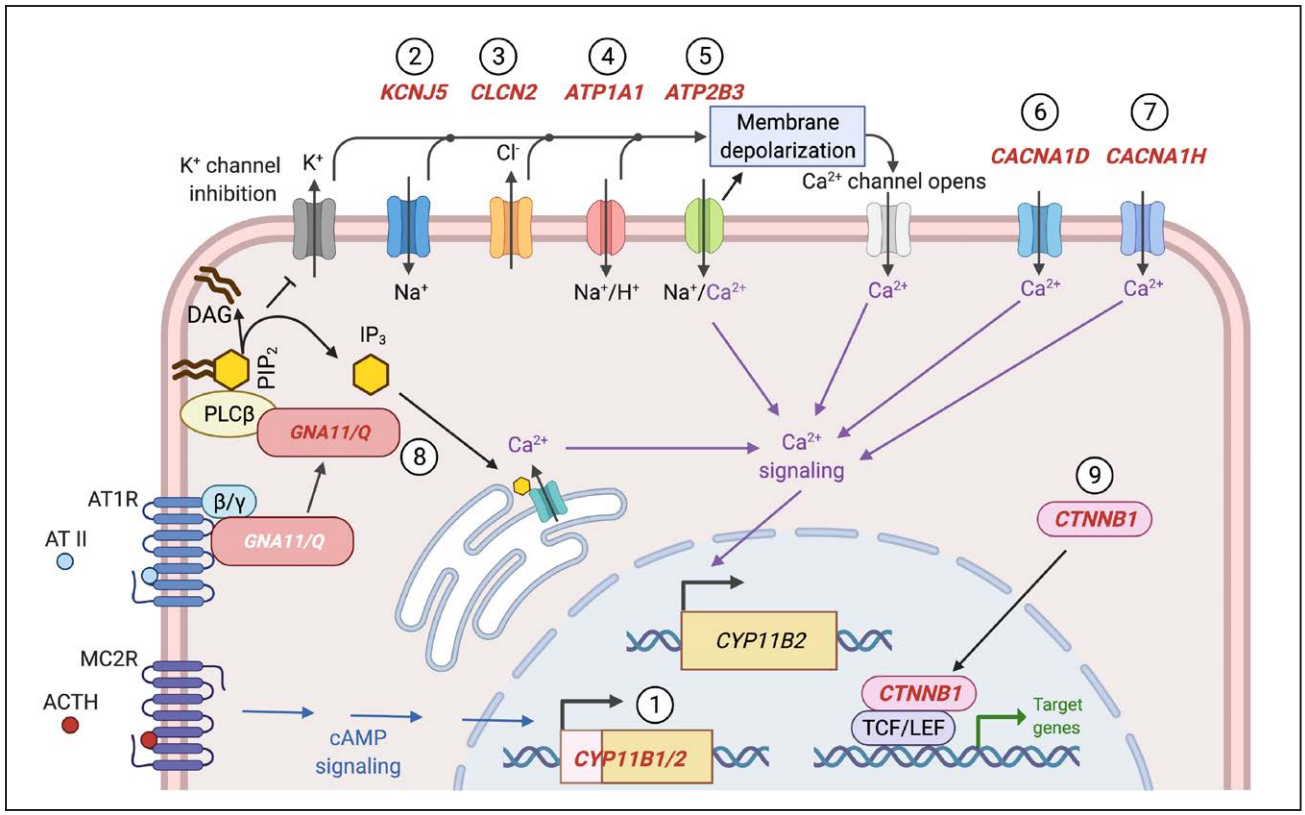

Figure 1. Somatic and germline mutations in primary aldosteronism (PA).
In familial hyperaldosteronism (FH)-I, the *CYP11B1/2* hybrid gene (1) is activated by adrenocorticotropic hormone (ACTH) via the MC2R (melanocortin 2 receptor) and cAMP signaling. *KCNJ5* mutations (2) in aldosterone-producing adenomas (APAs) and in FH-III lead to abnormal Na^+ influx, *CLCN2* mutations (3) in APAs and in FH-II to higher Cl^- efflux, and *ATP1A1* (4) and *ATP2B3* (5) mutations in APAs to channel like-permeabilities for Na^+, H^+, and Ca^{2+}, as well as impaired pump function. These effects cause membrane depolarization, activation of voltage-gated calcium channels, calcium influx and increased calcium signaling, stimulating *CYP11B2* expression and aldosterone production. Acidification may also play a role in *ATP1A1* pathophysiology (not shown). Mutations in *CACNA1D* (6) and *CACNA1H* (7) directly increase calcium permeability. *GNA11* and *GNAQ* mutations (8) in APAs prevent termination of G-protein signaling downstream of the AT1R (angiotensin 1 receptor), leading to increased calcium release from intracellular stores. They cooccur with *CTNNB1* mutations (9) that prevent β-catenin degradation with increased signaling via the TCF/LEF (T-cell factor/lymphoid enhancer factor) family. Created with BioRender.com. DAG indicates diacyl glycerol; PLC, phospholipase C; IP_3, inositol trisphosphate; and PIP_2, phosphatidylinositol 4,5-bisphosphate.

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SEX AND ETHNICITY ASPECTS IN APA SOMATIC MUTATION FREQUENCIES

Silent adrenal masses (incidentalomas, Figure 2) are common, especially in the elderly,[37] and routine histopathology cannot distinguish between nonproducing and aldosterone-producing lesions. The most reliable data on mutations frequencies are from recent studies that identify the culprit lesion by immunohistochemistry for aldosterone synthase (Figure 2) and perform highly sensitive panel sequencing for mutation identification. When all known genes were sequenced, mutations were found in at least 95% of APAs. As noted for *KCNJ5* in earlier studies,[13] mutation frequencies differ between sexes and ethnicities[8] (Table 1). *KCNJ5* mutations are more frequent in women, whereas *CACNA1D* and *ATP1A1* mutations are more frequent in men. *KCNJ5* mutations have the highest prevalence in Asian populations,[38–40] followed by those of European origin[9] and African ancestry.[11] In contrast, *CACNA1D* mutations are more frequent in blacks, outnumbering *KCNJ5* mutations.[11] Differences in diagnostic algorithms, salt or phytoestrogen ingestion cannot be excluded; however, biological differences among sexes and ethnicities, such as those in circulating hormone levels, could well underlie these effects.[8,41] Of note, female mice show a 3-fold higher adrenocortical tissue turnover than males. Female mice also use a stem cell/progenitor cell compartment in the adrenal capsule for renewal that is unused by males.[42] Higher turnover and thus a higher probability of acquiring somatic mutations may thus underlie the higher frequency of adrenocortical tumors in women.[43] It is also tempting to speculate that the mutational profile of APAs depends on their cells of origin, which may differ between sexes and, possibly, ethnicities.

SOMATIC MUTATIONS IN ALDOSTERONE-PRODUCING (MICRO)NODULES AND IN BILATERAL DISEASE

Small nodules of cells with increased expression of aldosterone synthase that protrude beyond the zona glomerulosa into the zona fasciculata occur in healthy individuals and PA patients. They were first described as aldosterone-producing cell clusters (APCCs) in 2010[44] and are now, according to a recently published international consensus, referred to as aldosterone-producing

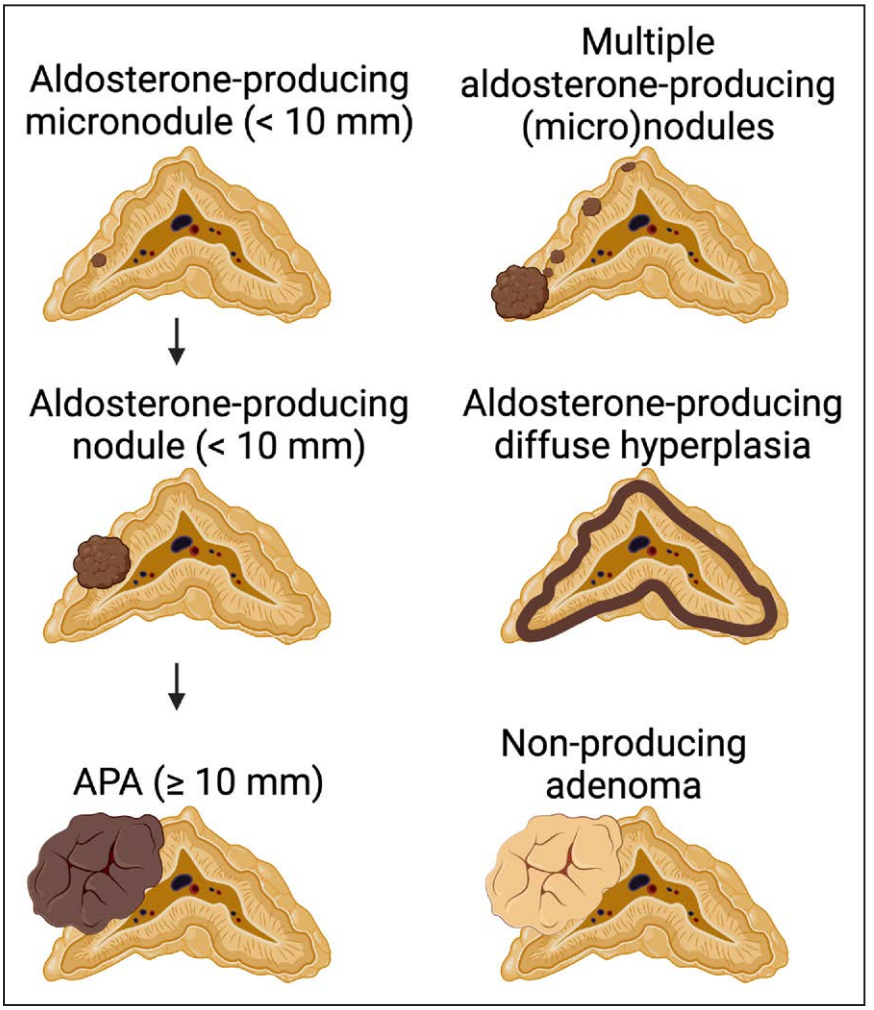

Figure 2. Adrenal lesions.[36] Aldosterone-producing micronodules (formerly aldosterone-producing cell clusters [APCCs]) are not recognizable by hematoxylin and eosin staining but stain positive for aldosterone synthase expression. Aldosterone-producing nodules are visible on hematoxylin and eosin staining and are distinguished from aldosterone-producing adenoma (APA) by size. Multiple (micro)nodules can cooccur within a single gland. Aldosterone-producing diffuse hyperplasia shows a broad, uninterrupted strip of aldosterone synthase-positive cells. Nonproducing adenomas stain negative for aldosterone synthase. Brown color indicates aldosterone synthase positivity on immunohistochemistry. Created with BioRendor.com.

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micronodules (Figure 2).[36] Using panel sequencing of APA disease genes, Nishimoto et al[45] in 2015 discovered that APCCs from healthy kidney donors carried somatic mutations in *CACNA1D* (6/23) and *ATP1A1* (2/23).[45] Nanba et al[46] expanded on these findings by demonstrating that the APCC area increases with age in healthy kidney donors, contrary to normal zona glomerulosa CYP11B2 expression. Interestingly, in a separate clinical study, they detected an increase of the aldosterone/renin ratio with age on a high-sodium diet, whereas aldosterone stimulation on a low-sodium diet was blunted with age, suggesting that autonomous aldosterone production may increase with age due to the development of aldosterone-producing (micro)nodules, whereas normal zona glomerulosa recedes.[46] Micronodules resembling those in normal individuals were also found in adrenals from patients with unilateral PA without adenoma; they carried somatic mutations in *CACNA1D*, *KCNJ5*, *ATP1A1*, and *ATP2B3*, whereas no such mutations were found in nonnodular diffuse hyperplasia.[47] Furthermore, 15 cases with bilateral disease who, as an unusual measure, underwent unilateral adrenalectomy, showed more and larger APCCs than normotensive controls. Sequencing of aldosterone-producing lesions revealed mutations in *CACNA1D* in 58% of APCCs, and of *KCNJ5* in 1% (in a lesion considered by the authors as micro-APA),[48] suggesting that somatic mutations in aldosterone-producing (micro)nodules may underlie bilateral disease. Interestingly, adrenal hyperplasia, including (micro)nodules, can also occur in adrenals carrying a circumscribed APA. De Sousa et al[18] investigated such adrenal glands using CYP11B2 immunohistochemistry and next-generation sequencing. Sequencing of 57 APCCs adjacent to an APA revealed mutations in known APA driver genes in 15, distinct from the mutation in the APA, with different mutations among different APCCs within the same gland. Besides mutations in *CACNA1D* and *ATP1A1*, interestingly, mutations in *KCNJ5*, previously considered rare or absent in APCCs, but also mutations in *CACNA1H*, *PRKACA*, and *CTNNB1* were observed.[18] Taken together, these results suggest that somatic mutations causing autonomous aldosterone production frequently occur in human adrenal glands. They contribute to variable degrees of autonomous aldosterone secretion.

IMPACT OF SOMATIC MUTATIONS ON PROLIFERATION AND SECOND-HIT HYPOTHESIS

A controversy in the field has been whether mutations in aldosterone-driver genes are sufficient only to cause increased/at least partially autonomous aldosterone production or also increased proliferation. In the APCC model, a somatic mutation in a single cell is assumed to cause increased aldosterone production and proliferation, leading to an aldosterone-producing micronodule, aldosterone-producing nodule, and eventually APA. In the alternative concept of a second-hit hypothesis, genetic or environmental factors cause proliferation, with additional somatic mutations leading to APA formation.[49] Whether *KCNJ5* mutations cause proliferation has been debated. The overexpression of mutant *KCNJ5* in cultured cells increases lethality rather than proliferation, likely due to massive Na^+ influx.[50,51] In vivo, however, *KCNJ5*-positive tumors show particularly low KCNJ5 expression.[52] Evidence from germline *KCNJ5* mutations (see below) and, specifically individuals with mosaicism for *KCNJ5* mutations suggest that these mutations either increase proliferation or block transdifferentiation, leading to hyperplasia in vivo.[50,53,54] APA-associated *ATPA1A1* mutations would likely be lethal if present in the germline. However, a recent study provided evidence for proliferative effects of an *ATPA1A* mutation in the HAC15 adrenocortical cancer cell line in vitro, an effect that was serum dependent.[21] Evidence for the second-hit hypothesis includes an individual with bilateral macronodular adrenal hyperplasia due to familial adenomatous polyposis with *APC* mutation and *KCNJ5*-positive APA.[55] Zona glomerulosa hyperplasia but also increased nodulation and decreased vascularity and expression of stem/progenitor cell markers in areas surrounding APAs has also been suggested to support the second-hit hypothesis,[56,57] however, such lesions typically carry independent somatic mutations.[18]

GERMLINE MUTATIONS IN FH

Genetic testing for FH subtypes (Table 2) is recommended in affected individuals with early onset and a positive family history of PA.[58] Familial aggregation of PA was first described in 1966 in a father and a son whose phenotype was relieved by administration of dexamethasone (glucocorticoid-remediable aldosteronism, later also FH-I).[59] This autosomal-dominant disorder is characterized by early-onset hypertension, often with a positive family history; hemorrhagic stroke and ruptured intracranial aneurysm are common in individuals without appropriate therapy.[60] Incomplete penetrance or mild forms have been reported.[61,62] In one study of 300 consecutive PA patients, 2 had FH-I. FH-I is caused by a hybrid gene that occurs due to a recombination event between *CYP11B1* (11β-hydroxylase, involved in cortisol synthesis under the control of adrenocorticotropic hormone) and *CYP11B2* (aldosterone synthase). This leads to ectopic expression of aldosterone synthase in the zona fasciculata under the control of adrenocorticotropic hormone and the formation of hybrid steroids 18-oxocortisol and 18-hydroxycortisol,[63] indicating the abnormal colocalization of enzymes involved in aldosterone and cortisol synthesis. Treatment with glucocorticoids suppresses adrenocorticotropic hormone and, sometimes in

Table 2. FH Subforms

Subform	Disease gene	Specific features	Therapy
FH-I	*CYP11B1/CYP11B2*	Responds to dexamethasone, hybrid steroids	Dexamethasone, MRA
FH-II	*CLCN2*	None	MRA
FH-III	*KCNJ5*	Variable hyperplasia, hybrid steroids	MRA, bilateral adrenalectomy
FH-IV	*CACNA1H*	None	MRA
PASNA syndrome	*CACNA1D*	Seizures, neurological abnormalities, heart defects, transient hypoglycemia (variable)	MRA, calcium antagonists (?)

FH indicates familial hyperaldosteronism; MRA, mineralocorticoid receptor antagonist; and PASNA, primary aldosteronism, seizures and neurological abnormalities.

combination with mineralocorticoid receptor antagonists, normalizes aldosterone and blood pressure. Unlike other FH genes, the chimeric *CYP11B1/2* gene has not been found in APAs.[64]

FH-II initially referred to all non-FH-I cases of FH.[65] An often-quoted prevalence of 6% in PA[66] is likely a considerable overestimate due to chance associations of sporadic PA cases in families. Because a heterozygous R172Q *CLCN2* mutation was discovered in a family from the original report,[65] FH-II now denotes individuals with germline mutations in the *CLCN2*.[67] Mutations were found in 7 additional families, among them 2 de novo. FH-II patients typically had early-onset hypertension with elevated aldosterone/renin ratios, with age at diagnosis typically before age 20 years, although, as in FH-I, incomplete penetrance was reported.[67] Hypertension responded to therapy with mineralocorticoid receptor antagonists or other antihypertensive agents. Another de novo germline N-terminal mutation was reported in a patient diagnosed with PA at age 9 years.[68] The affected girl showed severe hypertension and persistent hypokalemia and responded to medical therapy. Due to a positive dexamethasone suppression test, she was initially suspected to have glucocorticoid-remediable aldosteronism, but genetic analysis for the chimeric *CYP11B1/2* gene was negative. ClC-2, the chloride channel encoded by *CLCN2*, mediates net efflux of chloride in the zona glomerulosa.[67] *CLCN2* mutations lead to increased chloride efflux, cellular depolarization, calcium influx, and aldosterone production (Figure 1).[67,68]

FH-III refers to patients with heterozygous germline mutations in the *KCNJ5* gene. The initially published kindred showed severe therapy-resistant PA in childhood. Hybrid steroids were even higher than in FH-I, but treatment with dexamethasone failed to normalize blood pressure or serum aldosterone.[69] Severely enlarged adrenal glands were found upon bilateral adrenalectomy, with a large zone of lipid-laden cells that expressed aldosterone synthase and enzymes involved in cortisol synthesis, explaining hybrid steroid synthesis.[69,70] Genetic analysis identified a KCNJ5 T158A mutation.[6] Additional kindreds, often with bilateral adrenal hyperplasia, were subsequently identified, for example, patients with G151R mutation that is common as somatic mutation in APAs.[50] Interestingly, patients with another mutation, G151E, do not show massive hyperplasia and can be treated medically[50,71]; this mutation appears to cause extreme Na^+ influx that may interfere with cellular survival. This mutation does not occur in APAs, but, interestingly, has been identified in an APCC.[18]

FH-IV denotes patients with heterozygous germline mutations in the *CACNA1H* gene. One recurrent identical mutation, M1549V, was identified in 5 families with early-onset PA without other remarkable characteristics.[72] An additional early-onset case with de novo M1549I mutation had PA and multiplex developmental disorder. The pathogenicity of additional variants is less certain.[73,74] *CACNA1H* mutations lead to gain of channel function, with increased calcium influx and aldosterone production.[72,75]

A complex syndrome comprising PA, seizures, and neurological abnormalities was described in 2 individuals with de novo heterozygous gain-of-function *CACNA1D* mutations.[16] Both were diagnosed with cerebral palsy. Variable associated abnormalities included transient hypoglycemia and cardiac defects. Expression of the channel in several other organs, including brain, pancreas, and heart, likely accounts for associated abnormalities; PA is variable.[76–78] Several individuals with de novo *CACNA1D* mutations have been diagnosed with autism spectrum disorder without major endocrine abnormalities.[79–81]

Finally, any roles of *ARMC5* and phosphodiesterase germline variants in PA remain to be confirmed.[82,83] ATPase mutations have not been reported in FH, suggesting incompatibility of such germline mutations with survival.

GENETIC MOUSE MODELS OF PA

Several mouse models of human PA have been generated. A mouse model with transgenic expression of human *CYP11B2* under the control of the human *CYP11B1* promoter (resembling FH-I), shows elevated aldosterone levels and hypertension on a high-salt diet.[84] A knockin mouse ($Clcn2^{R180Q/+}$) models the commonest FH-II mutation, with normal adrenal weight and morphology, mildly elevated aldosterone levels and mildly elevated blood pressure. Intracellular Ca^{2+} oscillatory

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activity in the adrenal zona glomerulosa is elevated.[85] A second model carries a gain-of-function N-terminal deletion of 8 amino acids. In the homozygous state, the zona glomerulosa cells in this model are depolarized, with increased intracellular Ca^{2+} levels, elevated aldosterone and decreased renin, elevated blood pressure, hypokalemia, and moderate albuminuria.[86] *Kcnj5* is not expressed in rodent adrenal glomerulosa.[87] A mouse model expressing wild type or mutant human *KCNJ5* under the *Akr1b7* promoter, reported only as an abstract, appears to lack adrenal hypertrophy.[88] A knockin mouse with *Cacna1h*$^{M1560V/+}$ mutation (FH-IV model) shows normal adrenal morphology, elevated adrenal *Cyp11b2* expression and elevated blood pressure. Adrenals from these animals have elevated baseline and peak intracellular Ca^{2+} levels.[89] Last, a transgenic mouse with adrenocortical expression of a Gq-coupled designer receptor develops disorganization of adrenal zonation and hyperaldosteronism,[90] as in *GNAQ* mutations in APAs. Additional models with mutations in KCNK3 and KCNK9 potassium channels or cryptochrome genes have been reviewed elsewhere.[91]

DIAGNOSTIC AND THERAPEUTIC ADVANCES BASED ON GENETIC DISCOVERIES

Of particular interest regarding clinical diagnosis are APAs with *KCNJ5* mutations. Beyond their high prevalence, they are associated with early diagnosis, high aldosterone levels, large tumors,[13] high cure rates,[92] and lower precontrast Hounsfield units on computed tomography[93] due to lipid-rich fasciculata-like tumor cells.[17,93,94] Like FH-III patients, patients with *KCNJ5*-positive APAs have elevated concentrations of hybrid steroids,[95] allowing prediction of unilateral disease based on steroid profiling.[92] This, together with imaging, may in the future help to bypass adrenal venous sampling in individuals with *KCNJ5* mutations.

In addition, blockers of mutant KCNJ5 channels could serve as diagnostic or therapeutic tools in PA. A drop in blood pressure or aldosterone in response to short-term treatment may help to identify KCNJ5-positive tumors. Long-term therapy might lead to tumor shrinkage.[96] Sensitivity of mutant KCNJ5 channels to blockers of Na^+ and Ca^{2+} transporting proteins such as verapamil (for G151R and L168R) and amiloride (only L168R analyzed) has been reported.[7] In a high-throughput screen, macrolide antibiotics such as roxithromycin and clarithromycin were identified as specific blockers of both G151R and L168R, but not WT KCNJ5. Similarly, the nonantibiotic macrolide motilin receptor agonist idremcinal and synthesized macrolide derivatives without antibiotic or motilide activity specifically inhibited mutant KCNJ5 channels.[96] Macrolide compounds decrease the excessive aldosterone production associated with expression of mutant KCNJ5 channels in an aldosterone-producing cell line in vitro[96] and in APA cells carrying *KCNJ5* mutations ex vivo.[97] Results of proof-of-concept studies in humans evaluating macrolides for the diagnosis of PA are pending.[98]

The finding that intracellular calcium is the key signal for aldosterone also raises the question whether calcium channel blockers could be used to inhibit aldosterone production. Therapeutic use of calcium channel blockers in PA was considered long before the discovery of calcium channel mutations[99] but approved compounds target vascular calcium channels, and their antihypertensive effect is mostly aldosterone-independent.[5] Normal aldosterone values in *Cacna1h* knockout mice[89,100] and the phenotype of *Cacna1d* knockout mice (deafness and sinoatrial node dysfunction with bradycardia and arrhythmia)[101] argue against these channels as therapeutic targets.

Finally, the finding of a likely genetic continuum between hyperplastic lesions and APAs, combined with the availability of highly specific *CYP11B2* antibodies, has led to a new histopathology consensus for the description of unilateral disease[36] (Figure 2).

SUMMARY AND FUTURE PERSPECTIVES

Genetic studies, published over the last decade, have—perhaps unexpectedly—established PA as a largely genetic disorder. The initial discovery that APAs are due to somatic mutations, mostly in ion channels and pumps, was followed by the finding that similar mutations are highly prevalent in what are now called aldosterone-producing (micro)nodules, particularly in bilateral disease. There is probably a biologic continuum between somatic mutations in single cells in otherwise healthy individuals, (micro)nodule formation and eventually APA development,[102] making cutoffs for diagnosis of PA somewhat arbitrary. Indeed, there is clinical evidence that subclinical PA is present in normotensive individuals and is associated with an increased risk for the development of hypertension.[103] One study, based on urinary aldosterone and oral sodium suppression tests, found a continuum of renin-independent aldosterone production, paralleling the severity of hypertension.[4] Small lesions could be the histopathologic correlate of subclinical PA with low renin,[103] in particular in individuals with resistant hypertension, as suggested by the good response to spironolactone in the PATHWAY-2 study.[104]

The recognition of this biological continuum also to some extent questions the distinction between unilateral and bilateral disease; hyperplastic or nodular areas are common in APA patients and can lead to contralateral recurrence.[105] In addition, asymmetrical aldosterone production with bilateral lesions can prevent cure after adrenalectomy.[106]

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Unanswered questions regarding the genetics and pathophysiology of PA include:

In which cell(s) does the initial somatic mutation occur—stem/progenitor cells[107] and/or glomerulosa cells? What are the mechanisms underlying proliferation of aldosterone-producing lesions? Are there additional mutations to discover? Additional disease genes will explain only small fractions of APAs, but it is tempting to speculate that aldosterone-producing micronodules could carry mutations without major effects on proliferation, similar to *GNA11/Q* mutations discussed above, and that more FH cases will be solved. Last, how these discoveries will translate into clinical application is a major unresolved question. The major challenges in PA clinical care are low rates of screening and diagnosis,[108] which, beyond a lack of awareness, are due to complex, expensive and often invasive screening procedures[5] that are unavailable outside tertiary care centers. Furthermore, medical treatment options are scarce and include spironolactone, which is associated with severe side effects at higher doses, and eplerenone, which is not approved for the therapy of hypertension in many countries. Research discoveries have contributed to raised PA awareness in the academic world, but improved care will require less complicated diagnostic algorithms and new treatment options. Steroid profiling and drugs targeting mutant ion channels as discussed above are first steps. It is also conceivable that tracers could in the future recognize mutant surface proteins in imaging, but this again would likely be limited to large centers. The insight that PA, clinical or subclinical, occurs across normotension and all stages of hypertension suggests that simple screening tests, such as that for plasma renin should be performed in most hypertensive patients.

ARTICLE INFORMATION

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Rockefeller University holds a patent Compositions and methods for diagnosing and treating diseases and disorders associated with mutant KCNJ5 listing U.I. Scholl as an inventor.

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