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Somatic Mutations in Primary Aldosteronism – The Most Common Cause for Secondary Hypertension

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Abstract

Hypertension is highly prevalent in Malaysia and even the rest of the world. Primary aldosteronism (PA) is one of the most common treatable cause of secondary hypertension. PA commonly occurs due to a unilateral aldosterone-producing adenoma (APA) or due to bilateral adrenal hyperplasia. Up to one in five resistant hypertension cases are due to PA. Therefore, there are a high number of individuals who have the potential to be cured of their hypertension. In the past decade, five genes have been found to cause excess aldosterone production in APAs which are *KCNJ5*, *ATP1A1*, *ATP2B3*, *CACNA1D* and *CTNNB1*. These somatic mutations have been found to activate the intracellular signalling pathway that regulates aldosterone production. Herein, we review the genetic causes of PA as a result of aldosterone-stimulating somatic mutations, the expression profile of the aldosterone-driver gene, and the associated mechanism of actions.

Keywords: Primary aldosteronism, hypertension, hyperaldosteronism, mutation

Introduction

Hypertension, or commonly known as high blood pressure, can lead to comorbidities and mortality if uncontrolled as high blood pressure is a major risk factor for stroke and heart disease. Primary aldosteronism (PA) is one of the causes of hypertension that can be treated by controlling or blocking the secretion of aldosterone. PA is also known as Conn syndrome in conjunction with the endocrinologist from America, Jerome W Conn who first characterized the disease (Conn 1966). Clinical manifestations of PA include increased blood pressure which can lead to impaired vision, hypokalemia, muscles cramps, back pain, numbness, tingling sensation and excessive urination. The two main causes of PA are adrenal aldosterone-producing adenomas (APA), commonly unilateral, and adrenal hyperplasia, commonly bilateral (Nishikawa et al. 2011). In these adrenal lesions the excessive secretion of aldosterone is autonomous.

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The physiology of aldosterone is to regulate the homeostasis of water and minerals in the body while maintaining the level of potassium ions (K^+) and sodium ions (Na^+) in the blood. The hormone is secreted by the zona glomerulosa (ZG) in the adrenal gland and regulated by the renin-angiotensin-aldosterone system (Morgan et al. 1996; Tanabe et al. 1998). The renin-angiotensin system begins with the conversion of angiotensinogen to angiotensin I by the proteolytic enzyme renin. Angiotensin I is then cleaved by the angiotensin-conversion enzyme (ACE) into angiotensin II. Angiotensin II then interacts with the angiotensin receptor on the cell surface of the adrenal cortex stimulating aldosterone synthesis in the ZG. The secreted hormone aldosterone then binds to the mineralocorticoid receptor in the renal tubules and activates the expression of the epithelial Na^+ channel (ENaC), Na^+-K^+-ATPase and NaCl co-transporter which causes the reabsorption of Na^+ and chloride ions (Cl^-) (Garty & Palmer 1997; Rossier et al. 2013; Czogalla et al. 2016). The reabsorption contributes to an increase in blood pressure as it causes the reabsorption of water in the kidneys. The adrenocorticotrophic hormone (ACTH) also stimulates the secretion of aldosterone from the adrenal glands, but at a

lower level to angiotensin II. Similarly, the level of K^+ ion in the blood can also stimulate the synthesis of aldosterone (Tanabe et al. 1998). When hypokalemia is present, depolarization of the cell membranes occur which open up the voltage-gated calcium channels (Ca^{2+}) (Spat, 2004). This increase of intracellular Ca^{2+} concentration provides the signal for aldosterone synthesis.

In PA patient, the synthesis of aldosterone hormone is autonomous of the renin-angiotensin-aldosterone system (Funder, 2012). Therefore, the aldosterone to renin-ratio (ARR) can be used as a screening test as the renin is suppressed due to the raised aldosterone which thus leads to an elevated ARR. If detected to have a high ARR, autonomous aldosterone production can be assessed using an oral sodium loading test, saline infusion test (SIT), fludrocortisone test (FST) and captopril challenge test to confirm PA diagnosis (Funder et al. 2016). In the Endocrine Society (USA) clinical practice guideline for the management of PA, adrenal computed tomography (CT) is then recommended as the initial study in subtype testing and to exclude adrenocortical carcinoma (Funder et al. 2016). However, the gold standard to establish or exclude unilateral PA is through bilateral adrenal venous sampling (AVS) by an experienced radiologist. Once confirmed to be unilateral, laparoscopic adrenalectomy of the adrenal affected should be recommended for patients suitable for surgery. Whereas for patients with bilateral adrenal hyperplasia (BAH) or those unsuitable for surgery, the PA should be treated primarily with a mineralocorticoid receptor antagonist (Funder et al. 2016).

Investigations on excised adrenal lesions from PA patients have identified several mutations that can cause PA and BAH diseases. Using the latest sequencing technology on APA samples, aldosterone-driving mutations in *KCNJ5*, *ATP1A1*, *ATP2B3*, *CACNA1D*, and *CTNNB1* genes were found in the last decade (Azizan et al. 2012; Azizan et al. 2013; Beuschlein et al. 2013; Scholl et al. 2015). A summary of the signal and channels involved is illustrated in the schematic of Figure 1. In this review paper, we present and highlight the known mutation findings that contribute to PA.

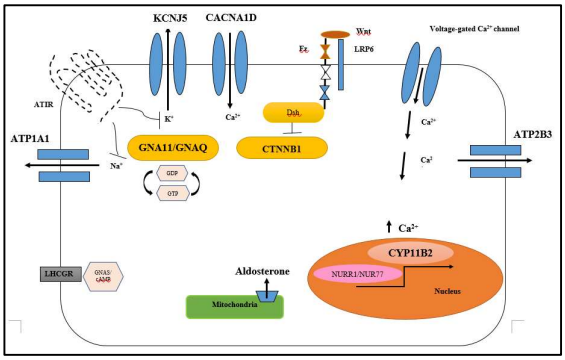

Figure 1. The pathways and channels involved in physiological and pathological biosynthesis of aldosterone. In the physiological state (angiotensin II binding to angiotensin receptor type I (AT1R) and in pathological conditions (gene mutations of the aldosterone-driver genes), the intracellular Ca^{2+} concentration increases which stimulates CYP11B2 expression and production of aldosterone (adapted from Dutta et al. 2016 and Zhou et al. 2021).

Somatic mutations of KCNJ5

The KCNJ5 gene, located at chromosome position 11q24.3, encodes for the Kir3.4 channel which is expressed in the ZG of the adrenal glands. It forms a heterotetramer with Kir3.1 (encoded by genes KCNJ3) which contributes to the maintenance of K^+ ions across the cell membrane. Monticone et al. (2012) stated that this channel is widely expressed in several different tissues including the heart, neurons, and various endocrine tissues. This channel usually carries K^+ out of the cell and thus helps to maintain the hyperpolarization of the ZG membrane. Mutations leading to channel malfunction have been linked to long QT syndrome (type 13) (Monticone et al. 2012).

In a genetic study of 22 APA samples, Choi et al. (2011) showed that there are two mutations located at KCNJ5 gene, p.Gly151Arg (G151R) and p.Leu168Arg (L168R), involving eight APA cases (38%). p.G151R and p.L168R Kir3.4 channel have now been identified as the two most frequently mutated locations in APA samples. These mutations cause a change in the selection of K^+ ions to Na^+ ions in the Kir3.4 channel and at the same time cause an increase in the conductivity of Na^+. Influxion of Na^+ activates the cell depolarization resulting in the entry of Ca^{2+} into the cell. The increase of intracellular Ca^{2+} levels leads to the excessive secretion of aldosterone (Choi et al. 2011). Overexpression of the mutant KCNJ5 in adrenocortical cells result in increased production of aldosterone (Monticone et al. 2012).

Currently, there are several other somatic mutations in KCNJ5 that occur frequently in APAs such as p.Thr158Ala (T158A) (Mulatero et al. 2012), p.Ile157del (I157del) (Azizan et al. 2012), p.Trp126Arg (W126R) (Williams et al. 2014), p.Glu145Gln (E145Q) (Akerstrom et al. 2015), and p.Glu145Lys (E145K) mutations (Azizan et al. 2013). However, in total, nearly 99% APAs that have somatic mutations in the KCNJ5 gene are G151R or L168R mutations, compared to other KCNJ5 somatic mutations (Table 1). Interestingly, KCNJ5 somatic mutations are also discovered among APA patients with a family history of PA (Mulatero et al. 2012) and also found as a germline mutation in familial forms of primary aldosteronism (Choi et al. 2011).

Somatic mutations of ATP1A1

ATP1A1 genes encoded alpha-1 Na^+/ K^+ ATPase subunit which is the ion carrier of the family ATPase type-P. It is located at the chromosome position 1p13.1 and consist of alpha- and beta-subunit. Alpha-1 subunit is found to be abundant in the kidney and epithelial cells. Na^+, K^+ and the ATP binding is located in the alpha-1 subunit, meanwhile beta subunit is responsible for directing the alpha-1 subunit into the plasma membrane (Einholm et al. 2007). Na^+/K^+ ATPases are expressed in the adrenal cortex and the highest expression of mRNA is in the ZG adrenal glands. Na^+/K^+-ATPases carries three Na^+ ions to be exchanged with two K^+ ions. This process is driven by ATP hydrolysis. In year 2013, Beuschlein et al. conducted a genetic study on non-mutated KCNJ5 APA from nine men who had PA and hypokalemia. The study found that three of them had somatic mutations in ATP1A1 gene; two cases of p.Leu104Arg (L104R) and one case of p.Val332Gly (V332G). A further six mutant cases were detected in 100 APA samples of which four of them had L104R mutations, while the other two had a deletion in the same region—p.Phe100_Leu104del (F100_L104del). Azizan et al. (2013), had also reported a complex mutation whereby there was the deletion of amino acids in position 960-963 with a replacement of 963 amino acid by serine (pEETA963S). Other mutations found in Italy was p.Gly99Arg (G99R). Stindl et al. (2015) proved that mutant expression of G99R, L104R and V332G in H295R cell adrenocortical led to an increase in aldosterone secretion. Among all mutations, L104R exhibits the highest depolarization of cell membranes within H295R cells. However, all mutations result in an increase of intracellular Na^+ and a decrease of K^+ ions. Studies in 199 APA samples have estimated that ATP1A1 mutations are present in ~5% of APA patients (Beuschlein et al. 2013). Interestingly, ATP1A1 mutations have been reported to occur in APAs that have ZG-like cell phenotype (Azizan et al. 2013).

Somatic mutations of ATP2B3

The ATP2B3 gene encodes for the plasma membrane calcium-transporting ATPase 3 (PMCA3) which belongs to the family of P-type primary ion transport ATPases. It is found in the adrenal cortex and located at chromosome X (Xq28). ATP2B3 is necessary for secretions of Ca^{2+} from cell cytoplasm and therefore has an essential role in Ca^{2+} homeostasis. Ca^{2+}-ATPases exchange one Ca^{2+} ion for one H^+ ion. p.Leu425_Val425del (L425_V426del) and p. Val426_Val427del (V426_V427del) were the first mutations found in ATP2B3 to be associated with APAs at the same time that ATP1A1 mutant APAs were first found (Beuschlein et al. 2013). In addition to Leu425, Val426, and Val427 mutations, Dutta et al. (2013) had also found ATP2B3 mutation involving Arg428 and Val429, LVVAV425-429 ATP2B3, associated with PA. This region is considered important for the binding of Ca^{2+} ions to PMCA3 and thus deletion of amino acids at this location would impair the function and activity of the Ca^{2+}-ATPase. In adenoma cell cultures, ATP2B3 mutation led to a significantly higher level of cell depolarization compared to control cells (Beuschlein et al. 2013). Thus far, ATP2B3 mutations has a low prevalence in APA patients with approximately 0.9%-1.6% (Dutta et al. 2013). Nevertheless, similar to ATP1A1, several ATP2B3 somatic mutations have been found in aldosterone-producing cell clusters (APCC) detected by the expression of the aldosterone synthase enzyme CYP11B2 (Table 1). To note, germline mutation in ATP2B3 has been found in ataxia spinocerebellar congenital patient (Cali et al. 2015).

Somatic mutation of CACNA1D

CACNA1D gene encodes for the alpha-1D subunit of the voltage-dependent L-type calcium Channel, Cav 1.3, located at chromosome position 3p14.3. CACNA1D is a large gene with 49 exons and belongs to the Cav 1 family channels. Cav 1 channels consist of five subunit which are α1, α2, β, γ and δ. α1 is the subunit for the formation of pore for the Cav 1.3 channel. CACNA1D is expressed in ZG and various other tissues such as liver, neurons and others. Cav1.3 channels are involved with cardiac pacemaking, hearing, hormone secretion and neuron plasticity

(Pinggera et al. 2015). This L-type $Ca2^{+}$ channel is commonly expressed by endocrine tumors and sensitive to selective inhibition by dihydropyridine (Catterall. 2011). Previous studies separately identified de novo mutations in CACNA1D gene when exome sequencing aldosterone-producing adenomas (Azizan et al. 2013; Scholl et al. 2013; Omata et al. 2017; Omata et al. 2018). The mutations that were identified are p.Val259Asp (V259D), p.Gly403Arg (G403R), p.Phe747Leu (F747L), p.Ile750Met (I750M), p. Ile770Met (I770M), p.Arg990His (R990H), p.Pro1336Arg (P1336R), and p.Met1354Ile (M1354I). Electrophysiology tests have revealed that in mutant channel the voltage dependence of activation is shifted to more hyperpolarized potentials and have continuous activation (Scholl et al. 2013). Furthermore, activation and inactivation of mutant channels are both shifted to more hyperpolarized potentials which leads to the increase influx of Ca^{2+} which is connected to the higher production of aldosterone (Azizan et al. 2013).

Similar to APAs with ATP1A1 and ATP2B3 mutations, CACNA1D mutant APAs are characterized to have ZG-like cells (Azizan et al. 2013). However, in contrast to ATP1A1, ATP2B3, and even more so KCNJ5, the mutations that occur in CACNA1D are not found in specific domains but are found in the whole gene (Table 1). Most of CACNA1D mutations are found in APAs excised from male PA patients and it is estimated about 3–11% APA exhibit this mutation (Akerstrom et al. 2015; Scholl et al. 2015). Interestingly, through exome sequencing study, Pinggera et al. (2015) have identified de novo mutations, p. Ala749Gly (A749G) and p. Gly407Arg (G407R) in CACNA1D, in patients with autism and intellectual disabilities.

Somatic Mutations of CTNNB1

CTNNB1, encodes for the protein β-catenin that activates the Wnt signaling pathway. To date, the Frizzled (Fz) family for G protein-coupled receptor (GPCRs), ROR1, ROR2, the receptor tyrosine kinases (RTKs), as well as RTK-like protein RYK have been identified as receptors involved in the Wnt signal (Angers & Moon 2009). This receptor-mediated signal pathway is the target of the highly preserved glycoproteins Wnt ligands (Angers & Moon 2009). Previous study has reported that active Wnt signalling is clearly seen in APAs even though the frequency of CTNNB1 mutations in APA is very low, between 3.7 % - 5.1% of all APAs (Bjorklund et al. 2008; Azizan et al. 2013; Scholl et al. 2013; Wu et al. 2017). The mutations in CTNNB1 in APAs occur in Exon-3 of the gene which affects serine and threonine residues in which phosphorylation marks β-catenin to degrade. This leads to inhibition of β-catenin phosphorylation and thus aberrant activation of the Wnt pathway as degradation has been inhibited. In the adrenal cortex of transgenic mice, Durand et al. (2011) reported that the activation of β-catenin leads to increased steroidogenesis, adrenal hyperplasia, and excessive secretion of aldosterone. However, CTNNB1 mutations have also been reported in adrenal adenomas secreting cortisol or that do not secrete any hormones (Boulkroun et al. 2011). Meanwhile, excessive expression of β-catenin in the adrenal cortical carcinoma (ACC) has been related to the severe prognosis (Durand et al. 2011). Together, this may suggest that β-catenin signalling plays a role in the formation of adenoma rather than hormone synthesis.

Interestingly, some APA cases with β-catenin mutations have presented with elevated levels of the luteinizing hormone/choriogonadotropin hormone receptor and the gonadotropin-releasing hormone (GnRH) receptor (Teo et al. 2015). These APAs were taken from three women who were diagnosed with PA during pregnancy or at the beginning of menopause. In the study, it was suggested that the activation of Wnt by the CTNNB1 mutation caused the adrenocortical cells to differentiate toward the adrenal-gonadal precursor cells (Teo et al. 2015). Subsequent studies have now shown that it is actually the synergism between GNA11/Q and CTNNB1 somatic mutations that upregulates LHCGR expression and aldosterone production in these three women (Zhou et al., 2021). The mutation in GNA11/Q that occurs in a CTNNB1 mutant APA affects p.Gln209 of the Gα11/Gαq protein. This amino acid is important for the GTPase activity. In total, Zhou et al. (2021) found 16/27 (59%) CTNNB1 mutant APAs to have a GNA11/Q Q209 somatic mutation, p.Gln209His (Q209H), p.Gln209Pro (Q209P) or p.Gln209Leu (Q209L). Transfection of adrenocortical cells suggested that CTNNB1 and GNA11 mutations have additive effects on aldosterone production and upregulation of genes specifically expressed in double mutant APAs such as LHCGR.

Table 1. Somatic mutations associated with autonomous aldosterone production

Gene	Mutation	Reference
KCNJ5	R115W	Cheng et al. 2015
	W126R	Williams et al. 2014
	I144_E145insAI	Scholl et al. 2015
	E145K	Azizan et al. 2012
	E145Q	Akerstrom et al. 2012
	E147Q_T149_I150insTTT	Wang et al. 2015
	T148_T149insR	Zheng et al. 2015
	T149_I150insT	Kuppusamy et al. 2014
	I150_G151insM	Scholl et al. 2015
	G151E	Scholl et al 2012
	G151R	Choi et al. 2011
	G153_G164dup	Wang et al. 2015
	F154C	Choi et al. 2011
	I157del	Azizan et al. 2012
	I157K	Scholl et al. 2015
	T158A	Mulatero et al. 2012
	L168R	Choi et al. 2011
	E246G	Choi et al. 2011
ATP1A1	EETA963S	Azizan et al. 2013
	G99R	Nishimoto et al. 2015; Stindl et al. 2015
	F100_L104del	Beuschlein et al. 2013
	L104R	Beuschlein et al. 2013; Stindl et al. 2015; Omata et al. 2017
	L104V	Nishimoto et al. 2015
	V332G	Beuschlein et al. 2013; Nishimoto et al. 2015; Stindl et al. 2015
	E687K	Omata et al. 2017
	M734L	Nishimoto et al. 2015
ATP2B3	D77N	Omata et al. 2017
	E135K	Omata et al. 2017
	G270D	Omata et al. 2017
	G325D	Omata et al. 2017
	R345Q	Nishimoto et al. 2015

	L425_V426del	Beuschlein et al. 2013
	V426_V427del	Beuschlein et al. 2013
	A790V	Omata et al. 2017
	G931D	Nishimoto et al. 2015
	S1137F	Omata et al. 2017
	P1150L	Omata et al. 2017
	A1157V	Omata et al. 2017
CACNA1D	E124K	Omata et al. 2018
	L248F	Omata et al. 2018
	V259D	Omata et al. 2018
	V259G	Omata et al. 2018
	L272R	Omata et al. 2018
	G323R	Omata et al. 2018
	V401L	Omata et al. 2018
	G403R	Nishimoto et al. 2015; Omata et al. 2017; Omata et al. 2018
	G407R	Pinggera et al. 2015
	S410L	Omata et al. 2017; Omata et al. 2018
	G457R	Omata et al. 2017
	R510X	Omata et al. 2017
	P548L	Omata et al. 2017
	L613Q	Nishimoto et al. 2015
	R619W	Nishimoto et al. 2015
	S652L	Omata et al. 2018
	S653P	Omata et al. 2018
	L655P	Fernandes-Rosa et al. 2014
	S672L	Omata et al. 2018
	L675P	Omata et al 2017
	S724L	Omata et al. 2018
	V728I	Wang et al. 2015
	Y741C	Fernandes-Rosa et al. 2014
	F747C	Omata et al. 2017
	F747L	Nishimoto et al. 2015; Omata et al. 2017; Omata et al. 2018
	F747V	Nishimoto et al. 2015; Omata et al. 2017; Omata et al. 2018

L748S	Omata et al. 2018
V748I	Nishimoto et al. 2015
A749G	Pinggera et al. 2015
I750F	Fernandes-Rosa et al. 2014
I750M	Azizan et al. 2013
755_757del	Omata et al. 2018
Y761C	Nishimoto et al. 2015
F767L	Nishimoto et al. 2015
I770F	Omata et al. 2018
I770M	Omata et al. 2018
S969L	Omata et al. 2018
V979E	Fernandes-Rosa et al. 2014
K981N	Fernandes-Rosa et al. 2014
R990H	Omata et al. 2017; Omata et al. 2018
A998I	Fernandes-Rosa et al. 2014
A998V	Omata et al. 2017; Omata et al. 2018
V999D	Omata et al. 2018
K1001N	Nishimoto et al. 2015
A1011T	Omata et al. 2018
I1015V	Omata et al. 2018
A1018V	Nishimoto et al. 2015
F1147C	Omata et al. 2017
F1147L	Omata et al. 2017; Omata et al. 2018
V1151F	Fernandes-Rosa et al. 2014
I1152N	Fernandes-Rosa et al. 2014
V1171F	Nishimoto et al. 2015
I1172N	Nishimoto et al. 2015
R1183H	Omata et al. 2018
F1248L	Omata et al. 2017; Omata et al. 2018
D1273N	Omata et al. 2018
P1336R	Omata et al. 2017
V1338M	Nishimoto et al. 2015; Omata et al. 2017; Omata et al. 2018

	I1352T	Omata et al. 2017
	M1354I	Azizan et al. 2013
	P1371R	Nishimoto et al. 2015
	V1373M	Nishimoto et al 2015
	P1499L	Omata et al. 2017
	T1835I	Omata et al. 2018
	W1836X	Omata et al. 2017
CTNNB1	D32G	Teo et al. 2015
	D32N	Wu et al. 2017
	S33C	Teo et al. 2015
	S33F	Teo et al. 2015
	G34R	Teo et al. 2015
	H36P	Wu et al. 2017
	S37C	Teo et al. 2015
	T40A	Teo et al. 2015
	T41A	Teo et al. 2015
	T42S	Wu et al. 2017
	S45C	Wu et al. 2017
	S45F	Wu et al. 2017
	S45P	Wu et al. 2017
	S45Y	Wu et al. 2017
	K345R	Teo et al. 2015
	N387K	Teo et al. 2015
GNA11/GNAQ	Q209H	Zhou et al. 2021
	Q209P	Zhou et al. 2021
	Q209L	Zhou et al. 2021

Conclusion

In short, the information on the basis of genetic mechanisms related to APA has increased drastically in the last two decades. Although great progress has been made, there are still many questions need to be addressed. For instance, genetic variation in other additional genes that can explain a small fraction of APA whose genetic origin is not yet known and also better characterization involving phenotypes of APA according to their genotype. Not to mention the question of bilateral adrenal hyperplasia which is still poorly studied due to lack of tissue because it is rarely treated through surgery. Therefore, the genetic of PA should be further investigated to ensure the contribution of new discoveries particularly in the pathophysiology of PA and blood pressure.

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