



# The Genotype-Based Morphology of Aldosterone-Producing Adrenocortical Disorders and Their Association with Aging

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Primary aldosteronism (PA) is the most common cause of secondary hypertension, and is associated with an increased incidence of cardiovascular events. PA itself is clinically classified into the following two types: unilateral PA, mostly composed of aldosterone-producing adenoma (APA); and bilateral hyperaldosteronism, consisting of multiple aldosterone-producing micronodules (APMs) and aldosterone-producing diffuse hyperplasia. Histopathologically, those disorders above are all composed of compact and clear cells. The cellular morphology in the above-mentioned aldosterone-producing disorders has been recently reported to be closely correlated with patterns of somatic mutations of ion channels including *KCNJ5*, *CACNA1D*, *ATP1A1*, *ATP2B3*, and others. In addition, in non-pathological adrenal glands, APMs are frequently detected regardless of the status of the renin-angiotensin-aldosterone system (RAAS). Aldosterone-producing nodules have been also proposed as non-neoplastic nodules that can be identified by hematoxylin and eosin staining. These non-neoplastic CYP11B2-positive nodules could represent possible precursors of APAs possibly due to the presence of somatic mutations. On the other hand, aging itself also plays a pivotal role in the development of aldosterone-producing lesions. For instance, the number of APMs was also reported to increase with aging. Therefore, recent studies indicated the novel classification of PA into normotensive PA (RAAS-independent APM) and clinically overt PA.

**Keywords:** Adrenal glands; Aldosterone; Adenoma; Pathology

## INTRODUCTION

Primary aldosteronism (PA) accounts for 5% to 10% of hypertensive patients, usually accompanied with increased cardiovascular events including stroke, ventricular hypertrophy, fibrosis, vascular remodeling, and others [1-4]. PA is mainly classified

into neoplastic and non-neoplastic lesions based on the biological characteristics of lesions responsible for aldosterone excess. However, the histopathological identification of these lesions by hematoxylin and eosin (H&E) staining (morphology) is well known to be extremely difficult even when differentiating between neoplasms and non-neoplastic nodules. Due to the devel-

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opment of cytochrome P450 family 11 subfamily B member 2 (CYP11B2) immunohistochemistry, rather unexpected patterns of CYP11B2 have been revealed [5]. In contrast to the diffuse CYP11B2-positive cells in ZG, CYP11B2-positive and cytochrome P450 family 11 subfamily B member 1 (CYP11B1)- and cytochrome P450 family 17 subfamily A member 1 (CYP17A1)-negative adrenocortical cells were identified as clusters of CYP11B2-positive cells in the subcapsular areas previously termed as aldosterone-producing cluster cells (APCCs). Various nomenclature systems for the histopathological classification of aldosterone-producing lesions have been proposed at this juncture, but the international consensus of PA histopathology was very recently published, and the terms were re-defined and integrated [6].

PA is clinically classified into two subtypes, unilateral hyperaldosteronism (UHA) and bilateral hyperaldosteronism (BHA), according to the results of adrenal vein sampling [7,8]. In addition, UHA is histologically classified into aldosterone-producing adenoma (APA) and aldosterone-producing micronodules (APMs) using CYP11B2 immunohistochemistry [6,9]. BHA includes multiple APMs and aldosterone-producing diffuse hyperplasia (APDH) and occasionally bilateral APAs [6,10]. Those non-neoplastic nodules involved in aldosterone overproduction were also recently reported to harbor APA-driver somatic mutations of various ion channels including potassium inwardly rectifying channel subfamily J member 5 (*KCNJ5*), calcium voltage-gated channel subunit alpha1 D (*CACNAID*), ATPase Na<sup>+</sup>/K<sup>+</sup> transporting subunit alpha 1 (*ATP1A1*), and ATPase plasma membrane Ca<sup>2+</sup> transporting 3 (*ATP2B3*), which indicated that APM could represent the precursor lesion of APA [11,12]. In addition, the number of APMs was significantly positively correlated with aging of the subjects, although serum aldosterone level usually declines with aging [11,13]. Therefore, we herein review recent developments regarding the pathogenesis of PA and its association with aging and histopathological features of the lesions.

## GENETIC ALTERATIONS ASSOCIATED WITH PRIMARY ALDOSTERONISM

PA is caused by autonomous aldosterone production due to either hyperplasia or nodules. This aldosterone overproduction was demonstrated to be caused by genetic mutations encoding for various ion channels such as *KCNJ5*, *CACNAID*, *ATP1A1*, and *ATP2B3* [14-17]. All of those genes above are well known to regulate intracellular ionic homeostasis and cell membrane po-

tential, resulting in increased intracellular calcium levels and subsequently promoting aldosterone biosynthesis [14-17]. In addition, rare PA cases were reported to harbor somatic mutations of catenin beta 1 (*CTNNB1*) and chloride voltage-gated channel 2 (*CLCN2*) encoding for  $\beta$ -catenin and the chloride channel ClC-2 [18,19].

*KCNJ5* mutation is the most frequent somatic mutation reported in APA, although its prevalence differs among ethnicities [14,20]. *KCNJ5* encodes inward rectifier K<sup>+</sup> channel 4, and its mutations cause increased Na<sup>+</sup> permeability, subsequently resulting in a sustained depolarization of the cell membrane [14]. Aldosterone biosynthesis could also be promoted by increased cytosolic calcium levels through activated calcium channels by increased intracellular K<sup>+</sup> concentrations [21].

*CACNAID* encodes L-type calcium channel  $\alpha$ -subunit CaV1.3, which regulates intracellular calcium homeostasis [22]. Mutation of CaV1.3 also stimulated an influx of Ca<sup>2+</sup>, resulting in autonomous aldosterone production [16]. Mutations of *ATP1A1*, encoding  $\alpha 1$  subunit of the Na<sup>+</sup>/K<sup>+</sup>-ATPase, facilitated an influx of calcium by altering sodium and potassium homeostasis [15]. Mutation of *ATP2B3* directly influenced the calcium pump PMCA3, increasing cytosolic calcium levels [23]. Both mutations could result in aldosterone overproduction by increasing intracellular calcium levels.

Wnt is secreted protein, which regulates growth and stem cell renewal, and its signaling pathway has been well known to be activated in APAs [18]. The Wnt signaling pathway could activate  $\beta$ -catenin, which subsequently promotes the cell proliferation. In addition, mutations of the *CTNNB1* gene coding for  $\beta$ -catenin were recently reported in APAs [18]. *CTNNB1* mutations were also reported to lead to constitutive activation of  $\beta$ -catenin protein eventually resulting in tumorigenesis, and this mutation is not only restricted to APAs but has also been identified in cortisol-producing adenomas (CPAs) and adrenocortical carcinomas [24].

A germline calcium voltage-gated channel subunit alpha1 H (*CACNAIH*) mutation was reported to encode an abnormal voltage-dependent T-type channel CaV3.2, which could induce early-onset hyperaldosteronism and hypertension, although rare [25]. A mutation of *CACNAIH* is well known to be the main cause of familial hyperaldosteronism (FH) type IV [26]. FH is frequently caused by germline mutations of ion channels such as *KCNJ5*, *CACNAID*, and *CACNAIH* and further classified into types I-IV according to its clinical and biochemical features [16,26-28]. FH-I is also characterized by glucocorticoid-remediable aldosteronism caused by crossover of both *CY*-

*P11B2* and *CYP11B1* genes, resulting in ectopic expression of CYP11B2 [29,30]. The etiology of FH-II, however, has remained unknown, although its possible association with germline *CLCN2* mutations was previously proposed [31]. *CLCN2* mutations induced a loss of the voltagegating of the channel, leading to increased chloride influx, which subsequently resulted in cell membrane depolarization [32]. Intracellular calcium levels were eventually increased by depolarization of the cell membrane, as discussed above. On the other hand, FH-III is characterized by development of massive BHA as a result of germline mutations of *KCNJ5* [14]. FH-IV is characterized by germline mutations of *CACNAID* and *CACNAIH* [16,25,26].

## THE GENETIC SPECTRUM OF THE HISTOLOGICAL SUBTYPES OF PA

### Aldosterone-producing adenoma

In APAs, somatic mutations were detected at 88% to 90% of patients. Among those, *KCNJ5* is the most frequent genotype regardless of ethnicity. However, 38% of Caucasian patients were reported to harbor *KCNJ5* mutations [22,33,34], but a prevalence of 70% was reported among Japanese patients [35]. *CACNAID* mutations are the next frequent genotype following *KCNJ5* mutations, and 9.3% of European and 2.5% of Japanese APA patients were reported to harbor this somatic mutation in the tumor tissue [22,35]. Mutations of *ATP1A1*, *ATP2B3*, and *CTNNT1* were rare, constituting approximately less than 5% of APAs regardless of ethnicity [18,22,35].

### Aldosterone-producing non-neoplastic nodules

#### Non-neoplastic nodules in non-pathological adrenal glands

These non-neoplastic nodules were reported to harbor somatic mutations like those of APAs [10,11,36,37]. We previously reported somatic mutations in 21 of 61 nodules (34%) from normally-functioning adrenal glands with normotension [11]. Among 21 mutated nodules, 14 harbored *CACNAID* mutations; three had *ATP2B3* mutations, two had *ATP1A1* mutations, and two had both *CACNAID* and *ATP2B3* mutations. Eight of 23 nodules (35%) were also reported to harbor somatic mutations (six *CACNAID* and two *ATP1A1*) in normal adrenal glands [37]. In addition, another study reported somatic mutations of *KCNJ5* in five APMs in adjacent non-pathological adrenal glands [34].

#### Non-neoplastic nodules in pathological adrenal glands harboring PA

Non-neoplastic CYP11B2 positive nodules in BHA also fre-

quently harbor somatic mutations of the genes above. We previously reported that 58% of 99 nodules in clinically diagnosed BHA cases harbored *CACNAID* mutations and 1% had a *KCNJ5* mutation [9]. We also revealed in another study that 65% had *CACNAID* mutations, 8% *KCNJ5* mutations, and 4% both *ATP1A1* and *ATP2B3* mutations among a total of 21 nodules identified in the adrenal glands of BHA patients [10]. Of particular interest, no somatic mutations of any of the genes above were detected in the CYP11B2 positive non-nodular hyperplastic zona glomerulosa cells in APDH [10]. These results indicated that there were substantial differences in the etiology and pathogenesis of autonomous aldosterone production between hyperplastic zona glomerulosa cells exhibiting nodular and non-nodular growth, but further investigations are warranted to clarify the details.

## HISTOPATHOLOGY AND CELLULAR MORPHOLOGY OF PA

Adrenocortical cells are basically classified into compact and clear cells, also termed ZG/ZF-like cells from the standpoint of histology [38-41]. Compact cells have a high nuclear to cytoplasm ratio with an eosinophilic lipid-poor cytoplasm containing relatively abundant cell organelles, including mitochondria. On the other hand, clear cells have a low nuclear to cytoplasm ratio with a lipid-rich cytoplasm containing numerous lipid droplets. However, the correlation between these morphological features and functional aspects has remained rather unexplored. Recent developments regarding the relationship between these morphological features and functional findings of PA lesions are summarized in Figs. 1, 2.

### Aldosterone-producing adenomas

APAs are solitary neoplasms usually measuring more than 10 mm in their greatest dimension and histologically composed of both compact and clear cells with immense intratumoral heterogeneity in their morphological features. The definition of APAs should be based upon both clinical information and histopathological features, including CYP11B2-positive immunoreactivity [6]. We previously reported that *KCNJ5* mutated APAs mostly consisted of clear cells [38]. CYP11B2 immunoreactivity was significantly positively correlated with clear phenotype of tumor cells, especially in *KCNJ5* mutated APAs [38]. *KCNJ5*-mutated APAs were also significantly larger than wild-type (WT) ones, consistent with results that *KCNJ5*-mutated APAs were predominantly composed of more abundant clear tumor cells. We

Histological entity of aldosterone-producing lesions		Diameter	Visible by H&E	Genotype	Cellular morphology	Immunohistochemical findings
Non-neoplastic lesion	Aldosterone-producing micronodule (APM) (previously termed as APCC)	<10 mm	✗	<i>CACNA1D</i> <i>KCNJ5</i> <i>ATP2B3</i> <i>ATP1A1</i>	Compact cells	Aldosterone synthesis: ✓ CYP11B2 (polar preserved)
	Aldosterone-producing nodule (APN)	<10 mm	✓	?	Clear cells	Cortisol synthesis: ✗
	Aldosterone-producing diffuse hyperplasia (APDH)	-	△	✗	Zona glomerulosa cells	Aldosterone synthesis: ✓ CYP11B2 (a broad and uninterrupted strip) Cortisol synthesis: ✗
Neoplastic lesion	microAPA	<10 mm	△	<i>KCNJ5</i> <i>CACNA1D</i> <i>ATP1A1</i> <i>ATP2B3</i> <i>CTNNB1</i> <i>CLCN2</i>	Compact cells	Aldosterone synthesis: ✓ ( <i>KCNJ5</i> > WT)
	APA	>10 mm	✓		Clear cells	Cortisol synthesis: ✓ ( <i>KCNJ5</i> > WT)

△: Sometimes visible

**Fig. 1.** Histopathological entities of aldosterone-producing lesions. Aldosterone-producing lesions are initially classified into neoplastic (microaldosterone-producing adenoma [microAPA] and APA) and non-neoplastic lesions (aldosterone-producing micronodule [APM], previously termed as aldosterone-producing cluster cell [APCC] and aldosterone-producing nodule [APN]). APMs could physiologically exist in the normal adrenal gland and pathologically in bilateral hyperaldosteronism. Both APMs and APNs are considered to harbor both compact and clear cells with polarity of cytochrome P450 family 11 subfamily B member 2 (CYP11B2) expression pattern and no cortisol-synthesizing capability. APMs frequently harbor predominant calcium voltage-gated channel subunit alpha1 D (*CACNA1D*) mutations. APA, including microAPA, has frequent potassium inwardly rectifying channel subfamily J member 5 (*KCNJ5*) mutations and consists of predominant clear cells. Both autonomous aldosterone and cortisol overproduction are more pronounced in *KCNJ5*-mutated APAs. *ATP2B3*, ATPase plasma membrane  $\text{Ca}^{2+}$  transporting 3; *ATP1A1*, ATPase  $\text{Na}^+/\text{K}^+$  transporting subunit alpha 1; *CTNNB1*, catenin beta 1; *CLCN2*, chloride voltage-gated channel 2; WT, wild-type.

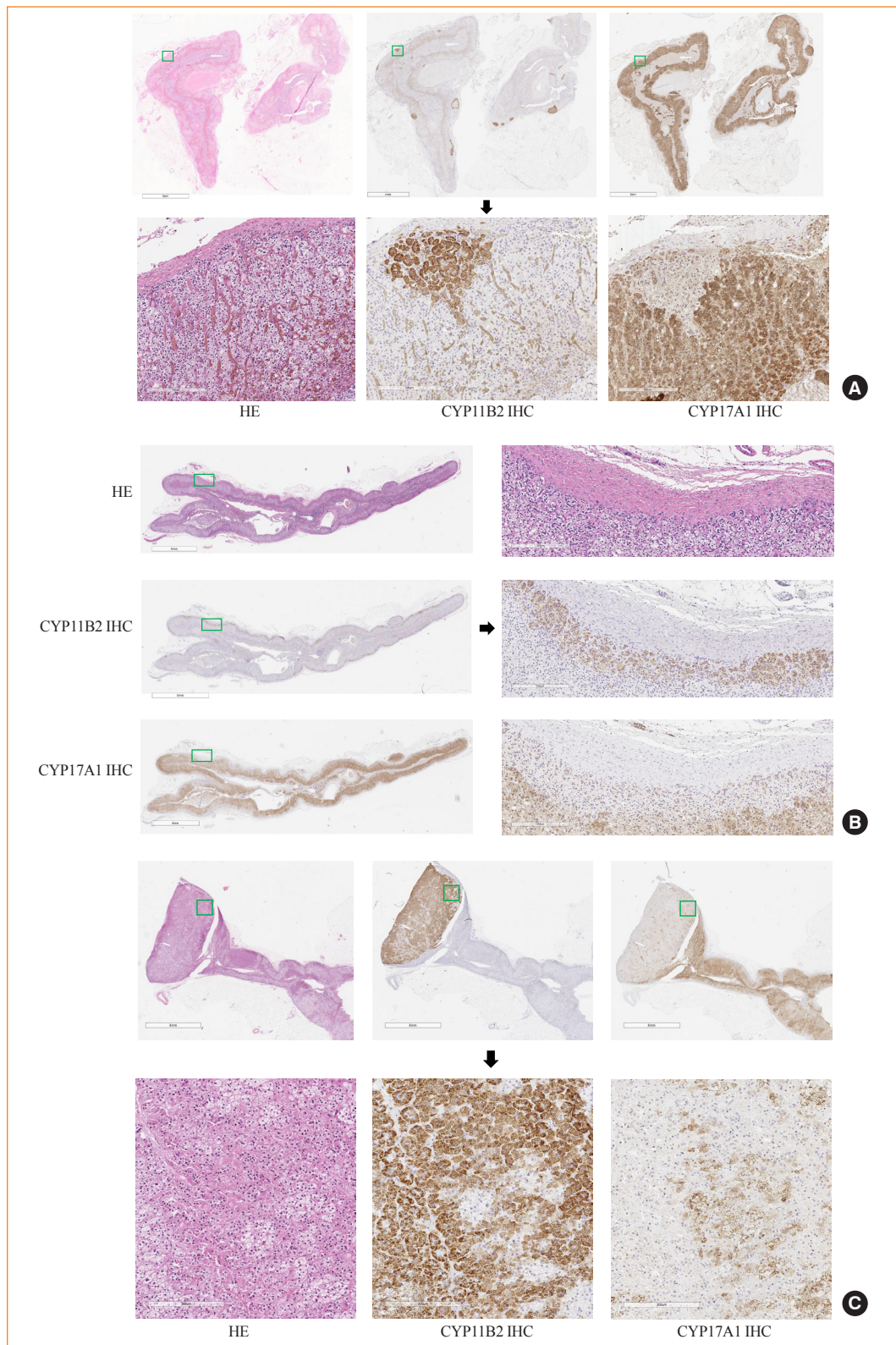
also compared ultrastructural features between *KCNJ5*-mutated and WT APAs in order to further explore the details of endocrine features between compact and clear cell tumor cells [38]. In WT APAs, a rather low intracellular density of lipid droplets and smaller but more mitochondria were identified by electron microscopic evaluations. In contrast, relatively larger mitochondria with a small number and higher density of lipid droplets were detected in *KCNJ5*-mutated APAs [38]. This implied that hormonal reactivity in clear cells of *KCNJ5*-mutated APAs was higher than that of WT ones. We also demonstrated in another study that both *ATP1A1*- and *CACNA1D*-mutated APAs harbored a higher ratio of compact tumor cells than clear ones [42]. *ATP2B3*-mutated APAs were predominantly composed of clear tumor cells, as in *KCNJ5*-mutated APAs [42]. In addition, CYP17A1 immunoreactivity was more abundant in *KCNJ5*-mutated APAs, which indicated that *KCNJ5*-mutated APAs were more active in their hormonal activities synthesizing both aldosterone and cortisol [42]. However, it is also true that intratumoral heterogeneity of compact and clear tumor cells within the

same adrenocortical cells harboring the same genotypes has remained unexplored. mRNA expression levels of both CYP17A1 and CYP11B1 were also reported to be significantly higher in compact tumor cells than in clear ones, whereas that of CYP11B2 tended to be higher in clear tumor cells regardless of genotype [43]. This is consistent with results of our previous study that the immunoreactivity of CYP11B1 and CYP17A1 was significantly higher in compact tumor cells of CPAs [39]. Therefore, the hybrid steroidogenesis or simultaneous biosynthesis of both glucocorticoids and mineralocorticoids in the same tumor cells of APAs represented the zonation-deviated phenotype and compact or ZG-like cells had higher cortisol-producing ability, whereas clear or ZF-like cells harbored higher aldosterone-producing ability.

#### *Aldosterone-producing non-neoplastic nodules (APMs and APNs)*

Based on the statement of international consensus on PA histopathology, aldosterone-producing non-neoplastic nodules are further classified into APMs and aldosterone-producing nodules





**Fig. 2.** Representative microscopic images of H&E, cytochrome P450 family 11 subfamily B member 2 (CYP11B2) immunohistochemistry (IHC), and cytochrome P450 family 17 subfamily A member 1 (CYP17A1) IHC. (A) Aldosterone-producing micronodules, previously termed aldosterone-producing cluster cells. (B) Aldosterone-producing diffuse hyperplasia. (C) Aldosterone-producing adenoma.

(APNs) according to whether the nodule is visible (detected) by H&E section (APN was defined as visible nodules) or not, although both types preserve the polarity of zonation, including the gradient of the pattern of CYP11B2 immunoreactivity from the outer to inner part [6]. The previously proposed classifications of “APCC” and/or “micronodule” have been reported to be difficult to distinguish from each other by histopathological findings including CYP11B2 immunohistochemistry alone, and required relevant clinical information such as the presence or absence of hypertension and/or PA. However, the recently proposed international consensus has made it possible to distinguish those non-neoplastic nodules (APM and APN) by histopathological findings alone even without patients’ clinical information. However, we should note that the threshold between APM and APN remains unclear and further discussion is definitively warranted to clearly distinguish these entities from each other. A further improved classification, combined with the clinical information listed above, should clarify the difference between the “physiological” and “pathological” status of those aldosterone-producing adrenocortical non-neoplastic lesions.

In the normal adrenal gland, CYP11B2 is intensely immunolocalized in the subcapsular area and its immunoreactivity gradually decreases [9,44]. This polarization of aldosterone production detected in normal adrenal glands is maintained in APMs but lost in APAs possible due to the nature of their neoplastic transformation [10,44]. This could contribute to the differential diagnosis of neoplasms versus non-neoplastic nodules in the resected adrenal glands of PA patients. Neither CYP11B1 nor CYP17A1 was expressed, indicating the preservation of development of adrenocortical differentiation. APMs are present not only in the physiologically normal adrenal gland, but of particular interest also in adjacent non-neoplastic adrenal glands with suppression of the renin-angiotensin-aldosterone system (RAAS) of PA patients [5,45]. In addition, APMs in normal adrenal glands also harbored APA-driver somatic mutations as described above, implying their association with APAs.

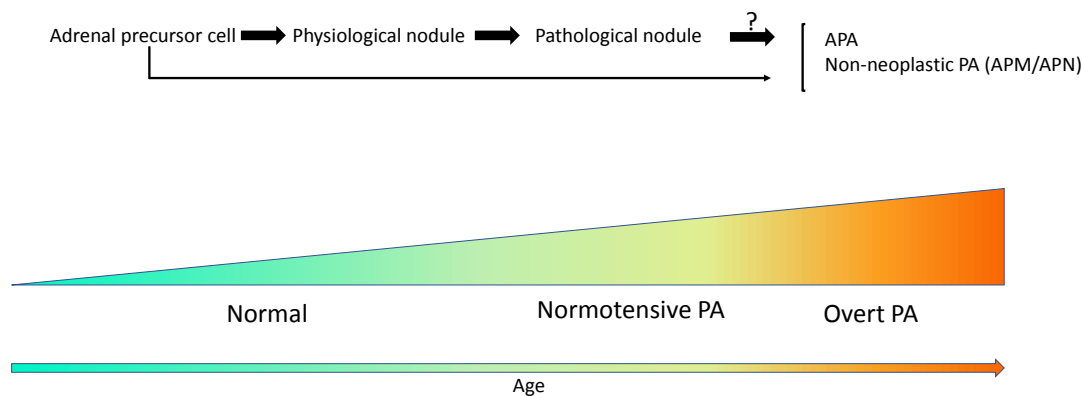
APMs or APNs could be detected in BHA cases with multiple numbers and in unilateral PA. In our previous study, 32 nodules were examined in clinically identified BHA cases [10]. Eighteen nodules were composed predominantly of clear cells, 13 of compact cells, and one did not have the information available [10]. The ratio of clear and compact cells was significantly lower in APMs than that in APAs. This is possibly due to the genetic effects on cellular morphology, as *KCNJ5* mutations are closely associated with the development of clear cell morphology in autonomous aldosterone-producing adrenocortical lesions and

*CACNAID* mutations are closely associated with that of compact cells as described above. In clinical practice, the best practical approach to differentiate between APA and non-neoplastic nodule is based on their diameters because of the reasons above, but CYP11B2 immunohistochemistry could also contribute to this differentiation in routine clinical settings.

## POTENTIAL ASSOCIATION OF APM AND APA

APMs have been reported to harbor APA-driver mutations and APMs have been proposed as potential precursors in the development of APA, in addition to the fact that non-neoplastic CYP11B2-positive nodules in adrenal glands in PA patients could be developed from physiological ones, acquiring the ability of excessive autonomous aldosterone production in patients with BHAs due to the predominant genotype of *CACNAID* in both entities and the presence of similar histological features [10,12].

In general, some tumors are considered to originate from stem cells with increased ability of self-renewal following oncogene activation. These stem cells in adrenal glands are considered responsible for continuous replenishment of steroidogenic cells in order to compensate for the cell death required to maintain normal adrenal size and function [46]. Adrenal stem/precursor cells have been detected in both APAs and adjacent ZG cells with weak sonic hedgehog signaling molecule (SHH) expression, which is one of the precursor markers and activated  $\beta$ -catenin indicating their origin from adrenocortical stem cells [46]. ZG cells not only produce aldosterone but also have a relatively high capacity of cellular renewal, and this renewal process follows a centripetal migration: from ZG to ZF [47,48]. In addition, CYP11B2-positive ZG cells can be also differentiated into ZF and CYP11B2 and CYP11B1 double-positive cells present at the ZG-ZF boundary, although extremely rare, resembling the hybrid steroidogenesis in APA cells [48]. In addition, melanocortin 2 receptor (MC2R) expression was detected in APMs, indicating that these cells could potentially differentiate into ZF cells through the protein kinase A signaling pathway [48]. MC2R was also reported to regulate SHH signaling, which further demonstrated its potential differentiation ability [48]. Collectively, these findings above all indicate that APMs in normal adrenal glands could possibly differentiate into ZF-like cells and play important roles in development of APAs. However, at this juncture, the transition from APMs to APAs remains unestablished.



**Fig. 3.** Development of aldosterone-producing lesions along with aging. Aldosterone-producing micronodule (APM) develops from adrenal precursor cells or progenitor ZG cells. Along with aging, APM contributing to normal renin-angiotensin-aldosterone system (RAAS) has the potential of developing to APM that is independent of RAAS and accounts for normotensive primary aldosteronism (PA). APM may continue to develop with aging, which clinically accounts for many cases of PA, but its association with APA remains unclear. In addition, aging could play a pivotal role in the formation of aldosterone-producing micronodules.

### SUBCLINICAL OR NORMOTENSIVE PA

The diagnosis of overt PA is generally based upon the value of the aldosterone-to-renin ratio in severe or resistant hypertension. Overt PA is mostly composed of APA and BHA and it is clinically not difficult to differentiate between these two entities. However, it is also true that some individuals have normotension and normal electrolyte values accompanied by suppressed plasma renin activity with normal or slightly elevated plasma aldosterone levels [49]. These subjects demonstrated a tendency for renin-independent aldosteronism, which could place them at a higher risk of developing clinically meaningful hypertension [49]. Therefore, we tentatively termed this condition as subclinical PA [49]. One of the potentially frequent causes of subclinical PA is considered the presence of APMs in the normal adrenal cortex [11,37]. APMs frequently harbor APA-driver somatic mutations and the number of mutated APMs was found to increase with aging [11,37]. We also demonstrated the presence of APMs in normal adrenal gland adjacent to APAs with autonomous aldosterone production [37]. These findings above all indicate that the development of APMs in the normal adrenal glands is indeed independent of the status of RAAS in subjects (Fig. 3). This formation of RAAS-independent APMs and APA or APN is therefore reasonably postulated to result from aging, but further investigations are warranted for clarification.

### AGING AND ALDOSTERONE

Aging is a natural process characterized by a progressive loss of

physiological function, which is especially marked in the endocrine system. Many earlier studies demonstrated an age-dependent decline in the activity of the RAAS in normal human subjects regardless the status of sodium repletion [50,51]. This decline in the RAAS with increased aging is generally considered to result from decreased plasma renin activity [52]. One possible effect of this phenomenon may be the deterioration of renal function with aging [53]. In addition, the response of aldosterone to angiotensin II (ANGII) was more sensitive in younger adult rats, but decreased in older ones [54]. This was further confirmed in human female subjects, but of particular interest, not in males [55]. The progressive decrement of plasma renin activity despite unchanged plasma and urinary aldosterone levels was reported in elderly subjects without concomitant evidence of PA in the supine position [56]. Physiological RAAS-independent autonomous aldosterone production was proposed to develop possibly due to changes of ZG with aging [56].

The age-dependent decline of aldosterone levels is not only due to decreased renin or ANGII levels, but also due to age-associated structural changes. For instance, the ZG becomes atrophic in older people compared with young adults [57,58]. The number of APMs in the normal ZG was also reported to increase during aging [11,56]. However, with the increased number of APMs, the total CYP11B2-expressing area in APMs was significantly negatively correlated with aging [56]. Based on those results, RAAS-independent development of APMs with aging leads to more autonomous aldosterone production, but less physiological aldosterone production [56]. In addition, adrenocortical nodule formation is well known to increase with



aging by classical autopsy studies [57]. Moreover, the ZG cells lost in elderly people were replaced by abundant progenitor cells, which implied the potential differentiation to APMs or APA [58]. Collectively, aging is a risk factor in the transformation of “physiological” to “pathological” adrenocortical nodules and possibly to APAs, which all could contribute to autonomous aldosterone production. This autonomous aldosterone production leads to a potentially high risk of incident hypertension in elder people.

## CONCLUSIONS

Aldosterone-producing disorders are the major cause of PA or normotensive PA with autonomous aldosterone secretion. These disorders frequently harbor somatic mutations in ion channels or pumps including *KCNJ5*, *CACNA1D*, *ATP1A1*, and *ATP2B3*. Somatic mutations may induce a secondary differentiation of adrenocortical cells into compact or clear cells. All of these contribute to normotensive PA and overt PA depending on the subtype of the disorder (APMs, APNs, and APAs). The formation of these disorders remains unclear, but may possibly be due to somatic mutations or aging, which converts physiological adrenocortical morphology to pathological disorders. In addition, these disorders in elder people secrete RAAS-independent aldosterone, which accounts for the high risk of incident hypertension or other cardiovascular events.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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