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Primary aldosteronism (PA) was first reported by Jerome Conn from the University of Michigan[1] and is characterized by hypertension with excessive, autonomous production of

aldosterone relative to suppressed renin levels.[2] Once thought to be a rare condition, it is now known to be the most common form of endocrine hypertension. The overall prevalence in the general hypertensive population was recently demonstrated to be 5.9% increasing with the severity of hypertension (3.9-11.8%, grade I-III hypertension)[3] to as much as 20% in patients with resistant hypertension.[4] An early diagnosis followed by correct treatment has an important impact on clinical outcome because it can reverse the increased risk of cardiovascular and cerebrovascular complications associated with PA.[5,6]

PA can be familial caused by either of the 4 currently recognized subtypes (Familial Hyperaldosteronism types I-IV) or arise sporadically in which the aldosterone excess is unilateral [usually caused by an aldosterone-producing adenoma (APA)] or bilateral [usually caused by bilateral adrenal hyperplasia (BAH)]. The unilateral and bilateral forms of PA should be differentiated because a patient with unilateral APA is most appropriately treated by adrenalectomy,[2] which normalizes or improves blood pressure in 84% of cases,[7] and a patient with BAH is treated in almost all cases with a mineralocorticoid antagonist.[2] Details for the diagnosis of PA and recommendations for the protocol and interpretation of adrenal venous sampling are described in detail elsewhere.[8-10]

Until 2011, the genetic basis of PA was largely unknown when in an unprecedented development germline mutations causing new familial forms of PA and somatic mutations driving aldosterone excess in the majority of sporadic unilateral APAs were uncovered. The development of specific monoclonal antibodies to the highly homologous human CYP11B2 and CYP11B1 enzymes identified novel histological structures and evolved our understanding of the pathophysiology of aldosterone overproduction. Herein we comprehensively cover what is old and what is new in the rapidly developing field of the

pathogenesis of PA and focus on discoveries from genetics to histopathology of the last 3 years.

Familial forms of hyperaldosteronism

Familial hyperaldosteronism is estimated to account for less than 6% of all referred cases of PA.[11] Familial hyperaldosteronism type I (FH type I, previously referred to as glucocorticoid-remediable aldosteronism) was the first genetically-defined form of human hypertension.[12] The molecular basis of FH type II is unknown whereas the genetic determinants of FH types III and IV were identified with the advent of next-generation sequencing methods. The genetic basis and clinical phenotype of each of the known familial forms of PA are summarized in table 1.

Familial hyperaldosteronism type I

Familial hyperaldosteronism type I (FH type I) accounts for less than 1% of diagnosed cases of PA.[11] This disorder displays a high degree of inter- and intra-familial phenotypic variability but is usually an early-onset form of hypertension associated with a high incidence of stroke. FH type I is caused by a chimaeric gene that comprises the promoter of the 11β-hydroxylase gene (*CYP11B1*) fused to the coding region of the aldosterone synthase gene (*CYP11B2*).[12] Gene expression of the *CYP11B1*/*CYP11B2* chimera is therefore regulated by adrenocorticotropic hormone, as for *CYP11B1*, and not by angiotensin II like *CYP11B2*. Unlike wild-type *CYP11B2*, the expression of aldosterone synthase from the chimaeric gene is suppressed by glucocorticoids, such as dexamethasone, because they suppress adrenocorticotropic hormone. *CYP11B2* expression is normally restricted to the *zona glomerulosa* (zG) whereas patients with FH type I express the chimaeric gene, and the coding region of aldosterone synthase in the *zona fasciculata* (zF).[12] Aldosterone synthase activity is

thus co-localized with cortisol that can be metabolized further by the 18-hydroxylase and 18-oxidase activities of aldosterone synthase to overproduce the hybrid steroids 18-hydroxycortisol and 18-oxocortisol as is characteristic of this condition.[12]

Familial hyperaldosteronism type II

The clinical and biochemical phenotype of FH type II is indistinguishable from sporadic PA[11] and is not suppressible by glucocorticoids.[13] FH type II is diagnosed when at least two family members present with PA who are negative for the chimeric gene which causes FH type I, and for FH type III and FH type IV germline mutations (described in more detail later). The prevalence of FH type II is difficult to evaluate considering the high prevalence of hypertension in the adult population[14] and the prevalence of PA in the general population with hypertension[3] and therefore the chance finding of 2 or more adult family members with a diagnosis of PA should be considered. Nonetheless, an estimated prevalence has been reported as 3.2-5.5% of all cases of PA.[10,11,15] Although an association of FH type II with a genetic locus on chromosomal region 7p22 has been reported for some affected families,[16-18] no mutations in candidate genes have been identified to date despite direct Sanger sequencing of multiple candidate genes and next generation sequencing of the linked locus and therefore the genetic basis of FH type II remains unknown.[10]

Familial hyperaldosteronism type III associated with *KCNJ5* mutations

Familial hyperaldosteronism type III (FH type III) was first described by Geller et al.[19] in 3 family members, the male index case and his 2 daughters, who presented with hyperaldosteronism and severe hypertension from childhood with hypokalemia and high levels of the hybrid steroids 18-oxocortisol and 18-hydroxycortisol.[19] The index case originally came to medical attention as a 5 year old some 50 years previously; treatment

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required bilateral adrenalectomy at 9 years of age which normalized blood pressure and the serum K^+ concentration within 1 week of surgery.[19,20] The daughters were treated similarly: the resected adrenals from all 3 patients were greatly enlarged and showed extensive hyperplasia of the zF.

The arrival of next-generation sequencing technology later revealed the presence of a germline heterozygous mutation in the *KCNJ5* gene (encoding the G protein-activated inward rectifier potassium channel 4, GIRK4) that associated with the disorder in both father and daughters.[21] The mutated K^+ channel GIRK4 p.Thr158Ala) displayed a loss of selectivity for K^+ and an increase in Na^+ conductance that depolarizes the cell membrane.[21] In zG cells, membrane depolarization causes the opening of voltage-dependent Ca^{2+} channels and Ca^{2+} influx which activates Ca^{2+} signaling pathways and leads to an increase in aldosterone production.[22,23] Since this original case, other families with FH type III and patients with germline *KCNJ5* mutations have been described with a clinical phenotype ranging from mild to severe (Table 1; Figure 1).

Familial hyperaldosteronism type IV associated with *CACNA1H* mutations

A new familial form of early-onset hyperaldosteronism has been described recently (Familial hyperaldosteronism type IV, FH type IV) and is caused by a germline mutation in the *CACNA1H* gene that encodes the T-type (low voltage activated) Ca^{2+} channel $Ca_v3.2$ ($Ca_v3.2$ p.Met1549Val).[24] Gain-of-function mutations in *CACNA1H* have previously been associated with epilepsy, seizures and autism.[25,26] Scholl et al. reported 5 unrelated individuals with early-onset PA with identical Met1549Val mutations.[24] Genetic analyses of the patients' families showed that 1 mutation occurred *de novo* and 4 occurred in the germline. In some kindreds, the $Ca_v3.2$ p.Met1549Val variant segregated with the presentation of early-onset PA with severe hypertension; in another, the phenotype

displayed an incomplete penetrance with an intra-familial clinical presentation that varied from asymptomatic adults to the early-onset phenotype.[24,27] This apparent incongruence with the autosomal dominant pattern of inheritance is presumably due to differences in age or to other genetic and environmental factors.[27]

The physiology of the Ca$_v$3.2 p.Met1549Val channel was studied by patch clamp recordings of whole human embryonic kidney 293T cells expressing either the mutated channel or the wild-type Ca$_v$3.2 channel. Ca$_v$3.2 p.Met1549Val displayed a markedly slower channel inactivation and a shift of activation to less depolarizing potentials.[24] The Met1549Val mutated form of Ca$_v$3.2 thus permits an increased Ca^{2+} influx that in the steroidogenic cell lines NCI H295R and HAC 15, as for mutated GIRK4 channels that likewise cause an influx of Ca^{2+}, result in an increased expression of the *CYP11B2* gene and aldosterone production.[28]

A *de novo* mutation affecting the same residue, Ca$_v$3.2 p.Met1549Ile, was identified shortly after in a study that reported 4 different germline *CACNA1H* mutations.[29] The Met1549Ile substitution was associated with early onset PA and multiplex developmental disorder (Table 1). The same study also reported Ca$_v$3.2 p.Ser196Leu and p.Pro2083Leu mutations associated with familial hyperaldosteronism and found a germline Ca$_v$3.2 p.Val1951Glu mutation in a patient with sporadic APA who was successfully treated by unilateral adrenalectomy.[29]

Germline *CACNA1D* mutations associated with primary aldosteronism with seizures and neurologic abnormalities (PASNA)

In an attempt to identify new germline mutations associated with PA, candidate genes with recurring somatic APA mutations were sequenced in 100 patients with early-onset PA.[30] This approach identified 2 patients with *de novo* mutations in *CACNA1D* that encodes Ca$_v$1.3, a subunit of an L-type (high-voltage activated) Ca^{2+} channel.[30] The two different mutations- Ca$_v$1.3 p.Gly403Asp and p.Ile770Met- affect amino acid residues of Ca$_v$1.3 that are also substituted in sporadic APA (described later) with the latter identical to a somatic APA mutation. Unlike the somatic variants, the germline mutations cause an extreme phenotype, with early onset severe hypertension and hyperaldosteronism and a diagnosis in both patients of neurologic abnormalities that include cerebral palsy and seizures referred to as PASNA.[27,30]

Missense *de novo* mutations in Ca$_v$1.3 p.Ala749Gly, p.Gly407Arg and p.Val401Leu have also been identified in patients with autism spectrum disorder. These patients additionally presented with epilepsy and had a clinical phenotype resembling that of patients with PASNA.[31,32] Characterization of the electrophysiological effects incurred by the mutations was demonstrated by patch clamp recordings *in vitro*. All 3 mutations confer a gain-of-function phenotype with a predicted increase in Ca^{2+} inward currents[31,32] which would be expected to result in elevated plasma aldosterone concentrations in these patients but regrettably, these data are unavailable.

Germline *KCNJ5* mutations associated with sporadic primary aldosteronism

Germline *KCNJ5* mutations were identified in 3 of 251 patients with a florid form of sporadic BAH.[33] The mutations (p.Arg52His, p.Glu246Lys and p.Gly247Arg) were located at some distance from the selectivity filter of the GIRK4 K$^+$ channel. Despite this, the Arg52His and Glu246Lys mutations caused cell depolarization and increased aldosterone production *in*

vitro in contrast to the Gly247Arg mutation that had no functional effect. A rare nonsynonymous single-nucleotide polymorphism rs7102584 was also identified in 12 of 251 white Australasian patients (5%) with PA compared with 22 of 1075 individuals (2%) in the international 1000 genomes cohort. In the 12 patients with PA carrying the rs7102584 (p.Glu282Gln) polymorphism, 9 were diagnosed with BAH and 3 with APA.[33]

Sporadic forms of hyperaldosteronism caused by somatic mutations

Over half of APAs carry somatic gain-of-function mutations in genes encoding ion channels or transporters (*KCNJ5*, *CACNA1D*, *ATP1A1*, *ATP2B3*) that can account for excessive production of aldosterone. Somatic mutations have also been described in APA that are found in cortisol-producing adenomas (*CTNNB1*, *PRKACA* and *GNAS*) although *PRKACA* and *GNAS* mutations are rare in APA. The somatic APA mutations reported to date are listed in Table S1.

Somatic *KCNJ5* mutations in APA

The original somatic APA mutations described by Choi[21] in the *KCNJ5* gene cause GIRK4 p.Gly151Arg and p.Leu168Arg substitutions in or near the pore of the channel, respectively. Both mutations, as for others described subsequently, disturb the selectivity properties of the channel as for the p.Thr158Ala mutation associated with FH type III described above. At least 17 somatic APA *KCNJ5* mutations have been described to date (Fig. 1, Table S1), but the p.Gly151Arg and p.Leu168Arg substitutions predominate accounting for all 34% of APA with GIRK4 mutations of 380 APA recruited through the European Network for the Study of Adrenal Tumours[34] (ENS@T, http://www.ensat.org) and 47% of APA in an international series of 351 APA.[35] Somatic *KCNJ5* mutations in APA are more frequent in

women than in men and compared to non-carriers, are associated with a more pronounced hyperaldosteronism, a younger age and larger tumor size.[34,36]

The overall prevalence of APA with *KCNJ5* mutations was determined in a meta-analysis of 1636 patients as 43% (range 12-80%) with a higher reported frequency in Japan and China compared to European countries.[36] Because patients with APA carrying a *KCNJ5* mutation have higher concentrations of the aforementioned hybrid steroid 18-oxocortisol in peripheral blood,[37] the reported predominance of APA with a *KCNJ5* mutation in patients from Japan explains the utility of using 18-oxocortisol in peripheral plasma as a potential biomarker to differentiate patients with APA from those with BAH[38] whereas this is not useful in patients from Europe.[39] However, the measurement of a panel of 7 steroids, that includes the hybrid steroids 18-oxocortisol and 18-hydroxycortisol, in peripheral plasma by liquid chromatography-tandem mass spectrometry correctly classified 92% of 79 patients with APA according to genotype with a correct call for 26 of 27 patients with APA with a *KCNJ5* mutation.[37]

In a recent development, macrolide antibiotics were identified as selective inhibitors of GIRK4 p.Gly151Arg and p.Leu168Arg compared with the wild-type GIRK4 channel and the macrolide compound roxithromycin ablated *CYP11B2* gene expression and inhibited aldosterone production induced by the expression of either GIRK4 p.Gly151Arg or p.Leu168Arg in steroidogenic cell lines.[40] Macrolides have a potential use in the diagnosis of patients with an APA carrying a mutated GIRK4 channel by measuring drug-induced decreases in aldosterone concentrations- possibly in tandem with peripheral venous steroid profiling- and in their treatment.

Somatic *ATP1A1* and *ATP2B3* mutations in APA

Shortly following the discovery of *KCNJ5* mutations, somatic APA mutations were described in the *ATP1A1* and *ATP2B3* genes, encoding Na$^+$/K$^+$-ATPase 1 and Ca^{2+}-ATPase 3, respectively,[41,42] that combined account for 7% of APA in patients of European ancestry and can result in an increase in *CYP11B2* gene expression and aldosterone production[43,44] (Table S1 lists the *ATP1A1/ATP2B3* mutations described to date). APA mutations in ion transporters and channels were thought to share a common mechanism of activation of Ca^{2+} signaling, but two mutations in the Na$^+$/K$^+$-ATPase, p.Leu104Arg and p.Val332Gly, reportedly result in intracellular acidification, with no detectable increase in intracellular Ca^{2+} concentration, that causes the overproduction of aldosterone.[45] Germline mutations in ATPases have not been described to date presumably because they are incompatible with life.

Somatic *CACNA1D* mutations in APA

Somatic APA mutations in *CACNA1D* that cause substitutions at conserved sites of Ca$_v$1.3 were first described in two reports.[30,41] The mutations determine an impairment of the ion channel gating mechanism such that both activation and inactivation of the channel are affected and an increase in Ca^{2+} influx results.[30,41] The number of different somatic APA mutations in *CACNA1D* are numerous with at least 31 substitution mutations of 25 different amino acid residues reported (Table S1). Somatic *CACNA1D* mutations are associated with APA of a smaller size[41,44] and, with a reported prevalence of 9.3%, comprise the second largest group of APA after those with a *KCNJ5* mutation.[44] A highly selective antagonist of Ca$_v$1.3, 1-(3-chlorophenethyl)-3-cyclopentylpyrimidine-2,4,6-(1H,3H,5H)-trione has been developed as a potential novel therapeutic approach for Parkinson's disease, displaying 600-times selectivity over Ca$_v$1.2[46] and selective antagonism of Ca$_v$1.3 may be a target for the treatment of aldosterone overproduction in patients with PA.[47]

Somatic *CTNNB1* mutations in APA

The canonical Wnt (Wnt/β-catenin) pathway controls key developmental gene expression programs and constitutive activation of this pathway is involved in the pathogenesis of many human cancers. In the absence of extracellular Wnt ligands, glycogen synthase kinase 3β, as part of the multi-protein Axin complex, phosphorylates specific serine and threonine residues in the N-terminal region of β-catenin which is then degraded via the ubiquitin-proteosome pathway.[48] Binding to the Frizzled/LRP (lipoprotein receptor-related protein) receptor inhibits the Axin complex and β-catenin is stabilized, accumulates and translocates to the nucleus to activate LEF/TCF (T-cell factor/lymphoid enhancer factor) transcription factors thereby activating Wnt target gene expression programs.[48]

Activating somatic mutations in the *CTNNB1* gene, that encodes β-catenin, have been reported at a similar prevalence in both adrenocortical adenomas (27% of 26 tumors, with a higher proportion in non-functioning adenomas compared with cortisol-producing adenomas) and adrenocortical carcinomas (31% of 13 tumors).[49] Most mutations affected a phosphorylation site (Ser45) for glycogen synthase kinase 3β.

A high proportion of APAs (70%) display constitutive activation of Wnt/β-catenin signaling that is caused, at least partly, by a decrease in the expression of SFRP2 (Secreted Frizzled Related Protein 2) an endogenous inhibitor of Wnt/β-catenin signaling.[50] Somatic mutations in the *CTNNB1* gene have been identified in 2-5% of APA.[51,52] All mutations target exon 3 and almost all affect serine and threonine residues at the β-catenin N-terminus resulting in an accumulation of β-catenin. Therefore, β-catenin mutations are present in APA as in other adrenocortical tumors where they play a role in adrenocortical tumorigenesis.

Somatic APA *CTNNB1* mutations have also been described in 2 pregnant women and a woman post-menopause with highly up-regulated adrenal expression of luteinizing hormone/choriogonadotropin receptor and gonadotropin releasing hormone receptor

leading to the suggestion that pregnancy could trigger the overexpression of these gonadotropin receptors via a mechanism mediated by constitutive activation of β-catenin.[53]

Histopathology of aldosterone production

The development of specific monoclonal antibodies against the key steroidogenesis enzymes CYP11B2 and CYP11B1[54] has transformed the interpretation of the histopathology of PA and identified a new histological structure called the aldosterone-producing cell cluster (APCC).[55-58] APCCs are distinct clusters of zG cells identified by strong CYP11B2 immunostaining and an absence of CYP17 staining and are located in the subcapsular portion of the adrenal cortex that penetrates into the cortisol-producing cells of the zF.[54,59] At least 1 APCC was identified in 69% of an autopsy series of 127 normal adrenals[59] and 50% of 22 adrenals had an APCC in the tissue adjacent to an APA.[60] The physiology of aldosterone production changes with age with a transition from continuous expression of CYP11B2 in the zG in the young to a discontinuous expression with an increased expression of APCCs in older subjects.[59] The adrenals of children aged below 12 years show a conventional zonation of the adrenal cortex without APCCs[61] with the earliest documented case of an APCC occurring in a 12 year old male.[59] Transcriptome analysis of APCC and the 3 zones of the adrenal cortex demonstrated the highest expression of *CYP11B2* in APCCs and the similarity of the APCC and the zG transcriptomes.[62]

A high proportion of APCCs in the normal adrenal glands from kidney donors carry mutations in genes that are mutated in APAs (35% of 23 APCCs have mutations in *CACNA1D* or *ATP1A1*) leading to the proposal that APCCs are precursors of APAs.[62] Although APCC with *KCNJ5* mutations, the most frequently recurring mutation in APAs, were not identified, a histological structure with a transitional phenotype from APCC (subcapsular region) to microAPA (outer region), called APCC-to-APA transitional lesions, have been described in

which *KCNJ5* mutations were identified in the microAPA portion of the larger lesions (>3mm). This supports the hypothesis of the APCC to microAPA transition and suggests that *KCNJ5* mutations could occur as a second genetic hit.[61,63] The number of the reported transitional lesions are however few with 6 known cases to date.

If present adjacent to an APA, the production of aldosterone from APCCs is presumably biochemically autonomous given the suppressed renin-angiotensin-system, but their contribution to aldosterone excess is likely to be negligible, since the APA is probably the main source of aldosterone production. However, in the normal adrenal cortex of elderly persons APCCs may be a driver of hypertension and contribute substantially to age-related cardiovascular risk.[59]

APA display different patterns of CYP11B2 and CYP11B1 immunostaining that can be homogeneous and dominant for CYP11B2, heterogeneous for CYP11B2 and interspersed with patches of CYP11B1, or CYP11B2-negative.[56,60] CYP11B2 immunoreactivity is significantly higher in microadenomas than in macroadenomas and patients with the smaller tumors display disproportionately higher levels of aldosterone production[64] and CYP11B1 immunostaining is associated with glucocorticoid overproduction in some APA.[65]

The adrenal tissue adjacent to an APA can display micronodules or macronodules that can carry a somatic mutation of a different genotype to the APA.[58,66,67] In some infrequent cases, a secondary nodule is the source of the excess aldosterone production and in these circumstances the adenoma is usually CYP11B2-negative.[56,68]

Conclusion

Advances in sequencing technologies and immunohistochemistry have advanced our understanding of the pathogenesis of PA and shown that the pathological production of

aldosterone in this most common form of endocrine hypertension is not as straightforward as classically contended.

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References

1) Conn, JW. Part I. Painting background. Part II. Primary aldosteronism, a new clinical syndrome. *J. Lab Clin Med*. 1955;45:3-17.

2) Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M & Young WF, Jr. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2016;101:1889-1916.

3) Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, Gabetti L, Mengozzi G, Williams TA, Rabbia F, Veglio F, Mulatero P. Prevalence and Clinical Manifestations of

Primary Aldosteronism Encountered in Primary Care Practice. *J Am Coll Cardiol.* 2017;69:1811-1820.

4) Douma S, Petidis K, Doumas M, Papaefthimiou P, Triantafyllou A, Kartali N, Papadopoulos N, Vogiatzis K, Zamboulis C. Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. *Lancet.* 2008;371:1921-1926.

5) Mulatero P, Monticone S, Bertello C, Viola A, Tizzani D, Iannaccone A, Crudo V, Burrello J, Milan A, Rabbia F, Veglio F. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. *J Clin Endocrinol Metab.* 2013;98:4826-4833.

6) Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular complications associated with primary aldosteronism: a controlled cross-sectional study. *Hypertension.* 2013;62:331-336.

7) Williams TA, Lenders JWM, Mulatero P et al. 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.

8) Monticone S, Viola A, Rossato D, Veglio F, Reincke M, Gomez-Sanchez C, Mulatero P. Adrenal vein sampling in primary aldosteronism: towards a standardised protocol. *Lancet Diabetes Endocrinol.* 2015;3:296-303.

9) Rossi GP, Auchus RJ, Brown M, Lenders JW, Naruse M, Plouin PF, Satoh F, Young WF Jr. An expert consensus statement on use of adrenal vein sampling for the subtyping of primary aldosteronism. *Hypertension.* 2014;63:151-160.

10) Stowasser M, Gordon RD. Primary Aldosteronism: Changing Definitions and New Concepts of Physiology and Pathophysiology Both Inside and Outside the Kidney. *Physiol Rev.* 2016;96:1327-1384.

11) Mulatero P, Tizzani D, Viola A, Bertello C, Monticone S, Mengozzi G, Schiavone D, Williams TA, Einaudi S, La Grotta A, Rabbia F, Veglio F. Prevalence and characteristics of

familial hyperaldosteronism: the PATOGEN study (Primary Aldosteronism in TOrino-GENetic forms). *Hypertension.* 2011;58:797-803.

12) Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, Ulick S, Lalouel JM. A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature*. 1992:355;262-265.

13) Stowasser M, Gordon RD. Primary aldosteronism: learning from the study of familial varieties. *J Hypertens.* 2000;18:1165-1176.

14) Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet.* 2005;365:217-223.

15) Stowasser M, Pimenta E, Gordon RD. Familial or genetic primary aldosteronism and Gordon syndrome. *Endocrinol Metab Clin North Am.* 2011;40:343-368.

16) Lafferty AR, Torpy DJ, Stowasser M, Taymans SE, Lin JP, Huggard P, Gordon RD, Stratakis CA. A novel genetic locus for low renin hypertension: familial hyperaldosteronism type II maps to chromosome 7 (7p22). *J Med Genet.* 2000;37:831-835.

17) So A, Duffy DL, Gordon RD, Jeske YW, Lin-Su K, New MI, Stowasser M. Familial hyperaldosteronism type II is linked to the chromosome 7p22 region but also shows predicted heterogeneity. *J Hypertens.* 2005;23:1477-1484.

18) Sukor N, Mulatero P, Gordon RD, So A, Duffy D, Bertello C, Kelemen L, Jeske Y, Veglio F, Stowasser M. Further evidence for linkage of familial hyperaldosteronism type II at chromosome 7p22 in Italian as well as Australian and South American families. *J Hypertens.* 2008;26:1577-1582.

19) Geller DS, Zhang J, Wisgerhof MV, Shackleton C, Kashgarian M, Lifton RP. A novel form of human mendelian hypertension featuring nonglucocorticoid-remediable aldosteronism. *J Clin Endocrinol Metab.* 2008;93:3117-3123.

20) Therien B, Mellinger RC, Caldwell JR, Howard PJ. Primary aldosteronism due to adrenal hyperplasia; occurrence in a boy aged 10 years. *AMA J Dis Child* 1959;98:90-99.

21) Choi M, Scholl UI, Yue P et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science.* 2011;331:768-772.

22) Hattangady NG, Olala LO, Bollag WB, Rainey WE. Acute and chronic regulation of aldosterone production. *Mol Cell Endocrinol.* 2012;350:151-162.

23) Oki K, Plonczynski MW, Luis Lam M, Gomez-Sanchez EP, Gomez-Sanchez CE. Potassium channel mutant KCNJ5 T158A expression in HAC-15 cells increases aldosterone synthesis. *Endocrinology.* 2012:153;1774-1782.

24) Scholl UI, Stölting G, Nelson-Williams C et al. Recurrent gain of function mutation in calcium channel CACNA1H causes early-onset hypertension with primary aldosteronism. *Elife.* 2015;4:e06315.

25) Eckle VS, Shcheglovitov A, Vitko I, Dey D, Yap CC, Winckler B, Perez-Reyes E. Mechanisms by which a CACNA1H mutation in epilepsy patients increases seizure susceptibility. *J Physiol.* 2014;592: 795-809.

26) Splawski I, Yoo DS, Stotz SC, Cherry A, Clapham DE, Keating MT. CACNA1H mutations in autism spectrum disorders. *J Biol Chem.* 2006;281:22085-22091.

27) Korah HE, Scholl UI. An Update on Familial Hyperaldosteronism. *Horm Metab Res*. 2015;47:941-946.

28) 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.

29) Daniil G, Fernandes-Rosa FL, Chemin J, Blesneac I, Beltrand J, Polak M, Jeunemaitre X, Boulkroun S, Amar L, Strom TM, Lory P, Zennaro MC. CACNA1H Mutations Are Associated With Different Forms of Primary Aldosteronism. *EBioMedicine.* 2016;13:225-236.

30) Scholl UI, Goh G, Stolting G et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet.* 2013:45;1050-1054.

31) Pinggera A, Lieb A, Benedetti B, Lampert M, Monteleone S, Liedl KR, Tuluc P, Striessnig J.CACNA1D de novo mutations in autism spectrum disorders activate Cav1.3 L-type calcium channels. *Biol Psychiatry.* 2015;77:816-822.

32) Pinggera A, Mackenroth L, Rump A, Schallner J, Beleggia F, Wollnik B, Striessnig J. New gain-of-function mutation shows *CACNA1D* as recurrently mutated gene in autism spectrum disorders and epilepsy. *Hum Mol Genet.* 2017;26:2923-2932.

33) Murthy M, Xu S, Massimo G, Wolley M, Gordon RD, Stowasser M, O'Shaughnessy KM. Role for germline mutations and a rare coding single nucleotide polymorphism within the KCNJ5 potassium channel in a large cohort of sporadic cases of primary aldosteronism. *Hypertension.* 2014;63:783-789.

34) Boulkroun S, Beuschlein F, Rossi GP et al. Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism. *Hypertension.* 2012;59:592-598.

35) Åkerström T, Crona J, Delgado Verdugo A 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.

36) 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.

37) Williams TA, Peitzsch M, Dietz AS, Dekkers T, Bidlingmaier M, Riester A, Treitl M, Rhayem Y, Beuschlein F, Lenders JW, Deinum J, Eisenhofer G, Reincke M. Genotype-Specific Steroid Profiles Associated With Aldosterone-Producing Adenomas. *Hypertension.* 2016;67:139-145.

38) Nakamura Y, Satoh F, Morimoto R, Kudo M, Takase K, Gomez-Sanchez CE, Honma S, Okuyama M, Yamashita K, Rainey WE, Sasano H, Ito S. 18-oxocortisol measurement in adrenal vein sampling as a biomarker for subclassifying primary aldosteronism. *J Clin Endocrinol Metab*. 2011;96:E1272-E1278.

39) Eisenhofer G, Dekkers T, Peitzsch M, Dietz AS, Bidlingmaier M, Treitl M, Williams TA, Bornstein SR, Haase M, Rump LC, Willenberg HS, Beuschlein F, Deinum J, Lenders JW, Reincke M. Mass Spectrometry-Based Adrenal and Peripheral Venous Steroid Profiling for Subtyping Primary Aldosteronism. *Clin Chem*. 2016;62:514-524.

40) Scholl UI, Abriola L, Zhang C, Reimer EN, Plummer M, Kazmierczak BI, Zhang J, Hoyer D, Merkel JS, Wang W, Lifton RP. Macrolides selectively inhibit mutant KCNJ5 potassium channels that cause aldosterone-producing adenoma. *J Clin Invest.* 2017;127:2739-2750.

41) Beuschlein F, Boulkroun S, Osswald A et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet.* 2013;45:440-444.

42) Azizan EA, Poulsen H, Tuluc P et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet.* 2013;45:1055-1060.

43) Williams TA, Monticone S, Schack VR et al. Somatic ATP1A1, ATP2B3, and KCNJ5 mutations in aldosterone-producing adenomas. *Hypertension.* 2014;63:188-195.

44) Fernandes-Rosa FL, Williams TA, Riester A et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension.* 2014;64;354-361.

45) Stindl J, Tauber P, Sterner C, Tegtmeier I, Warth R, Bandulik S. Pathogenesis of Adrenal

Aldosterone-Producing Adenomas Carrying Mutations of the Na(+)/K(+)-ATPase.
Endocrinology. 2015;156:4582-4591.

46) Kang S, Cooper G, Dunne SF, Dusel B, Luan CH, Surmeier DJ, Silverman RB. CaV1.3-selective L-type calcium channel antagonists as potential new therapeutics for Parkinson's disease. *Nat Commun*. 2012;3:1146.

47) Xie CB, Shaikh LH, Garg S, Tanriver G, Teo AE, Zhou J, Maniero C, Zhao W, Kang S, Silverman RB, Azizan EA, Brown MJ. Regulation of aldosterone secretion by Cav1.3. *Sci Rep*. 2016;6:24697.

48) MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell.* 2009;17:9-26.

49) Tissier F, Cavard C, Groussin L, Perlemoine K, Fumey G, Hagneré AM, René-Corail F, Jullian E, Gicquel C, Bertagna X, Vacher-Lavenu MC, Perret C, Bertherat J. Mutations of beta-catenin in adrenocortical tumors: activation of the Wnt signaling pathway is a frequent event in both benign and malignant adrenocortical tumors. *Cancer Res.* 2005;65:7622-7627.

50) Berthon A, Drelon C, Ragazzon B et al. WNT/beta-catenin signalling is activated in aldosterone-producing adenomas and controls aldosterone production. *Hum Mol Genet.* 2014;23**:**889-905.

51) Scholl UI, Healy JM, Thiel A et al. Novel somatic mutations in primary hyperaldosteronism are related to the clinical, radiological and pathological phenotype*. Clin Endocrinol*. 2015;83:779-789.

52) Åkerström T, Maharjan R, Sven Willenberg H et al. Activating mutations in CTNNB1 in aldosterone producing adenomas. *Sci Rep* 2016;6:19546.

53) Teo AE, Garg S, Shaikh LH, Zhou J, Karet Frankl FE, Gurnell M, Happerfield L, Marker A, Bienz M, Azizan EA, Brown MJ. Pregnancy, Primary Aldosteronism, and Adrenal CTNNB1

Mutations. *N Engl J Med.* 2015;373:1429-1436.

54) Gomez-Sanchez CE, Qi X, Velarde-Miranda C, Plonczynski MW, Parker CR, Rainey W,

Satoh F, Maekawa T, Nakamura Y, Sasano H, Gomez-Sanchez EP. Development of

monoclonal antibodies against human CYP11B1 and CYP11B2. *Mol Cell Endocrinol.*

2014;383:111-117.

55) Nakamura Y, Maekawa T, Felizola SJ, Satoh F, Qi X, Velarde-Miranda C, Plonczynski MW,

Ise K, Kikuchi K, Rainey WE, Gomez-Sanchez EP, Gomez-Sanchez CE, Sasano H. Adrenal

CYP11B1/2 expression in primary aldosteronism: immunohistochemical analysis using novel

monoclonal antibodies. *Mol Cell Endocrinol.* 2014;392:73-79.

56) Gioco F, Seccia TM, Gomez-Sanchez EP, Rossi GP, Gomez-Sanchez CE. Adrenal

histopathology in primary aldosteronism: is it time for a change? *Hypertension.* 2015;66:724-

730.

57) Nakamura Y, Kitada M, Satoh F, Maekawa T, Morimoto R, Yamazaki Y, Ise K, Gomez-

Sanchez CE, Ito S, Arai Y, Dezawa M, Sasano H. Intratumoral heterogeneity of

steroidogenesis in aldosterone-producing adenoma revealed by intensive double- and triple-

immunostaining for CYP11B2/B1 and CYP17. *Mol Cell Endocrinol.* 2016;422:57-63.

58) Nishimoto K, Nakagawa K, Li D, Kosaka T, Oya M, Mikami S, Shibata H, Itoh H, Mitani F,

Yamazaki T, Ogishima T, Suematsu M, Mukai K. Adrenocortical zonation in humans under

normal and pathological conditions. *J Clin Endocrinol Metab.* 2010;95:2296-2305.

59) Nanba K, Vaidya A, Williams GH, Zheng I, Else T, Rainey WE. Age-Related Autonomous

Aldosteronism. *Circulation* 2017;136:347-355.

60) Nanba K, Tsuiki M, Sawai K, Mukai K, Nishimoto K, Usui T, Tagami T, Okuno H, Yamamoto

T, Shimatsu A, Katabami T, Okumura A, Kawa G, Tanabe A, Naruse M. Histopathological

diagnosis of primary aldosteronism using CYP11B2 immunohistochemistry. *J Clin Endocrinol*

Metab. 2013;98:1567-1574.

61) Nishimoto K, Seki T, Hayashi Y, Mikami S, Al-Eyd G, Nakagawa K, Morita S, Kosaka T, Oya M, Mitani F, Suematsu M, Kabe Y, Mukai K. Human Adrenocortical Remodeling Leading to Aldosterone-Producing Cell Cluster Generation. *Int J Endocrinol.* 2016;2016:7834356. Epub 2016 Sep 18.

62) Nishimoto K, Tomlins SA, Kuick R, Cani AK, Giordano TJ, Hovelson DH, Liu CJ, Sanjanwala AR, Edwards MA, Gomez-Sanchez CE, Nanba K, Rainey WE. Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands. *Proc Natl Acad Sci USA* 2015;112:E4591-E4599.

63) Nishimoto K, Seki T, Kurihara I, Yokota K, Omura M, Nishikawa T, Shibata H, Kosaka T, Oya M, Suematsu M, Mukai K. Case Report: Nodule Development From Subcapsular Aldosterone-Producing Cell Clusters Causes Hyperaldosteronism. *J Clin Endocrinol Metab.* 2016;101:6-9.

64) Ono Y, Nakamura Y, Maekawa T, Felizola SJ, Morimoto R, Iwakura Y, Kudo M, Seiji K, Takase K, Arai Y, Gomez-Sanchez CE, Ito S, Sasano H, Satoh F. Different expression of 11β-hydroxylase and aldosterone synthase between aldosterone-producing microadenomas and macroadenomas. *Hypertension.* 2014;64:438-444.

65) Arlt W, Lang K, Sitch AJ et al. Steroid metabolome analysis reveals prevalent glucocorticoid excess in primary aldosteronism. *JCI Insight.* 2017;2:e93136.

66) Boulkroun S, Samson-Couterie B, Dzib JF, Lefebvre H, Louiset E, Amar L, Plouin PF, Lalli E, Jeunemaitre X, Benecke A, Meatchi T, Zennaro MC. Adrenal cortex remodeling and functional zona glomerulosa hyperplasia in primary aldosteronism. *Hypertension* 2010;56:885-892.

67) Fernandes-Rosa FL, Giscos-Douriez I, Amar L, Gomez-Sanchez CE, Meatchi T, Boulkroun

S, Zennaro MC. Different Somatic Mutations in Multinodular Adrenals With Aldosterone-Producing Adenoma. *Hypertension.* 2015;66:1014-1022.

68) Nanba AT, Nanba K, Byrd JB, Shields JJ, Giordano TJ, Miller BS, Rainey WE, Auchus RJ, Turcu AF. Discordance between Imaging and Immunohistochemistry in Unilateral Primary Aldosteronism. *Clin Endocrinol (Oxf).* 2017 Aug 8. doi: 10.1111/cen.13442. [Epub ahead of print]

69) Mulatero P, Tauber P, Zennaro MC et al. KCNJ5 mutations in European families with nonglucocorticoid remediable familial hyperaldosteronism. *Hypertension.* 2012;59:235-240.

70) Scholl UI, Nelson-Williams C, Yue P, Grekin R, Wyatt RJ, Dillon MJ, Couch R, Hammer LK, Harley FL, Farhi A, Wang WH, Lifton RP. Hypertension with or without adrenal hyperplasia due to different inherited mutations in the potassium channel KCNJ5. *Proc Natl Acad Sci USA.* 2012;109:2533-2538.

71) Charmandari E, Sertedaki A, Kino T, Merakou C, Hoffman DA, Hatch MM, Hurt DE, Lin L, Xekouki P, Stratakis CA, Chrousos GP. A novel point mutation in the KCNJ5 gene causing primary hyperaldosteronism and early-onset autosomal dominant hypertension. *J Clin Endocrinol Metab.* 2012;97:E1532-E1539.

72) Monticone S, Hattangady NG, Penton D, Isales CM, Edwards MA, Williams TA, Sterner C, Warth R, Mulatero P, Rainey WE. A novel Y152C KCNJ5 mutation responsible for familial hyperaldosteronism type III. *J Clin Endocrinol Metab.* 2013;98:E1861-E1865.

73) Monticone S, Bandulik S, Stindl J, Zilbermint M, Dedov I, Mulatero P, Allgaeuer M, Lee CC, Stratakis CA, Williams TA, Tiulpakov A. A case of severe hyperaldosteronism caused by a de novo mutation affecting a critical salt bridge Kir3.4 residue. *J Clin Endocrinol Metab.* 2015;s:E114-E118.

| PA subtype	Genetic variant	Affected protein	Amino acid substitution		Clinical phenotype	Treatment
FH type I	*CYP11B1/B2* chimaeric gene[12]	CYP11B2 regulated by ACTH in zF	-		PA of variable severity, early onset; high frequency of stroke; overproduction of hybrid steroids*	DEX
FH type II	Genetic linkage with chromosome 7p22[10]	Unknown	Unknown		Indistinguishable from sporadic PA	Unilateral ADX MRA
FH type III	*KCNJ5* mutations	GIRK4	Thr158Ala[21] Gly151Glu[69] Gly151Arg[70]	Ile157Ser[71] Tyr152Cys[72] Glu145Gln[73]	PA of variable severity, usually early onset	Bilateral ADX MRA
FH type IV	*CACNA1H* mutations	Ca$_v$3.2	†Met1549Val[24]	Ser196Leu[29] Pro2083Leu[29]	PA of variable severity, early onset with incomplete penetrance for Met1549Val variant	Unilateral ADX MRA
Sporadic PA	*KCNJ5* mutations	GIRK4	Arg52His[33] Glu246Lys[33]	Gly247Arg[33] #Glu282Gln[33]	Indistinguishable from sporadic BAH/APA	MRA Unilateral ADX
Sporadic APA	*CACNA1H* mutation	Ca$_v$3.2	Val1951Glu[29]		Indistinguishable from sporadic APA	Unilateral ADX
PA and multiplex developmental disorder	*CACNA1H* mutation	Ca$_v$3.2	‡Met1549Ile[29]		Early onset PA with mild mental retardation, social skills alterations and learning disabilities	MRA
PASNA	*CACNA1D* mutations	Ca$_v$1.3	‡Gly403Asp[30]	‡Ile770Met[30]	Early onset PA with neuromuscular abnormalities	CCB MRA

Table 1. Germline mutations associated with primary aldosteronism

FH, Familial hyperaldosteronism; PA, primary aldosteronism; APA, aldosterone-producing adenoma; BAH, bilateral adrenal hyperplasia;

PASNA, PA with seizures and neurologic abnormalities; *CYP11B1*, gene encoding 11β-hydroxylase; *CYP11B2*, gene encoding aldosterone

synthase; ACTH, adrenocorticotropic hormone; zF, *zona fasciculata*; GIRK4, G protein coupled inwardly rectifying potassium channel 4;

Ca$_v$3.2, Ca^{2+} channel, voltage-dependent, T type, α 1H subunit; Ca$_v$1.3, Ca^{2+} channel, voltage-dependent, L type, α 1D subunit; DEX, dexamethasone; ADX, adrenalectomy; MRA, mineralocorticoid receptor antagonist; CCB, Ca^{2+} channel blocker. *The hybrid steroids are 18-hydroxycortisol and 18-oxocortisol; †*de novo* mutation also identified, ‡*de novo* mutation; #rare nonsynonymous single-nucleotide polymorphism rs7102584 present in 12 of 251 patients (5%) with PA compared with 22 of 1075 individuals (2%) in the international 1000 genomes cohort. In the 12 patients with PA carrying the rs7102584 (Glu282Gln) polymorphism, 9 were diagnosed with BAH and 3 with APA.[33]
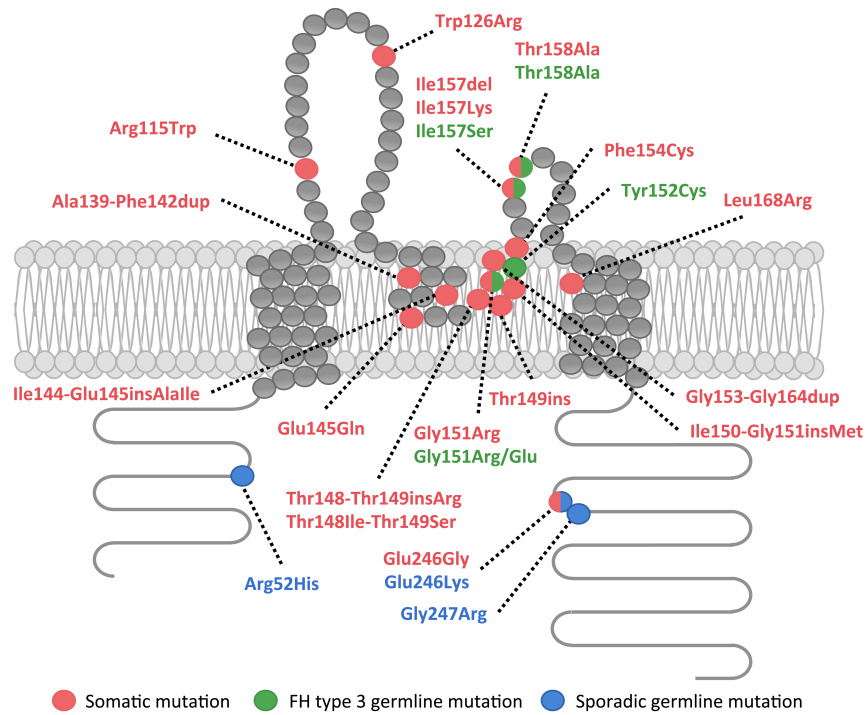
Figure 1. Germline and somatic mutations in GIRK4 associated with different forms of primary aldosteronism

Different somatic and germline mutations are indicated in GIRK4 encoded by the *KCNJ5* gene.

ONLINE SUPPLEMENT

Old and New Concepts in the Molecular Pathogenesis of Primary Aldosteronism

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References

1) Cheng CJ, Sung CC, Wu ST, Lin YC, Sytwu HK, Huang CL, Lin SH. Novel KCNJ5 mutations in sporadic aldosterone-producing adenoma reduce Kir3.4 membrane abundance. *J Clin Endocrinol Metab.* 2015;100:E155-E163.

2) Williams TA, Monticone S, Schack VR et al. Somatic ATP1A1, ATP2B3, and KCNJ5 mutations in aldosterone-producing adenomas. *Hypertension.* 2014;63:188-195.

3) Xu S, Hardege I, Murthy M, Gordon R, Stowasser M, O'Shaughnessy K. 9B.03: A novel insertional somatic KCNJ5 mutation in an Australian patient with an aldosterone producing adenoma. *J Hypertens*. 2015;33 Suppl 1:e120.

4) Scholl UI, Healy JM, Thiel A et al. Novel somatic mutations in primary hyperaldosteronism are related to the clinical, radiological and pathological phenotype. *Clin Endocrinol*. 2015;83:779-789.

5) Akerstrom T, Crona J, Delgado Verdugo A met 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.

6) Nanba K, Omata K, Tomlins SA, Giordano TJ, Hammer GD, Rainey WE, Else T. Double adrenocortical adenomas harboring independent KCNJ5 and PRKACA somatic mutations. *Eur J Endocrinol. 2*016;175:K1-K6.

7) Zheng FF, Zhu LM, Nie AF, Li XY, Lin JR, Zhang K, Chen J, Zhou WL, Shen ZJ, Zhu YC, Wang JG, Zhu DL, Gao PJ. Clinical characteristics of somatic mutations in Chinese patients with aldosterone-producing adenoma. *Hypertension.* 2015;65:622-628.

8) Kuppusamy M, Caroccia B, Stindl J, Bandulik S, Lenzini L, Gioco F, Fishman V, Zanotti G, Gomez-Sanchez C, Bader M, Warth R, Rossi GP. A novel KCNJ5-insT149 somatic mutation close to, but outside, the selectivity filter causes resistant hypertension by loss of selectivity for potassium. *J Clin Endocrinol Metab.* 2014;99:E1765-E1773.

9) Choi M, Scholl UI, Yue P et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science.* 2011;331:768-772.

10) Wang B, Li X, Zhang X, Ma X, Chen L, Zhang Y, Lyu X, Tang Y, Huang Q, Gao Y, Fan Y, Ouyang J. Prevalence and characterization of somatic mutations in Chinese aldosterone-producing adenoma patients. *Medicine.* 2015;94:e708.

11) 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.

12) Mulatero P, Tauber P, Zennaro MC et al. KCNJ5 mutations in European families with nonglucocorticoid remediable familial hyperaldosteronism. *Hypertension.* 2012;59:235-240.

13) Beuschlein F, Boulkroun S, Osswald A et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet.* 2013;45:440-444.

14) Åkerström T, Willenberg HS, Cupisti K et al. Novel somatic mutations and distinct molecular signature in aldosterone-producing adenomas. *Endocr Relat Cancer.* 2015;22:735-744.

15) Nishimoto K, Seki T, Kurihara I, Yokota K, Omura M, Nishikawa T, Shibata H, Kosaka T, Oya M, Suematsu M, Mukai K. Case Report: Nodule Development From Subcapsular Aldosterone-Producing Cell Clusters Causes Hyperaldosteronism. *J Clin Endocrinol Metab.* 2016;101:6-9

16) Azizan EA, Poulsen H, Tuluc P et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet.* 2013;45:1055-1060.

17) Wu VC, Huang KH, Peng KY, Tsai YC, Wu CH, Wang SM, Yang SY, Lin LY, Chang CC, Lin YH, Lin SL, Chu TS, Wu KD. Prevalence and clinical correlates of somatic mutation in aldosterone producing adenoma-Taiwanese population. *Sci Rep.* 2015;5:11396.

18) Fernandes-Rosa FL, Williams TA, Riester A et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension* 2014;64:354-361.

19) Kitamoto T, Suematsu S, Yamazaki Y, Nakamura Y, Sasano H, Matsuzawa Y, Saito J, Omura M, Nishikawa T. Clinical and Steroidogenic Characteristics of Aldosterone-Producing Adenomas With

ATPase or CACNA1D Gene Mutations. *J Clin Endocrinol Metab.* 2016;101:494-503.

20) Yamazaki Y, Nakamura Y, Omata K, Ise K, Tezuka Y, Ono Y, Morimoto R, Nozawa Y, Gomez-Sanchez CE, Tomlins SA, Rainey WE, Ito S, Satoh F, Sasano H. Histopathological Classification of Cross-Sectional Image-Negative Hyperaldosteronism. *J Clin Endocrinol Metab.* 2017;102:1182-1192.

21) Nanba K, Chen AX, Omata K, Vinco M, Giordano TJ, Else T, Hammer GD, Tomlins SA, Rainey WE. Molecular Heterogeneity in Aldosterone-Producing Adenomas. *J Clin Endocrinol Metab.* 2016;101:999-1007.

22) Scholl UI, Goh G, Stolting G et al. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. *Nat Genet.* 2013;45:1050-1054.

23) Tan GC, Negro G, Pinggera A et al. Aldosterone-Producing Adenomas: Histopathology-Genotype Correlation and Identification of a Novel *CACNA1D* Mutation. *Hypertension.* 2017;70:129-136.

24) Teo AE, Garg S, Shaikh LH, Zhou J, Karet Frankl FE, Gurnell M, Happerfield L, Marker A, Bienz M, Azizan EA, Brown MJ. Pregnancy, Primary Aldosteronism, and Adrenal CTNNB1 Mutations. *N Engl J Med*. 2015;373:1429-1436.

25) Akerstrom T, Maharjan R, Sven Willenberg H et al. Activating mutations in CTNNB1 in aldosterone producing adenomas. *Sci Rep.* 2016;6:19546.

26) Rhayem Y, Perez-Rivas LG, Dietz A, Bathon K, Gebhard C, Riester A, Mauracher B, Gomez-Sanchez C, Eisenhofer G, Schwarzmayr T, Calebiro D, Strom TM, Reincke M, Beuschlein F. PRKACA Somatic Mutations Are Rare Findings in Aldosterone-Producing Adenomas. *J Clin Endocrinol Metab.* 2016;101:3010-3017.

27) Nakajima Y, Okamura T, Horiguchi K, Gohko T, Miyamoto T, Satoh T, Ozawa A, Ishii S, Yamada E, Hashimoto K, Okada S, Takata D, Horiguchi J, Yamada M. GNAS mutations in adrenal aldosterone-producing adenomas. *Endocr J.* 2016;63:199-204.

Gene	Protein	Mutations*		
KCNJ5	GIRK4	Arg115Trp[1] Trp126Arg[2] Ala139_Phe142dup[3] Ile144_Glu145insAlaIle[4] Glu145Gln[5] Thr148Ile_Thr149Ser[6]	Thr148_Thr149insArg[7] Thr149ins[8] Ile150_Gly151insMet[4] †Gly151Arg[9] Gly153_Gly164dup[10] Phe154Cys[4]	Ile157del[11] Ile157Lys[4] Thr158Ala[12] †Leu168Arg[9] Glu246Gly[1]
ATP1A1	Na$^+$/K$^+$ ATPase 1	Gly99Arg[2] Phe100_Leu104del[13] Met102_Leu103del[14] Met102_Ile106del[14] Leu103_Leu104del[14]	Leu104Arg[13] Ile322_Ile325del[15] Ile327Ser[15] Val332Gly[13]	Phe956_Glu961del[14] Phe959_Glu961del[14] Glu960_Leu964del[14] GluGluThrAla963Ser[16]
ATP2B3	Ca^{2+}ATPase 3	Tyr410Asp[17] Thr423_Leu425del[14] Val424_Leu425del[18] Val424_Val426del[14] Leu425_Val426del[13]	Val426_Val427del[13] Val426_Val429del[14] Val426Gly_Val427Gln_Ala428_Leu433del[4] Val427_Ala428del[19]	
CACNA1D	Cav1.3	Val259Asp[16] Val401Leu[14] Gly403Arg[16] Gly403Arg[4] Glu412Asp[20] Ser652Leu[18] Leu655Pro[18] Tyr741Cys[18] Phe747Leu[16] Phe747Val[20] Phe747Cys[21]	Val748Ile[10] Ile750Met[16] Ile750Phe[18] Val752Gly[20] Phe767Val[4] Ile770Met[22] Val979Asp[18] Val981Asn[18] Arg990His[16] Arg993Ser[20]	Ala998Ile[18] Ala998Val[18] Ala998Val[20] Val1151Phe[18] Ile1152Asn[18] Val1153Gly[23] Pro1336Arg[16] Val1338Met[22] Val1353Met[14] Met1354Ile[16]
CTNNB1	β-catenin	Ser33Cys[24] Gly34Arg[24] Ala39Glu.fs*3[4]	Thr41Ala[25] Ser45Phe[24] Ser45Pro[25]	
PRKACA	cAMP dependent protein kinase α	‡Leu206Arg[26] His88Asp[26]		
GNAS	Guanine Nucleotide Binding Protein α	‡Arg201Cys[27]		

Table S1. Somatic mutations associated with aldosterone-producing adenomas

GIRK4, G protein coupled inwardly rectifying potassium channel 4; Ca$_v$1.3, Ca^{2+} channel, voltage-dependent, L type, α1D subunit; Ca$_v$3.2, Ca^{2+} channel, voltage-dependent, T type, α1H subunit

*To our knowledge, the above table shows all somatic mutations in aldosterone-producing adenomas reported to date.

†Most frequently occurring mutations in aldosterone-producing adenomas
‡Mutations also present in adenomas co-secreting cortisol