Published in final edited form as:

Mol Cell Endocrinol. 2015 June 15; 408: 213–219. doi:10.1016/j.mce.2014.12.004.

Somatic Mutations of the ATP1A1 Gene and Aldosterone-Producing Adenomas

Celso E. Gomez-Sanchez^{1,2}, Maniselvan Kuppasamy², and Elise P. Gomez-Sanchez³

¹Division of Endocrinology, G.V. (Sonny) Montgomery VA Medical Center, Jackson, MS, USA

²Department of Medicine-Endocrinology, University of Mississippi Medical Center, Jackson, MS, USA

³Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS, USA

Abstract

Primary aldosteronism is the most common form of secondary hypertension. It affects approximately 10% of patients with hypertension and causes greater cardiovascular morbidity and mortality compared to essential hypertension of similar severity and duration. The cause of primary aldosteronism in about half of these patients is an aldosterone-producing adenoma; over half of these adenomas have mutations in one of several ion channels and pumps, including the potassium channel KCNJ5, calcium channel $\text{Ca}_v1.3$, $\alpha 1$ subunit of the sodium potassium ATPase, and membrane calcium ATPase 3. This review concentrates on the molecular and physiological mechanisms by which mutations of the *ATP1A1* gene increase aldosterone production.

Keywords

Primary aldosteronism; Aldosterone-producing adenomas; Sodium/potassium ATPase

1. Introduction

Primary aldosteronism was first described by Jerome Conn in 1955 (Conn, 1955) in patients presenting with hypertension and hypokalemia. A review of his experience at the University of Michigan demonstrated that approximately 20% of hypertensives had primary aldosteronism due to an aldosterone-producing adenoma (Conn et al., 1964). Subsequent studies suggested that this high incidence of primary aldosteronism was due to a selection bias, as this was a referral clinic and one of the few institutions where aldosterone could be measured. The incidence was found to be as low as 0.1% (Berglund et al., 1976) based on a retrospective review of Swedish patients with hypertension. A major cause of this

Address Correspondence: Celso E. Gomez-Sanchez, G.V. (Sonny) Montgomery VA Medical Center, 1500 E. Woodrow Wilson Dr., Jackson, MS 39216, USA, Cgomez-sanchez@umc.edu, 601 368 3844.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

discrepancy may have been the use of hypokalemia as a major part of the criteria for screening for primary aldosteronism using serum of plasma obtained in the standard methods of using a tourniquet for blood sampling that increases potassium in the blood (Abdelhamid et al., 2003). The most potent regulator of aldosterone secretion is the renin-angiotensin system. Renin secreted from the kidney in response to a decrease in blood pressure generates angiotensin II, the most potent stimulator of aldosterone synthesis and secretion by adrenal glomerulosa cells. Aldosterone actions in the kidney, vessels, heart and sympathetic nervous system synergizes with those of angiotensin II to increase blood pressure and suppress renin. Hiramatsu (Hiramatsu et al., 1981) devised a screening test based on this feed-back mechanism of the renin-angiotensin-aldosterone system (RAAS): autonomous secretion of aldosterone in PA produces an increase in the aldosterone:plasma renin activity ratio. This aldo:renin ratio, now the standard screening test to select patients for further confirmation studies, has established that PA is the most common form of secondary hypertension, causing about 1% of hypertension in the general population and around 6-10% in the hypertensive population (Fardella et al., 2000, Funder et al., 2008, Hannemann et al., 2012, Mosso et al., 2003). Primary aldosteronism was classified into several groups, the most common due a unilateral benign aldosterone-producing adenoma and idiopathic hyperaldosteronism, or bilateral zona glomerulosa hyperplasia, with aldosterone production coming from both adrenals. Less common forms of PA are unilateral hyperplasia, adrenal carcinoma producing aldosterone, and rare familial cases. For many years research concentrated on the differentiation of aldosterone-producing adenoma from other forms of primary aldosteronism, as the tumors could be treated with surgery resulting in either cure or significant improvement in the hypertension and usually full correction of the hypokalemia. Idiopathic hyperaldosteronism is treated medically. Primary aldosteronism is associated with a significant increase in cardiovascular morbidity and mortality. Atrial fibrillation, congestive heart failure and ischemic heart disease are 2-5 times more prevalent in patients with PA (Savard et al., 2013) and there was an increase in 14 yr. mortality in aldosterone-producing adenoma patients over matched patients with essential hypertension in the German Conn's Registry (Reincke et al., 2012).

2.1 Molecular pathogenesis of aldosterone-producing adenoma

Studies of pathogenesis of aldosterone-producing adenomas up to 2011 concentrated in the search for altered expression of genes using microarray technology, particularly of aberrant G-protein-coupled receptors that might regulate aldosterone production. Aberrant receptors were found in many aldosterone-producing adenomas including GnRH (Ye et al., 2007), LH (Saner-Amigh et al., 2006), vasopressin (Perraudin et al., 2006), serotonin (Ye et al., 2007), endothelin receptor type B-like (GPR37) and glutamate receptor metabotropic 3 (Ye et al., 2007). These receptors may play a role in occasional patients with PA (Zwermann et al., 2009), but do not explain the pathogenesis of aldosterone-producing adenomas in most patients (Ye et al., 2007). During the 1970-80s there was a futile search for unidentified aldosterone-stimulating factors to explain the pathogenesis of idiopathic hyperaldosteronism or even aldosterone-producing adenomas.

2.2 Somatic mutation of the *KCNJ5* gene

A major advance occurred in 2011 when the Lifton group reported the results of whole exome sequencing of a group of aldosterone-producing adenomas and the identification of somatic mutations of the gene for G-protein-activated potassium inward rectifying channel *KCNJ5*, with mutations G151R and L168R, in approximately 30% of the aldosterone-producing adenomas studied and an additional mutation at T158A a family of patients with familial hyperaldosteronism type 3 (Choi et al., 2011). These mutations occur in or next to the selectivity filter of the channel of *KCNJ5*, also called the Kir3.4 channel. Since then multiple other mutations of the *KCNJ5* have been found (reviewed in (Gomez-Sanchez and Oki, 2014). These mutations within the same region of the selectivity filter and some outside the selectivity filter (Murthy et al., 2014) result in a significant decrease in potassium selectivity and leakage of sodium to the cell, depolarizing the membrane and opening of calcium channels, resulting in activation of the calcium-calmodulin kinase pathway and transcriptional activation of the enzymatic machinery for the biosynthesis of aldosterone (Choi et al., 2011, Oki et al., 2012, Tauber et al., 2014). In European cohorts somatic mutations of the *KCNJ5* in aldosterone-producing adenomas have been found preferentially in younger females with a total incidence of around 30-40 % of aldosterone-producing adenomas (Akerstrom et al., 2012, Azizan et al., 2012, Boulkroun et al., 2012, Fernandes-Rosa et al., 2014, Kuppasamy et al., 2014, Mulatero et al., 2012, Scholl et al., 2012, Taguchi et al., 2012, Williams et al., 2014), while in Japan the incidence is around 60% with no sexual predominance (Taguchi et al., 2012). Germ line mutations of the *KCNJ5* gene may not be rare. A single nucleotide polymorphism that adversely affected inward-rectification and selectivity of the *KCNJ5* channel was found in 5% of a cohort of 251 patients with primary aldosteronism in the United Kingdom and Australia (Murthy et al., 2014). A significant number of these patients had bilateral hyperaldosteronism (Murthy et al., 2014). The adrenal glands with an aldosterone-producing adenoma expressing *KCNJ5* mutations often exhibit other abnormalities, including hyperplastic regions surrounding the adenoma and nodules that express stem/progenitor cell markers (Boulkroun et al., 2010), (Boulkroun et al., 2011). Not all of these nodules express the *KCNJ5* mutations but those that do also express the last enzyme in the steroidogenic cascade for aldosterone biosynthesis, the CYP11B2 enzyme (Dekkers et al., 2014).

Further exome sequencing of aldosterone-producing adenomas uncovered other somatic gene mutations, including those of the *ATP1A1* (sodium/potassium ATPase alpha subunit), *ATP2B3* (membrane calcium ATPase 3 or PMCA3) and *CACNA1D* (calcium channel, Ca_v1.3) genes (Azizan et al., 2013, Beuschlein et al., 2013, Scholl et al., 2013). Table 1 shows the relative incidence of the various mutations in the series reported.

2.3 Somatic mutations of the *ATP1A1* gene in aldosterone-producing adenoma

Exome sequencing of aldosterone-producing adenomas of males negative for a *KCNJ5* mutation led to the identification of mutations of the *ATP1A1* and *ATP2B3* genes (Beuschlein et al., 2013). Three somatic mutations were found in the *ATP1A1* gene, including L104R, V332G substitutions and a deletion of amino acids 100-104. Additional mutations of the *ATP1A1* gene were soon found, including a substitution of residues

960-963 by a serine (EETA963S) (Azizan et al., 2013); and an additional mutation at G99R (Fernandes-Rosa et al., 2014, Williams et al., 2014).

3 Sodium Potassium ATPase: ATPase structure and functions

3.1 Alpha subunit

Members of the P-type ATPase protein families are key players in the maintenance of electrochemical gradients across membranes. They share a common motif (DKTGT) which is autophosphorylated by ATP on the aspartic acid that is subsequently auto-hydrolyzed while exerting its function. The Na^+/K^+ -ATPase is located in the plasma membrane where it functions as a pump that transports three Na^+ out of the cells in exchange for two K^+ transported into the cell for each ATP hydrolyzed, creating the gradients for Na^+ and K^+ essential for cellular function (Einholm et al., 2007a). In 1957 J.C. Skou first reported ATP-hydrolyzing activity stimulated by altering concentrations of sodium and potassium in neuronal membrane fraction from crab nerve cells (Skou, 1957), a discovery that led to being awarded the Nobel Prize in 1997. The Na^+/K^+ ATPase pump is a heterodimer of two subunits, α and β and an auxiliary FXYD protein (also named γ subunit) bound to the membrane (Kanai et al., 2013, Lingrel et al., 2003). Four isoforms of the α subunit ($\alpha 1$ -4) and three isoforms for the β subunit ($\beta 1$ -3) occur in mammals. The α isoforms are expressed in a developmental- and tissue-specific manner (Kaplan, 2002, Lingrel et al., 2003). The $\alpha 1$ isoform is expressed in every tissue and is encoded by the *ATP1A1* gene. The catalytic α -subunit (~1,000 amino acid residues and 110kDa) consists of three cytoplasmic domains: actuator (A), nucleotide binding (N) and phosphorylation (P) domains and ten membrane-spanning helices (M1-M10), with intracellular N- and C- terminal domains. The α -subunit has four M1-M2, M3-M4, M5-M6, and M7-M8 helical pairs that are sequentially juxtaposed in the membrane. The N and P domains are formed by the M4, M5 loop, the A domain is formed by the N-terminal tail and the M2 M3 loop (Kaplan, 2002). Among 4 intracellular loops, these two intracellular loops are functionally more significant. The large loop of about 430 amino acids located between M4 and M5 facilitates ATP binding; its highly conserved sequence is a molecular signature of P-ATPases. The long N-terminal of about 90 amino acids and smaller loop of 120 in M2 and M3 facilitate conformational changes associated with energy transduction. Interactions of the transmembrane segment M1 with segment M4 promote the initiation of ion binding and the intracellular N-terminal domain might play a role in cation gating mechanism.

3.2 Beta subunit

The glycosylated regulatory β -subunits (~370 amino acids) are smaller and display a single membrane-spanning domain and a large extracellular domain (~300 amino acids) with three N-linked glycosylation sequences and three S-S bridges in the extracellular domain. Four isoforms have been described. The main site of interaction of the β -subunit (Cys46) with the α -subunit is the M7-M8 ectodomain. The β -subunit stabilizes the α -subunit and facilitates its routing and insertion into the membrane, as well as affects functional properties of the pump, including cation affinity. Although the β -subunit has no enzymatic or transport activity, its association with an α -subunit is an absolute requirement for ATPase and pump activities (Feraille and Doucet, 2001).

3.3 Mechanism of transport

P-type ATPases form a covalent acylphosphate enzyme intermediate resulting from the transfer of a γ -phosphoryl group of ATP to a conserved aspartyl residue within the protein. According to the Post-Albers reaction cycle, ion movement across the membrane is coupled to ATP hydrolysis via a cation-dependent inside-open E1 and outside-open E2 states, together with phosphorylated intermediates E1P and E2P (Post et al., 1972). During the ATP-driven transport cycle, 3 cytoplasmic Na^+ are exported and exchanged for 2 extracellular K^+ coupled to the $\text{E1/E1-P} \leftrightarrow \text{E2/E2-P}$ conformational transition, where E1 and E2 denote high affinity binding for Na^+ and K^+ ions and P denotes the phosphorylated state. Intracellular Na^+ and ATP binding stimulate phosphorylation of a conserved aspartyl residue forming the Na^+ -occluded E1P-ADP state. As ADP leaves the enzyme there is a conformation transition and the Na^+ ions leave the protein at the extracellular surface. This E2-P binds K^+ ions at the outer surface and dephosphorylates the enzyme, producing E2K2. Release of K^+ is catalyzed by ATP and the enzyme returns from E2 to E1 form and ready to initiate a new cycle (Einholt et al., 2007a, Einholt et al., 2007b, Kanai et al., 2013, Kaplan, 2002). Sodium/potassium ATPase ion pumping represents 20% to 80% of cellular resting energy metabolic rate in a tissue- and cell-specific manner, consuming 20-30% of ATP production to actively transport Na^+ out of and K^+ into the cell (Michiels, 2004). The cycle is summarized schematically in Figure 1.

3.4 Sodium Potassium ATPase interaction with other proteins

The Na^+/K^+ -ATPase interacts with many membrane and soluble proteins, including ankyrin and some others in caveolae (Liu and Xie, 2010), and, in addition to its ion transport function, is a classical receptor capable of binding to cardiotonic steroids like ouabain and serve as a signal transducer activating various protein kinase cascades (Aperia, 2012). The signaling cascade of the sodium potassium ATPase is activated by ouabain at concentrations below those required to inhibit the pump activity and promotes tissue-protective effects of growth and inhibition of apoptosis (Aperia, 2012). In epithelial cells the β -subunits from neighboring cells form bridges that are important for the integrity of intracellular junctions. In the kidney tubular cells, the bridges of the Na^+/K^+ -ATPase β -subunits between adjacent cells prevent the translocation of the enzyme to the apical membrane and maintain a polarized cell (Aperia, 2012). Studies from the Xie and Aperia laboratories have demonstrated that picomolar to nanomolar concentrations of ouabain, below those that inhibit the Na^+/K^+ -ATPase pump, produce a sustained increase of intracellular calcium concentration that could be attributed to a negative feedback on the sodium-calcium exchanger (Aperia, 2012, Aperia, 2007, Li and Xie, 2009, Liang et al., 2006, Xie and Xie, 2005). The ability for low concentrations of ouabain to stimulate proliferation and increase viability in many cell types including epithelial cells depend upon both the Src and IP3R pathways (Li et al., 2006, Tian et al., 2009). The interaction between the Na^+/K^+ -ATPase and Src keeps Src in an inactive state; binding to ouabain reduces the binding of the Src kinase domain, thus activating the Src signaling cascade (Li and Xie, 2009). Graded knockdown of Na^+/K^+ -ATPase in LLC-PK1 cells transfected with an $\alpha 1$ -specific siRNA resulted in a significant increase in basal Src activity and increased phosphorylation of FAK, a Src effector (Li and Xie, 2009). Pump activity and Src modulation are mediated through

different areas of the protein and it is possible to create a protein that retains pump activity, but not Src activity by mutagenesis (Lai et al., 2013).

3.5 Ouabain and aldosterone biosynthesis

Rat adrenal zona glomerulosa cells have a higher Na⁺/K⁺ ATPase pump activity than those in the rest of the adrenal. Angiotensin II inhibits the Na⁺/K⁺ ATPase in rat zona glomerulosa cells (Hajnoczky et al., 1992) and stimulates aldosterone secretion suggesting that inhibition of the Na⁺/K⁺ ATPase pump has a role in aldosterone secretion (Hajnoczky et al., 1992, Yingst et al., 1999). Ouabain in the rat also induces a nifedipine-sensitive stimulation of aldosterone secretion (Yingst et al., 1999) and chronic infusion of ouabain enhanced the growth and steroidogenic capacity of the rat zona glomerulosa (Neri et al., 2006). Knockout of the ATP1A1 in mice is lethal, however, heterozygotes survive and exhibit an increase in plasma aldosterone, however it is not clear if this is a direct effect of the haplo-insufficiency on the adrenal zona glomerulosa or an indirect effect through its effect on the kidney (Moseley et al., 2005). It is known that the ATP1A1 subunit of rats and mice is resistant to the effects of ouabain (Wansapura et al., 2011). In contrast, neither digoxin nor ouabain affected plasma aldosterone or Cortisol in monkeys (Kau et al., 2009) and ouabain inhibited aldosterone secretion by cultured human adrenal zona glomerulosa cells (Antonipillai et al., 1996). The amphibian cardiotonic steroids bufalin and cinobufagin inhibited synthesis of aldosterone and Cortisol associated with decreased expression of the CYP11B1 and CYP11B2 enzymes as well as StAR protein in the H295R cell (Kau et al., 2012). The stimulatory effect of ouabain on aldosterone biosynthesis in rodents might not be mediated through inhibition of the Na⁺/K⁺ pump as this is resistant to the effects of ouabain, but through other signaling functions of the Na⁺/K⁺ ATPase (Aperia, 2012, Xie and Xie, 2005).

4 Somatic mutations of the ATP1A1 gene in aldosterone-producing adenomas

Exome sequencing of tumor and adjacent adrenal cortex from nine males with aldosterone-producing adenoma with no *KCNJ5* mutations found several somatic variants in two members of the P-type ATPase family, *ATP1A1* coding for the α -1 subunit of the Na⁺/K⁺ ATPase and *ATP2B3* coding for the membrane calcium ATPase (PMC3) (Beuschlein et al., 2013). To date, five somatic mutations have been described for the *ATP1A1* gene, L104R, V332G, a deletion of F100-104L (Beuschlein et al., 2013), G99R (Williams et al., 2014) and a substitution of residues 960-963 by a serine (EETA963S) (Azizan et al., 2013). It was reported that the morphology of the aldosterone-producing adenoma cells with the *ATP1A1* mutations resemble zona glomerulosa cells, in contrast to those with the more common *KCNJ5* mutations that resemble zona fasciculata cells (Azizan et al., 2013), however this was not confirmed by another group (Fernandes-Rosa et al., 2014).

4.1 Potential mechanisms of hypersecretion of aldosterone and adenoma formation

The L104 residue in transmembrane helix M1 and the V332 in M4 are in close approximation to residue E334 in M4 which is crucial for K⁺ binding and gating of the binding pocket (Beuschlein et al., 2013, Kopec et al., 2014). Molecular dynamic simulations

with the L104R, the substitution of a hydrophobic amino acid, leucine, with a large charged amino acid, arginine, disturbs ion binding and coordination and promotes hydration of regions near the mutated residues (Kopec et al., 2014). The deletion F100-104L promotes similar hydration of adjacent residues, but the effect is more marked. Similar effects are expected for the adjacent G99R mutation (Williams et al., 2014). The insertion mutation EETA963S is in a different area, near the C-terminus affecting the III sodium-specific binding site and appears to open a new water pathway (Kopec et al., 2014). To examine the functional consequences of L104R and V332G, Beuschlein *et al* transfected COS cells with mutated ouabain-insensitive rat cDNA (Beuschlein et al., 2013). The addition of ouabain to inhibit the endogenous pump resulted in no viable colonies, indicating that the transfected mutant had no or insignificant Na⁺/K⁺ ATPase pump activity and could not rescue the cell from complete inhibition of the endogenous enzyme. They had to use siRNAs to knock down specifically the endogenous COS cell Na⁺/K⁺ ATPase α subunit and decrease the endogenous activity enough to allow enzymatic measurements of the transfected mutant enzyme to compare the wild-type and mutated enzyme. ATPase activity was undetectable for the L104R and very low for the V332G variant under optimal conditions for ATPase activity of the wild enzyme. The mutant enzymes were able to react with ATP and be phosphorylated in a Na⁺ ion-dependent reaction, although phosphorylation was somewhat reduced (Beuschlein et al., 2013). K⁺ inhibition of phosphorylation in the presence of 50 mM Na⁺ was slightly decreased in the mutant enzymes indicating a decreased affinity for the K⁺ ion. Electrophysiological examination showed that adenoma cells with ATP1A1 mutations had a higher degree of depolarization compared to cells adjacent to the adenoma. Removal of extracellular sodium hyperpolarized all cells, but the degree of hyperpolarization was greater in cells with *ATP1A1* mutations. This suggested that the strong depolarization was not primarily caused by higher Na⁺ ion conductance, but possibly by disturbed intracellular ion composition. ATPase phosphorylation is inhibited by K⁺ and *in vitro* can be used as a measure of K⁺ affinity (Williams et al., 2014). Compared to the wild type the G99R mutant also displayed a reduced affinity for Na⁺ activation of phosphorylation by the Mg ATP and was less sensitive to inhibition of phosphorylation by K⁺ (Williams et al., 2014). Membranes of HEK293 cells transfected with cDNA of the G99R mutated ATPase were depolarized compared to wild type. The difference in membrane voltage was also observed after removal of Na⁺ from the medium indicating that it was not caused by increased Na⁺ conductance, but rather by a disturbed ionic gradient and/or loss of net charge transport (Williams et al., 2014). These studies suggested that aldosterone secretion is mediated by a loss of functional ATP1A1 pump activity as a consequence of the haploinsufficiency (Beuschlein et al., 2013, Williams et al., 2014).

Azizan *et al* (Azizan et al., 2013) tested the effect of the mutations of the human Na/K ATPase enzyme on *Xenopus* oocytes and suggested that gain-of-function occurs with these mutations. In transfections with the wild type pump, extracellular K⁺ activates forward pumping with maximal current generated at positive membrane potentials. Pumping activity of the L104R and del100_104 and V332G mutants was not stimulated by K⁺, however at physiological membrane potentials they were marked ouabain-sensitive, producing a voltage-dependent inward current that was partially inhibited by K⁺. The EETA963S mutant also did not respond to K⁺ with forward pumping, but had a ouabain-sensitive inward

current, and under physiologically relevant conditions the net current was inward (Azizan et al., 2013). Varying extracellular pH and exchanging Na^+ for N-methyl-D-glucamine to determine which ions carry the currents, Azizan *et al* (Azizan et al., 2013) found that a change in pH of 1 changed the reversal potential by about 20 mV, whereas removal of extracellular Na^+ had little effect, suggesting that protons were the main carrier of the current in the L104R mutant enzyme (Fig 2C). In contrast, the del100_104 mutant responded poorly to changes in pH, whereas Na^+ removal shifted the reversal potential, suggesting that Na^+ was the main carrier of current by this mutated ATPase (Fig 2D). Transfection of the adrenal carcinoma cell line H295R with the rat ouabain-insensitive wild-type and rat mutant L104R cDNA showed an increased in aldosterone secretion and expression of the CYP11B2 mRNA when incubated with 10 μM ouabain, as well as an enhanced response to angiotensin II (Azizan et al., 2013). The mutated residue L104R is more hydrated than the wild type, which ultimately leads to a proton leak, while the V332G mutant does not promote hydration of surrounding residues, but mimics the structural behavior of the L104R (Kopeck et al., 2014). In the del100_104 a broader opening is observed because of rearrangements of the M1 helix, which might explain the sodium leak.

In summary, the mutations described for the ATP1A1 in aldosterone-producing adenomas produce complex changes in the function of the enzyme. They completely or significantly inhibit the activity of the enzyme as measured by the liberation of inorganic phosphate from ATP, inhibit phosphorylation of the enzyme mildly and decrease the inhibition of phosphorylation induced by K^+ (Beuschlein et al., 2013, Williams et al., 2014) indicating loss of pump function produced by the mutations. However, mutated human enzymes transfected into *Xenopus* oocytes, produce inward ouabain-sensitive Na^+ currents leading to depolarization of the membrane due either to an inward proton-dependent current for the L104R mutant or sodium-dependent current for the del100-104 mutant, which indicates a gain of function (Azizan et al., 2013). Future studies should clarify how alteration of the pump activity or other signaling functions of the ATP1A1 enzyme are responsible for the intracellular signals that increase aldosterone secretion and stimulate cell proliferation to form an aldosterone producing adenoma.

5. Summary

Somatic mutations of various ion channels or pumps including the potassium channel KCNJ5, the Na^+/K^+ -ATPase $\alpha 1$ subunit, the calcium ATPase 3 and the slowly inactivating calcium channel, $\text{Ca}_v1.3$, appear to be responsible for the increased mobilization of calcium and stimulation of aldosterone synthesis in a large proportion of aldosterone-producing adenoma. It is unclear as yet whether or how these mutations increase cellular proliferation. Judging by the rapid pace of discovery since the first aldosterone-producing adenoma mutations were described, the mechanisms leading to increased aldosterone biosynthesis and adenoma formation should soon be clarified.

Acknowledgments

This work was supported by NIH grants R01HL27255.

References

- Abdelhamid S, Blomer R, Hommel G, Haack D, Lewicka S, Fiegel P, Krumme B. Urinary tetrahydroaldosterone as a screening method for primary aldosteronism: a comparative study. *Am J Hypertens*. 2003; 16:522–30. [PubMed: 12850384]
- Akerstrom T, Crona J, Delgado Verdugo A, Starker LF, Cupisti K, Willenberg HS, Knoefel WT, Saeger W, Feller A, Ip J, Soon P, Anlauf M, Alesina PF, Schmid KW, Decaussin M, Levillain P, Wangberg B, Peix JL, Robinson B, Zedenius J, Backdahl M, Caramuta S, Iwen KA, Botling J, Stalberg P, Kraimps JL, Dralle H, Hellman P, Sidhu S, Westin G, Lehnert H, Walz MK, Akerstrom G, Carling T, Choi M, Lifton RP, Bjorklund P. Comprehensive Re-Sequencing of Adrenal Aldosterone Producing Lesions Reveal Three Somatic Mutations near the KCNJ5 Potassium Channel Selectivity Filter. *PLoS One*. 2012; 7:e41926. [PubMed: 22848660]
- Antonipillai I, Schick K, Horton R. Ouabain is a potent inhibitor of aldosterone secretion and angiotensin action in the human adrenal. *J Clin Endocrinol Metab*. 1996; 81:2335–7. [PubMed: 8964873]
- Aperia A. 2011 Homer Smith Award: To serve and protect: classic and novel roles for Na⁺, K⁺ - adenosine triphosphatase. *J Am Soc Nephrol*. 2012; 23:1283–90. [PubMed: 22745476]
- Aperia A. New roles for an old enzyme: Na,K-ATPase emerges as an interesting drug target. *J Intern Med*. 2007; 261:44–52. [PubMed: 17222167]
- Azizan EA, Murthy M, Stowasser M, Gordon R, Kowalski B, Xu S, Brown MJ, O'Shaughnessy KM. Somatic Mutations Affecting the Selectivity Filter of KCNJ5 Are Frequent in 2 Large Unselected Collections of Adrenal Aldosteronomas. *Hypertension*. 2012; 59:587–91. [PubMed: 22252394]
- Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, Maniero C, Garg S, Bochukova EG, Zhao W, Shaikh LH, Brighton CA, Teo AE, Davenport AP, Dekkers T, Tops B, Kusters B, Ceral J, Yeo GS, Neogi SG, McFarlane I, Rosenfeld N, Marass F, Hadfield J, Margas W, Chaggar K, Solar M, Deinum J, Dolphin AC, Farooqi IS, Striessnig J, Nissen P, Brown MJ. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet*. 2013; 45:1055–60. [PubMed: 23913004]
- Berglund G, Andersson O, Wilhelmsen L. Prevalence of primary and secondary hypertension: studies in a random population sample. *Br Med J*. 1976; 2:554–6. [PubMed: 963430]
- Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, Penton D, Schack VR, Amar L, Fischer E, Walther A, Tauber P, Schwarzmayr T, Diener S, Graf E, Allolio B, Samson-Couterie B, Benecke A, Quinkler M, Fallo F, Plouin PF, Mantero F, Meitinger T, Mulatero P, Jeunemaitre X, Warth R, Vilsen B, Zennaro MC, Strom TM, Reincke M. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet*. 2013; 45:440–4. [PubMed: 23416519]
- Boulkroun S, Beuschlein F, Rossi GP, Golib-Dzib JF, Fischer E, Amar L, Mulatero P, Samson-Couterie B, Hahner S, Quinkler M, Fallo F, Letizia C, Allolio B, Ceolotto G, Cicala MV, Lang K, Lefebvre H, Lenzini L, Maniero C, Monticone S, Perrocheau M, Pilon C, Plouin PF, Rayes N, Seccia TM, Veglio F, Williams TA, Zinamosca L, Mantero F, Benecke A, Jeunemaitre X, Reincke M, Zennaro MC. Prevalence, Clinical, and Molecular Correlates of KCNJ5 Mutations in Primary Aldosteronism. *Hypertension*. 2012; 59:592–8. [PubMed: 22275527]
- 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; 56:885–92. [PubMed: 20937967]
- Boulkroun S, Samson-Couterie B, Golib-Dzib JF, Amar L, Plouin PF, Sibony M, Lefebvre H, Louiset E, Jeunemaitre X, Meatchi T, Benecke A, Lalli E, Zennaro MC. Aldosterone-Producing Adenoma Formation in the Adrenal Cortex Involves Expression of Stem/Progenitor Cell Markers. *Endocrinology*. 2011; 152:4753–63. [PubMed: 21971159]
- Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Patel A, Men CJ, Lolis E, Wisgerhof MV, Geller DS, Mane S, Hellman P, Westin G, Akerstrom G, Wang W, Carling T, Lifton RP. K⁺ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science*. 2011; 331:768–72. [PubMed: 21311022]

- Conn JW. Primary aldosteronism, a new clinical syndrome. *J Lab Clin Med.* 1955; 45:3–7. [PubMed: 13233623]
- Conn JW, Knopf RF, Nesbit RM. Clinical characteristics of primary aldosteronism from an analysis of 145 cases. *Am J Surg.* 1964; 107:159–172. [PubMed: 14099489]
- Dekkers T, ter Meer M, Lenders JW, Hermus AR, Schultze Kool L, Langenhuijsen JF, Nishimoto K, Ogishima T, Mukai K, Azizan EA, Tops B, Deinum J, Kusters B. Adrenal nodularity and somatic mutations in primary aldosteronism: one node is the culprit? *J Clin Endocrinol Metab.* 2014; 99:E1341–51. [PubMed: 24758183]
- Einhorn AP, Andersen JP, Vilsen B. Importance of Leu99 in transmembrane segment M1 of the Na⁺, K⁺-ATPase in the binding and occlusion of K⁺ *J Biol Chem.* 2007a; 282:23854–66. [PubMed: 17553789]
- Einhorn AP, Andersen JP, Vilsen B. Roles of transmembrane segment M1 of Na⁺,K⁺-ATPase and Ca²⁺-ATPase, the gatekeeper and the pivot. *J Bioenerg Biomembr.* 2007b; 39:357–66. [PubMed: 18058007]
- Fardella CE, Mosso L, Gomez-Sanchez CE, Soto J, Montero J, Gomez L, Pinto M, Oestreicher E, Cortes P. Primary hyperaldosteronism in essential hypertensives: Prevalence, biochemical profile and molecular biology. *J Clin Endocrinol Metab.* 2000; 85:1863–1867. [PubMed: 10843166]
- Feraillie E, Doucet A. Sodium-potassium-adenosinetriphosphatase-dependent sodium transport in the kidney: hormonal control. *Physiol Rev.* 2001; 81:345–418. [PubMed: 11152761]
- Fernandes-Rosa FL, Williams TA, Riester A, Steichen O, Beuschlein F, Boulkroun S, Strom TM, Monticone S, Amar L, Meatchi T, Mantero F, Cicala MV, Quinkler M, Fallo F, Allolio B, Bernini G, Maccario M, Giacchetti G, Jeunemaitre X, Mulatero P, Reincke M, Zennaro MC. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension.* 2014; 64:354–61. [PubMed: 24866132]
- Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, Young WF Jr, Montori VM. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2008; 93:3266–81. [PubMed: 18552288]
- Gomez-Sanchez CE, Oki K. Minireview: potassium channels and aldosterone dysregulation: is primary aldosteronism a potassium channelopathy? *Endocrinology.* 2014; 155:47–55. [PubMed: 24248457]
- Hajnoczky G, Csordas G, Hunyady L, Kalapos MP, Balla T, Enyedi P, Spat A. Angiotensin-II inhibits Na⁺/K⁺ pump in rat adrenal glomerulosa cells: possible contribution to stimulation of aldosterone production. *Endocrinology.* 1992; 130:1637–44. [PubMed: 1311245]
- Hannemann A, Bidlingmaier M, Friedrich N, Manolopoulou J, Spyroglou A, Volzke H, Beuschlein F, Seissler J, Rettig R, Felix SB, Biffar R, Doring A, Meisinger C, Peters A, Wichmann HE, Nauck M, Wallaschofski H, Reincke M. Screening for primary aldosteronism in hypertensive subjects: results from two German epidemiological studies. *Eur J Endocrinol.* 2012; 167:7–15. [PubMed: 22495491]
- Hiramatsu K, Yamada T, Yukimura Y, Komiya I, Ichikawa M, Nagata H, Izumiyama T. A screening test to identify aldosterone-producing adenoma by measuring plasma renin activity. Results in hypertensive patients. *Arch Intern Med.* 1981; 141:1589–1593. [PubMed: 7030245]
- Kanai R, Ogawa H, Vilsen B, Cornelius F, Toyoshima C. Crystal structure of a Na⁺-bound Na⁺,K⁺-ATPase preceding the E1P state. *Nature.* 2013; 502:201–6. [PubMed: 24089211]
- Kaplan JH. Biochemistry of Na,K-ATPase. *Annu Rev Biochem.* 2002; 71:511–35. [PubMed: 12045105]
- Kau MM, Kan SF, Wang JR, Wang PS, Lau YT, Wang SW. Acute effects of digoxin on plasma aldosterone and Cortisol in monkeys. *Metabolism.* 2009; 58:55–61. [PubMed: 19059531]
- Kau MM, Wang JR, Tsai SC, Yu CH, Wang PS. Inhibitory effect of bufalin and cinobufagin on steroidogenesis via the activation of ERK in human adrenocortical cells. *Br J Pharmacol.* 2012; 165:1868–76. [PubMed: 21913902]
- Kopeck W, Loubet B, Poulsen H, Khandelia H. Molecular mechanism of Na⁽⁺⁾,K⁽⁺⁾-ATPase malfunction in mutations characteristic of adrenal hypertension. *Biochemistry.* 2014; 53:746–54. [PubMed: 24428543]

- 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:E1765–73. [PubMed: 25057880]
- Lai F, Madan N, Ye Q, Duan Q, Li Z, Wang S, Si S, Xie Z. Identification of a Mutant $\alpha 1$ Na/K-ATPase That Pumps but Is Defective in Signal Transduction. *J Biol Chem.* 2013; 288:13295–304. [PubMed: 23532853]
- Li J, Zelenin S, Aperia A, Aizman O. Low doses of ouabain protect from serum deprivation-triggered apoptosis and stimulate kidney cell proliferation via activation of NF-kappaB. *J Am Soc Nephrol.* 2006; 17:1848–57. [PubMed: 16707566]
- Li Z, Xie Z. The Na/K-ATPase/Src complex and cardiotonic steroid-activated protein kinase cascades. *Pflugers Arch.* 2009; 457:635–44. [PubMed: 18283487]
- Liang M, Cai T, Tian J, Qu W, Xie ZJ. Functional characterization of Src-interacting Na/K-ATPase using RNA interference assay. *J Biol Chem.* 2006; 281:19709–19. [PubMed: 16698801]
- Lingrel J, Moseley A, Dostanic I, Cougnon M, He S, James P, Woo A, O'Connor K, Neumann J. Functional roles of the α isoforms of the Na,K-ATPase. *Ann NY Acad Sci.* 2003; 986:354–9. [PubMed: 12763850]
- Liu J, Xie ZJ. The sodium pump and cardiotonic steroids-induced signal transduction protein kinases and calcium-signaling microdomain in regulation of transporter trafficking. *Biochim Biophys Acta.* 2010; 1802:1237–45. [PubMed: 20144708]
- Michiels C. Physiological and pathological responses to hypoxia. *Am J Pathol.* 2004; 164:1875–82. [PubMed: 15161623]
- Moseley AE, Huddleson JP, Bohanan CS, James PF, Lorenz JN, Aronow BJ, Lingrel JB. Genetic profiling reveals global changes in multiple biological pathways in the hearts of Na, K-ATPase $\alpha 1$ isoform haploinsufficient mice. *Cell Physiol Biochem.* 2005; 15:145–58. [PubMed: 15706699]
- Mosso L, Carvajal C, Gonzalez A, Barraza A, Avila F, Montero J, Huete A, Gederlini A, Fardella CE. Primary aldosteronism and hypertensive disease. *Hypertension.* 2003; 42:161–5. [PubMed: 12796282]
- Mulatero P, Tauber P, Zennaro MC, Monticone S, Lang K, Beuschlein F, Fischer E, Tizzani D, Pallauf A, Viola A, Amar L, Williams TA, Strom TM, Graf E, Bandulik S, Penton D, Plouin PF, Warth R, Allolio B, Jeunemaitre X, Veglio F, Reincke M. KCNJ5 Mutations in European Families With Nonglucocorticoid Remediable Familial Hyperaldosteronism. *Hypertension.* 2012; 59:235–240. [PubMed: 22203740]
- 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; 63:783–9. [PubMed: 24420545]
- Neri G, De Toni R, Tortorella C, Rebuffat P, Bova S, Cargnelli G, Petrelli L, Spinazzi R, Nussdorfer GG. Ouabain chronic infusion enhances the growth and steroidogenic capacity of rat adrenal zona glomerulosa: the possible involvement of the endothelin system. *Int J Mol Med.* 2006; 18:315–9. [PubMed: 16820940]
- 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; 153:1774–82. [PubMed: 22315453]
- Perraudin V, Delarue C, Lefebvre H, Do Rego JL, Vaudry H, Kuhn JM. Evidence for a role of vasopressin in the control of aldosterone secretion in primary aldosteronism: in vitro and in vivo studies. *J Clin Endocrinol Metab.* 2006; 91:1566–72. [PubMed: 16449345]
- Post RL, Hegyvary C, Kume S. Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. *J Biol Chem.* 1972; 247:6530–40. [PubMed: 4263199]
- Reincke M, Fischer E, Gerum S, Merkle K, Schulz S, Pallauf A, Quinkler M, Hanslik G, Lang K, Hahner S, Allolio B, Meisinger C, Holle R, Beuschlein F, Bidlingmaier M, Endres S.

- Observational Study Mortality in Treated Primary Aldosteronism: The German Conn's Registry. Hypertension. 2012; 60:618–624. [PubMed: 22824982]
- Saner-Amigh K, Mayhew BA, Mantero F, Schiavi F, White PC, Rao CV, Rainey WE. Elevated expression of luteinizing hormone receptor in aldosterone-producing adenomas. J Clin Endocrinol Metab. 2006; 91:1136–42. [PubMed: 16332935]
- Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular Complications Associated With Primary Aldosteronism: A Controlled Cross-Sectional Study. Hypertension. 2013; 62:331–6. [PubMed: 23753408]
- Scholl UI, Goh G, Stolting G, de Oliveira RC, Choi M, Overton JD, Fonseca AL, Korah R, Starker LF, Kunstman JW, Prasad ML, Hartung EA, Mauras N, Benson MR, Brady T, Shapiro JR, Loring E, Nelson-Williams C, Libutti SK, Mane S, Hellman P, Westin G, Akerstrom G, Bjorklund P, Carling T, Fahlke C, Hidalgo P, Lifton RP. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. Nat Genet. 2013; 45:1050–4. [PubMed: 23913001]
- 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; 109:2533–8. [PubMed: 22308486]
- Skou JC. The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochim Biophys Acta. 1957; 23:394–401. [PubMed: 13412736]
- Taguchi R, Yamada M, Nakajima Y, Satoh T, Hashimoto K, Shibusawa N, Ozawa A, Okada S, Rokutanda N, Takata D, Koibuchi Y, Horiguchi J, Oyama T, Takeyoshi I, Mori M. Expression and Mutations of KCNJ5 mRNA in Japanese Patients with Aldosterone-Producing Adenomas. J Clin Endocrinol Metab. 2012; 97:1311–9. [PubMed: 22278422]
- Tauber P, Penton D, Stindl J, Humberg E, Tegtmeier I, Sterner C, Beuschlein F, Reincke M, Barhanin J, Bandulik S, Warth R. Pharmacology and pathophysiology of mutated KCNJ5 found in adrenal aldosterone-producing adenomas. Endocrinology. 2014; 155:1353–62. [PubMed: 24506072]
- Tian J, Li X, Liang M, Liu L, Xie JX, Ye Q, Kometiani P, Tillekeratne M, Jin R, Xie Z. Changes in sodium pump expression dictate the effects of ouabain on cell growth. J Biol Chem. 2009; 284:14921–9. [PubMed: 19329430]
- Wansapura AN, Lasko VM, Lingrel JB, Lorenz JN. Mice expressing ouabain-sensitive alpha1-Na,K-ATPase have increased susceptibility to pressure overload-induced cardiac hypertrophy. Am J Physiol Heart Circ Physiol. 2011; 300:H347–55. [PubMed: 20952666]
- Williams TA, Monticone S, Schack VR, Stindl J, Burrello J, Buffolo F, Annaratone L, Castellano I, Beuschlein F, Reincke M, Lucatello B, Ronconi V, Fallo F, Bernini G, Maccario M, Giacchetti G, Veglio F, Warth R, Vilsen B, Mulatero P. Somatic ATP1A1, ATP2B3, and KCNJ5 mutations in aldosterone-producing adenomas. Hypertension. 2014; 63:188–95. [PubMed: 24082052]
- Xie Z, Xie J. The Na/K-ATPase-mediated signal transduction as a target for new drug development. Front Biosci. 2005; 10:3100–9. [PubMed: 15970564]
- Ye P, Mariniello B, Mantero F, Shibata H, Rainey WE. G-protein-coupled receptors in aldosterone-producing adenomas: a potential cause of hyperaldosteronism. J Endocrinol. 2007; 195:39–48. [PubMed: 17911395]
- Yingst DR, Davis J, Krenz S, Schiebinger RJ. Insights into the mechanism by which inhibition of Na,K-ATPase stimulates aldosterone production. Metabolism. 1999; 48:1167–71. [PubMed: 10484059]
- Zwermann O, Suttman Y, Bidlingmaier M, Beuschlein F, Reincke M. Screening for membrane hormone receptor expression in primary aldosteronism. Eur J Endocrinol. 2009; 160:443–51. [PubMed: 19131502]

Highlights

- Primary aldosteronism is the most common form of secondary hypertension.
- Mutations in adenomas of the *KCNJ5*, *ATP1A1*, *ATP2B3* and *CACNA1D* have been described.
- *ATP1A1* mutations result in proton or sodium leakage intracellularly.

Figure 1.

Schematic representation of the Post-Albers reaction cycle (Post *et al.*, 1972) of the sodium potassium ATPase. It demonstrates that ATP not only phosphorylates the enzyme, but it can bind the enzyme at various steps and have a regulatory role.

Figure 2.

Schematic representation of the binding and transport of Na⁺ and K⁺ for the wild enzyme and the abnormality in the mutated enzymes del100-104 and L104R whereas in the former the gap produced by the deletion allows leakage of sodium to the intracellular space and in the L104R the main leak is of protons that lead to the intracellular ion abnormality.

Table 1
KCNJ5, ATP1A1, ATP2B3 and CACNA1D mutations prevalence in aldosterone-
producing adenoma from different centers

Mutations	KCNJ5	ATP1A1	ATP2B3	CACNA1D
Beuschlein <i>et al</i> , 2013	-	16/308 (5.2%)	5/308 (1.6%)	-
Azizan <i>et al</i> , 2013	30/73 (41%)	12/152(7.9%)	-	12/152 (7.9%)
Scholl <i>et al</i> , 2013	-	-	-	7/64 (11%)
Williams <i>et al</i> , 2014	44/112 (39.3%)	7/112 (6.3%)	1/112 (0.9%)	-
Dutta <i>et al</i>, 2014	11/35 (31%)	2/35 (6%)	3/35 (9%)	-
Fernandes-Rosa <i>et al</i> , 2014	180/474 (38%)	25/474 (5.3%)	8/474 (1.7%)	44/474(9.3%)
Kuppusamy <i>et al</i> , 2014	48/195 (24.6%)	3/195 (1.5%)	1/195 (0.5%)	-