Aging and Adrenal Aldosterone Production

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Aldosterone, the primary mineralocorticoid, is synthesized in the outer zone of the adrenal cortex called the zona glomerulosa (ZG). The production of aldosterone is tightly regulated by angiotensin II (Ang II) and circulating potassium levels.[1] Physiologically, aldosterone plays a key role in the maintenance of intravascular volume and blood pressure through sodium retention in the kidney. Excess aldosterone causes hypertension and induces cardiovascular complications.[2–5] The autonomous secretion of aldosterone, independent of Ang II and sodium status, is known as primary aldosteronism (PA). PA is the most common cause of endocrine-related hypertension with a prevalence of 5% to 10% in hypertensive population[6–10] and ≈20% in resistant hypertension.[11–13] Because of the increased risk for cardiovascular complications in patients with PA, early detection of the disease and targeted treatment is recommended.[14]

The molecular pathogenesis of PA was largely unknown until recently. The development of specific antibodies against aldosterone synthase (CYP11B2), which is required for the final steps of aldosterone production, has allowed the detection of aldosterone-producing cells in resected adrenals.[15,16] Using these antibodies, non-neoplastic foci of CYP11B2-expressing cells called aldosterone-producing cell clusters (APCC)[15] have been identified in adrenal tissues adjacent to aldosterone-producing adenomas (APA) and in normal human adrenal glands without tumor or hyperplasia.[15–20] Studies have shown that APCC are a common occurrence in normal human adrenals.[21–23] Because circulating renin and Ang II levels are suppressed in patients with PA, the observation of APCC adjacent to APA suggested that APCC may represent a source of autonomous and renin-independent aldosterone secretion, perhaps even a precursor to APA.[24]

Over the past 6 years, somatic and germline mutations that cause inappropriate aldosterone production have been identified in patients with PA. Most of the mutations cause increased intracellular calcium (Ca^{2+}) levels, resulting in activated *CYP11B2* transcription and elevation of aldosterone production.[24–26] These somatic mutations have also been observed in APCC in normal adrenal glands.[23,27] Recent histopathologic studies demonstrated that older age is associated with greater adrenal APCC content[21–23] and a concomitant biochemical phenotype that supports progressive autonomous aldosteronism.[21] These findings provide a possible link between age-related histological changes in adrenal CYP11B2 expression and age-related physiological changes in aldosterone secretion. In this review, we cover recent topics on physiology and dysregulation of aldosterone production and discuss potential age-related effects on the aldosterone and adrenal physiology.

Human Adrenal Zonation and Steroid Production

Human adrenal glands are composed of an outer cortex and an inner medulla. The adrenal cortex is further divided into 3 functionally distinct zones; ZG, zona fasciculata, and zona reticularis (Figure 1A). In the ZG, the mineralocorticoid aldosterone is produced with the physiological role of maintaining fluid and electrolyte balance. The production of aldosterone is mainly regulated by Ang II, potassium (K^+), and adrenocorticotropic hormone.[1] Glucocorticoids are synthesized in the zona fasciculata under the regulation of adrenocorticotropic hormone. The zona reticularis is also mainly regulated by adrenocorticotropic hormone and produces adrenal androgens, including dehydroepiandrosterone and dehydroepiandrosterone sulfate. There is considerable evidence that aging is associated with a decline in circulating levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate.[28] The decrease in these adrenal steroids results from intra-adrenal changes that include a decrease in zona reticularis size and expression of steroidogenic enzymes required for dehydroepiandrosterone synthesis.[29,30] In contrast to dehydroepiandrosterone, little is known about the age-related adrenal changes related to aldosterone synthesis.

ZG synthesis of aldosterone occurs through the action of a series of enzymes, including cholesterol side-chain cleavage (CYP11A1), type 2 3β-hydroxysteroid dehydrogenase (HSD3B2), 21-hydroxylase (CYP21A2), and CYP11B2[1] (Figure 1B). Of these enzymes, CYP11B2 is expressed specifically in the ZG, whereas expression of 11β-hydroxylase (CYP11B1), which is required for the final step in the biosynthesis of cortisol, is limited to the zona fasciculata and zona reticularis.[31] This functional zonation acts to compartmentalize aldosterone production to the ZG.[32,33] On the basis of the critical nature of CYP11B2 in aldosterone production, we and others have studied the cell-signaling mechanisms regulating this enzyme in normal and pathological conditions within the adrenal.

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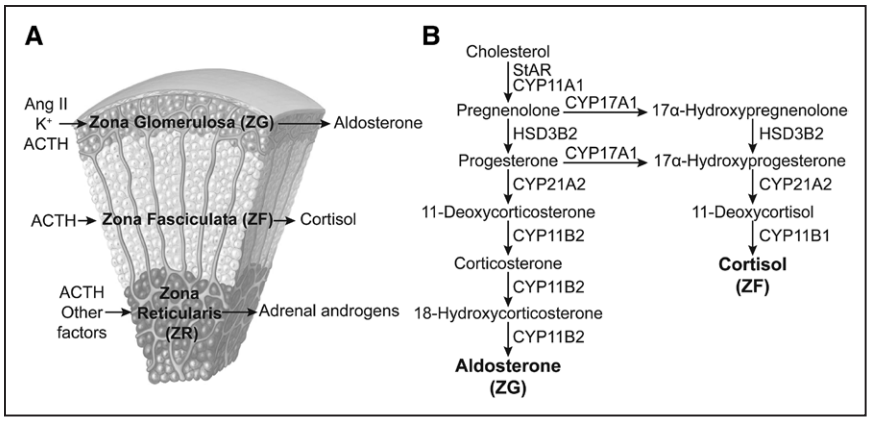

Figure 1. Adrenal zonation and steroidogenic pathway. **A**, Adrenal cortex zonation with the primary regulators of steroid production. **B**, Steroidogenic pathway for the production of aldosterone and cortisol. Aldosterone and cortisol production occur in the zona glomerulosa (ZG) and zona fasciculata (ZF), respectively. ACTH indicates adrenocorticotropic hormone; and StAR, steroidogenic acute regulatory protein.

Physiological and Pathological Regulation of Adrenal Cell Aldosterone Production

The primary physiological regulators of aldosterone production are Ang II, K^+, and adrenocorticotropic hormone. Other factors that have been proposed to modulate aldosterone production include serotonin,[34,35] estrogen,[36] parathyroid hormone,[37,38] vasopressin,[39,40] endothelin-1,[41,42] and very-low-density lipoprotein.[43–45] A recent study demonstrated that leptin induces *CYP11B2* expression and aldosterone production via Ca^{2+}-dependent mechanisms.[46] Several signaling pathways are involved in adrenal cell aldosterone production. The key intracellular pathways include Ca^{2+},[47,48] cAMP,[49,50] and phospholipase D signaling.[51–53] Of these, the Ca^{2+}-signaling pathway seems to be the most important in both physiological and pathological conditions. Both Ang II and K^+ use Ca^{2+} for physiological activation, whereas inappropriate aldosterone production, as seen in PA, is primarily regulated by somatic and germline mutations that dysregulate and raise intracellular Ca^{2+}.

Mutations in *KCNJ5*,[54] *ATP1A1*,[55] *ATP2B3*,[55] *CACNA1D*,[56,57] and *CACNA1H*[58] (aldosterone-driver genes) have been identified in APA and familial hyperaldosteronism. In vitro studies have shown that these mutations cause increased aldosterone production.[59–64] The *KCNJ5* gene encodes the inward-rectifying potassium channel GIRK4, and somatic mutations in this gene are considered to be the most prevalent genetic alteration in APA.[65–67] Mutations in *KCNJ5* gene cause a loss of ion selectivity, increased Na^+ conductance, cell membrane depolarization, and voltage-gated calcium channel opening, leading to increased intracellular Ca^{2+} concentration.[54,59,68] The *ATP1A1* gene encodes the α1-subunit of the Na^+/K^+ ATPase, which acts to maintain the resting cell membrane potential and electric excitability. *ATP1A1* mutations disrupt this function and cause cell membrane depolarization and cellular acidification because of a pathological H^+ leak, resulting in aldosterone overproduction.[55,61,69] The *ATP2B3* gene encodes the plasma membrane Ca^{2+} ATPase isoform 3, which regulates intracellular Ca^{2+} homeostasis by transporting cytoplasmic Ca^{2+} out of the cells. Mutations in *ATP2B3* gene seem to increase intracellular Ca^{2+} levels through a reduced Ca^{2+} export as a result of a loss of pump function and nonphysiological Na^+ and possible Ca^{2+} permeability to the cells.[62] The *CACNA1D* gene encodes the voltage-dependent L-type calcium channel subunit α-1D ($Ca_v1.3$). Mutations in *CACNA1D* gene cause channel activation at less depolarized potentials, leading to increased Ca^{2+} influx and aldosterone overproduction.[56,57,63,70] The *CACNA1H* gene encodes the voltage-dependent T-type calcium channel subunit α-1H ($Ca_v3.2$). In a recent study, a recurrent germline mutation in *CACNA1H* gene (p.Met1549Val) was identified in early-onset PA patients.[58] In vitro studies demonstrated that the *CACNA1H* mutation caused impaired channel inactivation and a shift of activation to less depolarized potentials, leading to increased Ca^{2+} entry and elevated aldosterone production.[58,64]

The cellular origins of APA with somatic mutations remain an area of active research. If the adrenal accumulation of somatic mutations follows other tissues, it would be expected that there would be an age dependence. We and others have approached this research by focusing on CYP11B2 because it is required for aldosterone synthesis and its expression is highly cell specific within the adrenal. Development of specific antibodies against human CYP11B2 and CYP11B1 has provided tools to better study adrenal functional histopathology.[15,16] Nishimoto et al[15] reported that there are 2 patterns of CYP11B2 expression within the adrenal: (1) conventional zonation with ZG cells expressing CYP11B2 and (2) variegated zonation consisting of a subcapsular non-neoplastic cell foci expressing CYP11B2 named APCC. The expression pattern of steroidogenic enzymes in APCC, that is, high expression of HSD3B2 and CYP11B2 and low expression of 17α-hydroxylase (CYP17A1) and CYP11B1 (needed for cortisol biosynthesis), supports the ability of APCC to produce aldosterone.[15] APCC have also been identified in adrenal tissues adjacent to APA[15,17–20]; given that renin and Ang II are suppressed in PA caused by APA, these observations supported APCC as non-neoplastic sources of autonomous and renin-independent aldosterone secretion. Of note, before the development of CYP11B2 antibodies, in situ hybridization methods allowed the identification of cell foci expressing *CYP11B2* mRNA but no *CYP11B1* or *CYP17* mRNA.[71]

Adrenal ZG and Aldosterone-Producing Cell Clusters in Aging

Age-related histological changes in adrenal CYP11B2 expression have been investigated. There is growing evidence for an age-dependent accumulation of APCC in adrenal glands.[21–23] Further, there is age-related loss of the classic continuous CYP11B2 expression pattern within the ZG that

is frequently observed in young adrenal glands (Figure 2).[21,72] Collectively, older adrenal glands have less normal CYP11B2 expression in the ZG and greater amount of APCC. Although there is no direct evidence to explain why such age-related changes in CYP11B2 expression patterns might occur, potential considerations may include genomic, epigenetic, or other environmental factors. The histological findings suggest a transition from continuous CYP11B2 expression in the ZG to APCC predominance with advancing age[21] and raise the hypothesis that with aging, there may be a progressive transition from normal physiological aldosterone regulation to autonomous and renin-independent aldosterone secretion. If true, this clinical phenotype associated with these age-related histopathologic changes should involve greater autonomous aldosteronism and higher risk for aldosterone-mediated cardiovascular disease.

To better define the cellular characteristics of APCC, we performed transcriptome analysis and targeted next-generation sequencing analysis on APCC isolated from normal adrenal glands from kidney donors.[27] Transcriptome analysis demonstrated that APCC mRNA profile had similar characteristics to those of ZG but with higher *CYP11B2* expression in APCC, indicating increased capacity to produce aldosterone in APCC.[27] Mutation analysis revealed that 8 out of 23 APCC (35%) had known aldosterone-driver somatic mutations in genes including *CACNA1D* and *ATP1A1*.[27] A recent study using a modified targeted next-generation sequencing protocol identified somatic mutations in 21 of 61 APCC (34%) in adrenals from normotensive Japanese autopsy cases.[23] No *KCNJ5* mutation, which is the most prevalent somatic mutation in APA, was identified in either American or Japanese cohorts.[23,27] In contrast to APA, both studies suggest that APCC are most likely to have aldosterone-driver mutations in the *CACNA1D* gene.[23,27] These findings suggest that L-type calcium channel blockers should be considered as a targeted treatment option that could reduce inappropriate autonomous aldosterone production from APCC. Interestingly, in 1 early-onset PA patient with germline de novo *CACNA1D* mutation (p.Gly403Asp), treatment with a calcium channel blocker, amlodipine, resulted in normalization of blood pressure and resolution of biventricular hypertrophy that had been present since birth.[57]

Although somatic mutations in APCC support the concept of renin-independent aldosterone production in APCC, genetic characteristics of majority of the APCC are still unknown. Further, because previous research on human APCC has been conducted from either postmortem adrenal specimen or adrenals containing APA, the biochemical phenotype of APCC has not been specifically quantified. Future clinical studies are needed to directly assess whether APCC autonomously secrete aldosterone.

Renin–Angiotensin–Aldosterone System in Aging

With aging, there is a decline in normal function of several physiological systems. Consistent with the histological findings of age-related changes in adrenal CYP11B2 expression patterns, physiological changes in the renin–angiotensin–aldosterone system seem to occur with advancing age. There have been several studies with relatively small sample size suggesting that older individuals may secrete less aldosterone and have lower plasma renin activity.[73–77] Specifically, studies have shown that older age is associated with a blunted ability to secrete aldosterone despite stimulation by its major regulators: Ang II with sodium restriction[74] and potassium during intravenous infusion.[78] We recently demonstrated the dynamics of aldosterone physiology in a large cross-sectional study that included a broad age continuum.[21] The most notable observations were a strong and significant association between older age with higher aldosterone:renin ratio and a blunted ability to secrete aldosterone with sodium restriction.[21] Taken together, older age was associated with greater autonomous aldosterone secretion and less physiological aldosterone secretion. Given the aforementioned age-related histopathologic changes in CYP11B2 expression, we hypothesized that this clinical phenotype may have been the consequence of parallel age-related increases in adrenal APCC content and concomitant decreases in normal adrenal CYP11B2 expression.

Clinical Perspectives

The concept of age-related autonomous aldosteronism may provide new avenues to investigate the pathogenesis and treatments for hypertension and cardiovascular diseases. Recent studies have demonstrated that autonomous aldosterone production and even overt PA can be detected in normotensive subjects[79,80]; in parallel with the finding that a subset of adrenal glands from normotensives have APCC-harboring somatic mutations in aldosterone-driver genes,[23] this may suggest that the spectrum of PA can range from mild and subclinical in normotension (possibly as

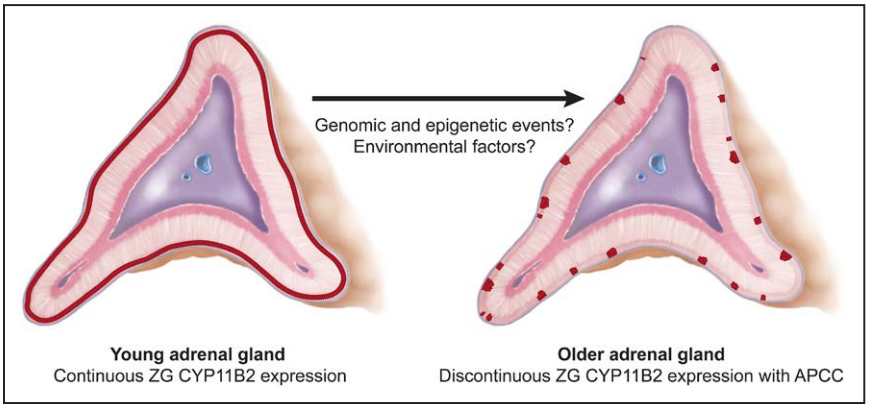

Figure 2. Age-related histological changes in human adrenal glands. Continuous CYP11B2 expression in the zona glomerulosa (ZG) is often seen in young adrenal glands, whereas older adrenals have less normal ZG CYP11B2 expression and more aldosterone-producing cell clusters (APCC). Potential effects that might cause the age-associated increase in CYP11B2-positive APCC include genomic, epigenetic events or environmental factors.

a result of autonomous aldosterone production from APCC) to more severe in hypertension (possibly as a result of a combination of APCC and neoplastic and hyperplastic processes such as APA and bilateral adrenal hyperplasia).[81] Consistent with this, normotensive patients with evidence for subclinical PA have a significantly higher risk for developing incident hypertension.[79,81,82] A recent population-based cohort study demonstrated that older normotensives were more likely to have a suppressed renin phenotype and that higher aldosterone levels in the context of this renin suppression were associated with a substantially higher risk for developing hypertension.[81] Further, physiological investigations have also shown age-related declines in 11β-hydroxysteroid dehydrogenase type 2 activity that result in a phenotype of renin suppression and cortisol-mediated mineralocorticoid receptor activation.[83] Given the accumulating evidence suggesting age-related histopathologic and biochemical changes consistent with renin-independent aldosteronism and MR activation, future studies may investigate to what extent the essential hypertension of aging may be mediated by mineralocorticoids, such as aldosterone and cortisol.

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Disclosures

None.

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