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Mouse models of primary aldosteronism: from physiology to pathophysiology

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Abstract

Primary aldosteronism (PA) is a common form of endocrine hypertension that is characterized by the excessive production of aldosterone relative to suppressed plasma renin levels. PA is usually caused by either a unilateral aldosterone-producing adenoma or bilateral adrenal hyperplasia. Somatic mutations have been identified in several genes that encode ion pumps and channels that may explain the aldosterone excess in over half of aldosterone-producing adenomas, whereas the pathophysiology of bilateral adrenal hyperplasia is largely unknown. A number of mouse models of hyperaldosteronism have been described that recreate some features of the human disorder although none replicate the genetic basis of human PA. Animal models that reproduce the genotype-phenotype associations of human PA are required to establish the functional mechanisms that underlie the endocrine autonomy and deregulated cell growth of the affected adrenal and for preclinical studies of novel therapeutics. Herein, we discuss the differences in adrenal physiology across species and describe the genetically-modified mouse models of PA that have been developed to date.

Introduction

Primary aldosteronism (PA) is the most common form of endocrine hypertension characterized by the excessive production of aldosterone relative to suppressed plasma renin and is associated with an increased risk of cardiovascular and cerebrovascular complications (1-2). PA is predominantly caused by either of two main subtypes: unilateral aldosterone excess due to an aldosterone-producing adenoma or bilateral aldosterone excess caused by bilateral adrenal hyperplasia. Unilateral PA is treated by adrenalectomy whereas patients with bilateral PA are usually treated pharmacologically with a mineralocorticoid receptor antagonist (3).

Major advances have been made in our understanding of the pathogenesis of aldosterone-producing adenomas with the discovery of somatic mutations in a number of genes that can explain the overproduction of aldosterone in over half of patients with a unilateral aldosterone-producing adenoma. The affected genes encode ion pumps and channels (*KCNJ5*, *ATP1A1*, *ATP2B3* and *CACNA1D*) or key players in the control of cell growth (*CTNNB1* and *PRKACA*) (4). Mutations in several of the affected genes (*KCNJ5*, *ATP2B3*, *CACNA1D*) have been shown to cause an increase in intracellular Ca^{2+} concentration that results in an increase in transcription of the aldosterone synthase gene (*CYP11B2*) via the activation of Ca^{2+} signaling (5-7). In contrast, mutations in *ATP1A1* (Na^+/K^+-ATPase 1-Leu104Arg and Val332Gly) result in intracellular acidification that may cause the overproduction of aldosterone (8). The pathogenesis of bilateral adrenal hyperplasia, however, remains obscure mainly because molecular studies are hampered due to the limited availability of resected adrenal specimens from patients with bilateral adrenal hyperplasia who are treated pharmacologically.

Animal models of PA would be useful to study mechanisms that control cell growth and autonomous aldosterone production and for preclinical testing of novel therapeutics. In this review, we discuss adrenal physiology in different animal species and describe the currently available mouse models of hyperaldosteronism.

Physiology of the adrenal gland in different species

The human adrenal cortex comprises 3 morphologically distinct zones, the *zona glomerulosa* (zG), *zona fasciculata* (zF) and *zona reticularis* (zR) that have specific functional properties for the production of mineralocorticoids, glucocorticoids and androgens, respectively (referred to as "functional zonation"). In the normal human adrenal gland, the restricted synthesis of aldosterone in the zG and of cortisol in the zF is due to the adrenal expression of *CYP11B2* exclusively in the zG for aldosterone synthesis and the expression of *CYP17* and *CYP11B1* in the zF for cortisol production (9, 10) (Figure 1).

The adrenal cortices of pigs, dogs and cattle are similarly functionally organized to those of humans with aldosterone biosynthesis restricted to cells of the zG and cortisol production in the zF. Mice display a different morphological zonation compared to humans. The *CYP17* gene encoding 17α-hydroxylase and 17,20-lyase is not expressed in mice, as in other rodents, and therefore mice produce corticosterone instead of cortisol and do not synthesize adrenal androgens. A functional zR is thus absent in mice and this zone is difficult to distinguish histologically (Figure 1, Table 1).

The adrenals of young mice have a layer of juxtamedullary cortical cells (the X-zone) with an undefined function (11). The X-zone degenerates during the first pregnancy of female mice, disappearing by the 12th day of gestation, and by puberty in male mice. In the absence of pregnancy, the X-zone nonetheless degenerates in females but slowly over a variable period that may last up to 200 days (11). The adrenals of female mice have a

notably higher weight and total volume of the cortex and medulla than those of males from the age of 5 weeks that is maintained into adulthood (12).

In humans, the final three steps in the biosynthesis of aldosterone involve the conversion of deoxycorticosterone by the successive action of the 11β-hydroxylase, 18-hydroxylase and 18-methyloxidase activities of a single enzyme, aldosterone synthase, encoded by *CYP11B2* (9, 10). An 11β-hydroxylase in the zF, encoded by a distinct but highly homologous gene to *CYP11B2* called *CYP11B1*, converts 11-deoxycortisol to cortisol. Humans, mice, rats, hamsters, guinea pigs and monkeys express two CYP11B enzymes whereas cows, sheep, horses, pigs and dogs express a single CYP11B enzyme that catalyzes the final step/s in the synthesis of both cortisol and aldosterone (13-18) (Table 1). Despite the presence of a single CYP11B in these species, functional zonation with aldosterone production in the zG and cortisol synthesis in the zF is maintained by an unknown mechanism, which presumably involves the presence of zone-specific factors that modulate CYP11B activity.

Regulation of aldosterone synthesis in humans and other species

The principal function of aldosterone is to maintain fluid and electrolyte balance promoting Na^+ retention and K^+ secretion in the distal nephron of the kidney, thereby controlling blood pressure (10). In zG cells, angiotensin II (Ang II) stimulates phospholipase C and triggers inositol triphosphate-dependent Ca^{2+} release from the endoplasmic reticulum, whilst high plasma K^+ concentrations or long-term stimulation by Ang II results in membrane depolarization and the activation of L- and T-type Ca^{2+} channels (19). The increase in intracellular Ca^{2+} leads to an increase in expression of the aldosterone synthase gene (*CYP11B2*) and aldosterone production (17). Small changes in plasma K^+, as low as 1 mM, can double aldosterone secretion (20). The basis of this sensitivity is the high background K^+

conductance with a membrane potential close to the K^+ equilibrium potential. At low plasma K^+ concentrations the membrane voltage is hyperpolarized and small increases in K^+ are sufficient to slightly depolarize the membrane and activate T-type Ca^{2+} channels (21-23). Therefore, mechanisms that regulate the membrane potential of the zG cell play a crucial role in the control of aldosterone production and usually depend on the equilibrium potential and ion conductance across the plasma membrane.

The negative membrane voltage is mainly determined by 2-pore domain K^+ channels, the TASK channels (TWIK-related acid sensitive K^+ channels or K^+-selective leak channels), that generate the aforementioned background or "leak" K^+ currents. K^+ inwardly rectifying channels (called G protein activated inwardly rectifying K^+ channels, GIRK) also regulate the membrane potential of zG cells and GIRK4 (encoded by *KCNJ5*) may function in normal adrenal physiology in addition to its pathological role in aldosterone production via gain-of-function mutations as described above.

Different species employ different K^+ channels to maintain the membrane potential of the zG cell (24) and distinct differences between species have been reported for the expression of some key K^+ channels (25). TASK-1 is expressed in the zG and zF in both humans and rats. TASK3 is localized to the zG in the human and rat adrenal with high expression in the rat and low expression in the human (25). GIRK4 (KCNJ5) is strongly expressed in the zG of the human adrenal but is undetectable in the rat zG and zF (25). Thus the distinct expression patterns of K^+ channels between species suggests an evolutionary divergence in the regulation of aldosterone production and indicates that rats and probably also mice may not be ideal models to study human pathological conditions of aldosterone excess.

Mouse models of primary aldosteronism

Several genetically modified mouse models of hyperaldosteronism have been reported that display the main biochemical features of PA but do not reproduce the adrenal tumourigenesis or hyperplasia associated with this condition. Mouse models of hyperaldosteronism are discussed further below and are summarized in Table 2.

Mouse models with deletions of TASK channels

Electrophysiological recordings of zG cells have emphasized the importance of leak-type K^+ channels of the 2-pore domain family in conferring background K^+ conductance. In rodents, two members of the 2-pore domain family, Task1 (Kcnk3) and Task3 (Kcnk9) are the dominant leak-type channels. Female mice with the *Task1* gene deleted display hyperaldosteronism with low plasma renin activity and a decrease in plasma K^+. Systolic blood pressure is increased and responds to administration of a mineralocorticoid receptor antagonist (26). The *Cyp11b2* gene is expressed aberrantly exclusively in the zF and the elevated aldosterone is suppressed by dexamethasone (26). Male *Task1*$^{-/-}$ mice and heterozygous females do not exhibit a phenotype. Mice of both sexes at postnatal day 18 exhibit an abnormal distribution of the Cyp11b2 enzyme. Castrated male *Task1*$^{-/-}$ display an abnormal expression of *Cyp11b2*, similar to females, and estradiol administration decreases the level of Cyp11b2 expression, but not the zonal distribution. Female *Task1*$^{-/-}$ injected with testosterone display a normal zonation indicating the role of androgens (26).

Gene expression analysis of the adrenals of male and female *Task1*$^{-/-}$ revealed a limited number of differentially expressed genes, mostly involved in signaling cascades, and one of these belongs to the dickkopf family, *Dkk3*, that is expressed in the zG of the adrenal and inhibits aldosterone secretion in cultured adrenal cells (27). Inactivation of *Dkk3* in male

Task1$^{-/-}$ extended the hyperaldosteronism phenotype to male mice. The zonal distribution of the double deleted of *Dkk3*$^{-/-}$ and *Task1*$^{-/-}$ was preserved in contrast to female *Task1*$^{-/-}$ mice.

TASK1 (KCNK3) is expressed in both mouse and human adrenals. In the human adrenocortical NCI H295R cell line, downregulation of TASK1 by a siRNA increased aldosterone production (28). Genome-wide association studies have identified KCNK3 single-nucleotide polymorphism (SNP) variants associated with blood pressure in humans (29). The KCNK3 SNP (rs1275988) is associated with hypertension in African-Americans and a nearby SNP (rs13394970) is associated with hypertension in Hispanics, which was found in the MESA (Multi-Ethnic Study of Atherosclerosis) that comprised 7,840 individuals. Aldosterone levels and plasma renin activity in a subset of 1,653 participants were also found associated with KCNK3 rs2586886 (29). The functional significance of these SNPs on channel function is unknown and while in the mouse complete deletion of *Kcnk3* in females is required for the phenotype, an alteration determined by a polymorphism is of unknown significance in humans.

Gene deletion of both the *Task1* and *Task3* in male mice ablates background K^+ currents resulting in marked depolarization of the membrane potential in zG cells (30). *Task1*$^{-/-}$, *Task3*$^{-/-}$ mice excrete higher urinary aldosterone at all levels of sodium intake. The mice also have normal or low renin activity and aldosterone production fails to normalize on high sodium intake or after the administration of the angiotensin receptor blocker candesartan (30). Adrenal zonation is unaltered and *Task1*$^{-/-}$, *Task3*$^{-/-}$ mice may represent a model of idiopathic primary aldosteronism.

Task3$^{-/-}$ mice display mild aldosterone overproduction, a decreased plasma renin concentration and fail to suppress aldosterone excretion on a high sodium diet. These mice are hypersensitive to Ang II and have high blood pressure (31, 32). Baseline membrane

potential of zG cells was not different from that of wild type mice, even though the mice were slightly hypokalemic. Although there was a small increase in *Cyp11b2* mRNA, there was no increase in aldosterone synthase expression. A recent study indicates that TASK3 is not located in the plasma membrane, but is located in the mitochondrial membrane and plays a role in mitochondrial membrane potential (33). It has been suggested that the lack of plasma membrane depolarization, unchanged expression of the Cyp11b2 protein, but increased production of aldosterone might result from an increase in the activity of the late pathway of aldosterone biosynthesis (34) (conversion of deoxycorticosterone to aldosterone in the mitochondria). Neonatal *Task3*$^{-/-}$ mice display a severe phenotype with a strong increase in plasma aldosterone, corticosterone and progesterone that decreases with age (35) with a significant increase in *Cyp11b2* mRNA for the neonatal mice that normalizes in adults. There is a marked increase in adrenal renin expression and concentration mainly in the zF that decreases with age (35).

Mouse models with deletions of large Ca^{2+} activated K^+ channel subunit deletions

Large Ca^{2+}-activating K^+ channels (BK) are composed of an α pore (BKα) and one to four β subunits. There are multiple splice variants of BKα and the cell-specific function of BK channels is determined by the BKα splice variant in association with one of the four β subunits (36). Under normal conditions, the BKα/β1 channel opens in response to local elevations in intracellular Ca^{2+} (Ca^{2+} sparks) leading to a compensatory vasorelaxation, but in a study performed with the BKβ1 gene inactivated (*Kcnmb1*$^{-/-}$) in male mice, channel activity is uncoupled from Ca^{2+} sparks leading to hypertension (37). The BK$\beta^{-/-}$ mice retain fluid, which is enhanced on a high K^+ diet and administration of the mineralocorticoid receptor antagonist eplerenone corrected the fluid retention and nearly normalized blood pressure

(37-38). Plasma aldosterone concentrations are increased and exacerbated by K^+ loading as the adrenal is highly sensitive to K^+ retention by the renal connecting tubules of the BK$\beta 1^{-/-}$ mice (37). The increased aldosterone secretion is due to an increased sensitivity of the zG to K^+ (37). While BKα is highly expressed in the zG and weakly in the adrenal medulla, BK$\beta 1$ appears to be expressed in the medulla only (37, 39). In the adrenal medulla BKα/β may function in hyperpolarizing the membrane potential of chromaffin cells to inhibit Ca^{2+}-mediated catecholamine release, analogous to the role of this channel in other tissues. In zG cells, catecholamines induce increases in cAMP levels that have been demonstrated to stimulate Ca^{2+} influx via L-type Ca^{2+} channels (40), a known pathway leading to increased aldosterone production. The hypertension in the BK$\beta 1^{-/-}$ model is due to both the elevation of aldosterone levels and the abnormal vasorelaxation of the vascular smooth muscle cells.

Gene deletion of the BKα (*Kcnma1*$^{-/-}$) resulted in a small, but significant increase in blood pressure with normal heart rate, a gender-independent decrease in plasma K^+ concentration and an elevation of plasma aldosterone with normal renin or serum corticotropin levels (39). The elevation of the blood pressure was due to the hyperaldosteronism and the vascular dysfunction that resulted from the gene deletion.

Transgenic mouse models with constitutively activated β-catenin

The Wnt/β-catenin signaling is essential for embryonic development and cell proliferation, but constitutive activation is associated with a variety of cancers (41). In the absence of extracellular Wnt ligands, the N-terminal domain of β-catenin is phosphorylated on serines/threonine residues by a multi protein destruction complex composed of casein kinase I, glycogen synthase kinase 3β, axin and APC (adenomatous polyposis coli), which results in ubiquitylation and proteosomal degradation. Binding to the Frizzled/LRP

(lipoprotein receptor-related protein) receptor inhibits the destruction complex and β-catenin is stabilized and undergoes nuclear translocation and activation of LEF/TCF (T-cell factor/lymphoid enhancer factor) transcription factors resulting in increased gene expression (42).

β-catenin plays a role in adrenal development (43) and activated β-catenin has been shown to occur in adrenal cortical carcinomas and adenomas (41) and activating mutations of β-catenin are found in some human aldosterone-producing adenomas (44). Transgenic mice with an adrenal-restricted constitutive activation of β-catenin (ΔCat) display adrenal hyperplasia and dysplasia that produces profound changes in zonal identity and causes hyperaldosteronism in females with some of the mice also developing adrenal cancers at 17 months of age (45). Disease progression was slow in male ΔCat mice and the study was largely performed in females; however, males also displayed adrenal hyperplasia but no signs of malignancy.

Transgenic mouse models with a truncated form of adenomatous polyposis coli

As described above, APC is a component of the destruction complex of β-catenin. Mice that express a defective mutant of Apc, which lacks the C-terminal portion of the gene (*apc*$^{Min/+}$), develop multiple intestinal tumors (46). These mice also exhibit hypertension with an increase in plasma volume, a marked increase in fractional urinary excretion of K^+, a decrease in urinary Na^+ excretion and an increase in plasma aldosterone and corticosterone concentrations (47).

The aldosterone-responsive SGK1 (serum/glucocorticoid regulated kinase 1) is also stimulated by β-catenin; therefore, stabilization of β-catenin would be expected to upregulate SGK1. Considerable evidence indicates that SGK causes an increase in the

expression of cell surface epithelial Na^+ channels (ENaC) that function in the regulation of Na^+ and fluid reabsorption in the kidney and colon (48, 49). *Apc*$^{Min/+}$/*sgk*$^{-/-}$ mice display increased aldosterone but not corticosterone levels compared to *apc*$^{Min/+}$ mice, and the hypertension of *apc*$^{Min/+}$ mice is ablated in the double transgenic model (47). This effect is attributed to the impaired Na^+ retention determined by the *sgk* deletion whereas aldosterone secretion is directly affected by Apc-dependent signaling (47). This suggests that the *apc*$^{Min/+}$ mice are a model of primary aldosteronism whereas the double *apc*$^{Min/+}$/*sgk*$^{-/-}$ transgenic model is a model of both primary and secondary aldosteronism. The normalization of hypertension by deletion of the *sgk* gene likely reflects the role of Sgk in ENaC expression. The above study on *apc*$^{Min/+}$ and *apc*$^{Min/+}$/*sgk*$^{-/-}$ mice was performed using sex-matched mice of 3 months of age. No gender-related phenotype differences were reported.

The potential relevance of APC variants in some patients with PA is indicated by a case report of a young patient with severe hypertension and PA in a background of familial adenomatous polyposis with a germline heterozygous APC mutation (50). The patient displayed bilateral macronodular adrenal hyperplasia with lateralized aldosterone secretion. Molecular analysis and histopathology of the resected adrenal showed 3 nodules with one expressing CYP11B2 and carrying a somatic *KCNJ5* mutation. This nodule and an additional nodule had a somatic biallelic APC inactivation that potentially triggered cell proliferation, with the *KCNJ5* mutation providing the second genetic hit to drive the CYP11B2 overexpression (50).

Mouse models with deletion of cryptochrome-1 and cryptochrome-2

Behaviour, metabolism and physiology are subject to a well-controlled daily rhythm regulated by a molecular oscillator called the circadian clock. Alteration of the circadian rhythm in shift workers, flight crews and individuals with sleep disorders have a higher than average incidence of cardiovascular disorders (51). Disruption of the circadian clock in *Cry*-null mice that lack the core clock components cryptochrome-1 and cryptochrome-2 (*Cry1*$^{-/-}$*/Cry2*$^{-/-}$) results in hyperaldosteronism with salt-sensitive hypertension and renal damage (53-54). Age- and gender-related variations were eliminated by the inclusion of only male mice aged 12 to 16 weeks. The hyperaldosteronism is reportedly due to dysregulation of the 3β-hydroxysteroid dehydrogenase b6 (Hsd3b6) which is overexpressed in the zG of the mouse adrenal. The expression of the aldosterone synthase is unchanged and it is believed that overexpression of the Hsd3b6 allows further substrate to become available for the synthesis of aldosterone (52-53). The Hsd3b6 homolog in humans is the HSD3B1 isozyme which is expressed in the zG of the human adrenal (54).

Period 1 (Per1) is another core component of the circadian clock and Per1 and Cry2 can mediate opposing effects on target genes (55). Mice with reduced Per1 levels *in vivo* display decreased plasma aldosterone concentrations and a reduction in *Hsd3b6* expression (56). This may be accounted for by an increase in Cry2 expression as Per1 levels decrease that causes a dysregulation of *Hsd3b6* gene expression.

Mouse models with differential levels of transforming growth factor β1 expression

Polymorphisms of the *TGFB1* gene located in the sequence coding the signal peptide are associated with a reduced risk of hypertension in a European population (57) and a different polymorphism that affects a different amino acid in the signal peptide has been

associated with hypertension in an Asian population (58). The 3'-untranslated region of the *Tgfb1* gene was manipulated to increase or decrease the stability of the encoded mRNA. Male mice were then created to express *Tgfb1* in five grades from 10-300% that of normal. Plasma aldosterone and corticosterone concentrations increased as *Tgfb1* gene expression levels decreased. Mice with the lowest *Tgfb1* expression ($Tgfb1^{L/L}$) had approximately 200% higher plasma aldosterone and corticosterone concentrations compared with wild type, whereas the plasma aldosterone concentration of mice with the highest expression of *Tgfb1* ($Tgfb1^{H/H}$) was approximately halved. Expression of *Cyp11b2* and *Cyp11b1* followed the changes in *Tgfb1* expression as predicted. The animals with decreased *Tgfb1* had higher plasma volumes and elevated blood pressure and lower levels of angiotensin II (59) and the hypertension was corrected with spironolactone.

Mouse models produced using a random mutagenesis screen

In attempt to find novel genetic loci associated with primary aldosteronism, a large-scale mutation screen was performed in mice injected with *N*-ethyl-*N*-nitrosourea (ENU, an alkylating agent that causes ethylation of nucleic acids resulting in point mutations) to randomly introduce mutations into the mouse genome (60). As expected, the resulting mutagenesis caused a phenotypic spectrum from total loss-of-function of some genes to gain-of-function and generated mouse lines with elevated plasma aldosterone concentrations in males but not in females. Exome next generation sequencing identified 8 mutated genes that were common between the F1 and F5 generations of the mice with high plasma aldosterone concentrations. Although animals carry more than one mutated gene, an attempt was made to correlate the expression of mutated genes with aldosterone levels and animals carrying mutations in the *Sspo, Dguok, Hoxaas2* and *Clstn3* genes displayed higher aldosterone levels (61). Histological examination of the adrenal did not reveal any

adenomas or hyperplasia, but exhibited an increased staining for the Cyp11b2 enzyme that was more pronounced in the middle and inner areas of the adrenal cortex compared to wild type animals suggesting an altered zonation of Cyp11b2. Further studies will be necessary to characterize each individual gene in the regulation of aldosterone.

Mouse models with an increased expression of the aldosterone synthase gene

The biosynthesis of aldosterone depends on the transcriptionally regulated last enzyme in the pathway, aldosterone synthase (CYP11B2) (19). Male and female mice were generated with increased stability of the *Cyp11b2* mRNA by substitution of the 3' untranslated region with the more stable 3' untranslated sequence of the bovine growth hormone mRNA (62). *Cyp11b2*$^{hi/hi}$ mice displayed a slight increase in *Cyp11b2* mRNA expression on a normal sodium diet with normal plasma aldosterone concentrations that were higher than those of wild type animals on a high sodium diet (62). The *Cyp11b2*$^{hi/hi}$ mice on a modest high salt diet infused with Ang II had a higher blood pressure, cardiac hypertrophy and oxidative stress than wild type animals (62). There were no reported phenotype differences related to gender. The modest elevation of aldosterone synthase expression in humans could result in the development of hypertension in societies that consume a high sodium diet.

Transgenic mice were produced with the promoter region of the human *CYP11B1* gene fused to the coding region of the human *CYP11B2* gene (63). The heterozygous mice called *hAS*$^{+/-}$ were shown to be hyperaldosteronemic, hypertensive when administered a high salt diet and as expected the plasma K^+ was lower and Na^+ higher than the wild type mice. The high salt induced hypertension was normalized by the administration of fadrozole, an aldosterone synthase inhibitor. These mice could be useful for the study of the cardiovascular and renal effects of endogenous elevated aldosterone.

Perspectives and Conclusions

Mouse models of human disease often fail to represent the whole clinical spectrum. Although several mouse models of hyperaldosteronism have been described over recent years, they have not reproduced a genetic alteration shown to cause PA in humans and do not display the tumour formation or hyperplasia that reflect the pathophysiology of this disease. These models are however valuable to study mechanisms of fluid and electrolyte homeostasis and the regulation of aldosterone production as well as providing potential targets for the pharmacological treatment of conditions where aldosterone production is elevated as in PA.

Humans and rodents display some notable differences in adrenal physiology with alternative patterns of adrenal steroid production and distinct differences in the expression of K^+ channels that maintain the membrane potential of zG cells. This divergence underscores the dubious suitability of available mouse models to resemble human pathophysiology. Further refined mouse models with inducible, zone specific introduction of point mutations that have been observed in human disease could represent one possible approach to fill this gap. Furthermore, the adrenal physiology of larger animals such as pigs, that are useful to model complex disease traits, could also resemble more closely that of humans and may offer the possibility to recapitulate genetic hits in ion pumps and channels that have been shown to cause the human disorder. Yet pigs express a single CYP11B enzyme for aldosterone and cortisol synthesis instead of the two enzymes expressed in humans (CYP11B2 and CYP11B1). Notwithstanding this complexity, the sequencing of the pig genome by the Swine Genome Sequencing Project, and advances in genome editing technologies render feasible the production of a pig knock-in model of PA with an adrenal

expressing human CYP11B1 and CYP11B2 to provide data that can be translated to the human condition.

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Figure legend

Cholesterol is mobilised from a store in the outer mitochondrial membrane (OMM) and is transferred by steroidogenic acute regulatory protein (StAR) to the inner mitochondrial membrane (IMM) where it is converted to pregnenolone by P450scc, the first and rate-limiting step of steroidogenesis (9, 10). Aldosterone biosynthesis is restricted to the *zona glomerulosa* (zG) where aldosterone synthase is localized. In humans, the activity of 17α-hydroxylase/17,20 lyase (17αOHase, CYP17A1) is a key enzyme for the synthesis of both cortisol and androgens; the main biosynthetic pathway for cortisol synthesis is via the 17α-hydroxylation of pregnenolone, indicated by thicker arrows (panel A). Mice, like other rodents, do not express CYP17A1 and as a result the major glucocorticoid synthesised is corticosterone (instead of cortisol) that is produced from deoxycorticosterone by 11β-hydroxylase (11βOHase, Cyp11b1) in the *zona fasciculata* (zF) (panel B). The absence of CYP17A1 expression in mice means that adrenal androgens cannot be synthesised as in humans in the *zona reticularis* (zR) and mice do not have a distinguishable zR (panels A and B). Young mice have an X-zone that degenerates by the first pregnancy in females and by sexual maturity in males. 3βHSD, 3β-hydroxysteroid dehydrogenase; 21OHase, 21-hydroxylase; Aldo synthase, aldosterone synthase; 17αOHase, 17α-hydroxylase; STS, steroid sulfatase; DHEAS, dehydroepiandrosterone sulfate. Including data from Stowasser M & Gordon RD (10).