

# Recent Advances in the Diagnosis and Molecular Aspects of Adrenal-Endocrine Hypertension

JMAJ 55(4): 334–338, 2012

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The scientific expertise of Endocrinology and Metabolism covers clinical metabolic abnormalities including hypertension, diabetes, hyperlipidemia and obesity, in addition to bone mineral disorders as well as diseases of the hypothalamus, pituitary, thyroid, parathyroid, pancreas, adrenal and sexual glands (**Fig. 1**). Over time, these disorders have become integrated, resulting in progressive advancement of diagnosis and treatment determinations. Because of the limitation of space in the manuscript, here we focus on the recent advances in the diagnosis and molecular characteristics of hypertension caused by primary aldosteronism (PA).

There are numerous patients with hypertension, in whom systolic and diastolic blood pressures are higher than 140 and 90 mmHg, respectively. The number of these patients is estimated to be 1.5 billion in the world and 40 million in Japan. Hypertension is a well-known risk factor for the development of cerebral-cardiovascular complications. Sodium has long been considered as an important environmental factor in the progression of hypertension.<sup>1</sup> Recent observations have resulted in considerable attention being paid to the association between aldosterone excess and hypertension. Aldosterone levels in the blood vary according to dietary sodium intake with a large community-based study, which shows that aldosterone concentrations in the upper limit of the normal range predispose to the development of hypertension.<sup>2</sup>

## Prevalence of PA in Japan

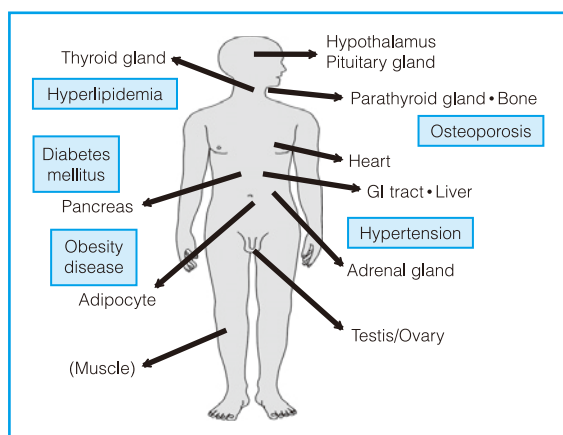
Patients with hypertension are categorized into two types; essential hypertension (unknown causes) and secondary hypertension. PA is the most frequent cause of secondary hypertension and is estimated to include approximately 10% of all hypertensive patients.<sup>3–6</sup> This equates to 4 million or more subjects with hypertension due to PA in Japan. PA includes several subtypes; aldosterone-producing adenoma (APA), bilateral hyperplasia (idiopathic hyperaldosteronism, IHA), unilateral adrenal hyperplasia, unilateral multiple adrenocortical nodules, bilateral APA, and hereditary glucocorticoid-reactive hyperaldosteronism. The first two subtypes are the most common.

## Why Does PA Have to Be Diagnosed and Treated?

Aldosterone is secreted from adrenal glomerulosa cells and exerts its characteristic functions through the mineral corticoid receptor (MR) that is expressed in the kidney, brain and heart.<sup>6</sup> In the kidney, aldosterone activates the sodium-chloride cotransporter in the distal tubule and epithelial sodium channel in the collecting duct, thereby promoting sodium retention and development of hypertension.<sup>1</sup> Aldosterone activates MAPK and CaM kinase II in the brain and heart to stimulate remodeling and fibrosis of endothelial smooth muscle cells, resulting in

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**Fig. 1 The organs covered by the scientific fields of Endocrinology and Metabolism**

organ damages.<sup>6-8</sup> We showed previously that patients with PA (mean age 52.0 years) had a significantly higher prevalence (28%) of cerebral-cardiovascular complications than subjects with essential hypertension (mean age 61.7 years, 4.2%).<sup>9</sup> Moreover, coexistence of PA and sub-clinical Cushing syndrome with autonomous hypersecretion of cortisol was shown to cause the rate of cerebral-cardiovascular events to increase markedly to over 50%.<sup>9</sup> Indeed, high serum levels of both aldosterone and cortisol have been observed to be independent predictors of increased mortality risk in patients with chronic heart failure.<sup>10</sup> Taken together, these results indicate that an accurate and distinguishable diagnosis for PA should be performed especially in younger (<60 years) patients with hypertension. It is also important to determine whether PA coexists with hypercortisolism, due to the considerably higher incidence of cerebral-cardiovascular events that occur when the two disorders coexist.

### Clinical Findings Related to PA

Aldosterone-induced activation of the MR stimulates the luminal sodium transporter in the kidney to reabsorb sodium and bicarbonate and enhance potassium secretion, resulting in kaliuresis. However, low potassium (<3.5 mEq/L) in the blood is found in only 18–20% of patients with PA,<sup>3,11</sup> indicating that a low blood potassium level is not a good predictor of the disorder. In

addition, it has been shown that the presence of resistant hypertension (blood pressure of >140/90 mmHg despite a three-drug regimen) is not useful for identifying PA in patients with hypertension.<sup>12</sup> Compared with that in Western countries, there is an increased frequency of APA (approximately 30% vs. 70%) associated with unilateral adrenal tumors and a relatively decreased frequency of IHA (approximately 70% vs. 30%) with bilateral adrenal swelling in Japan.<sup>13,14</sup> Nevertheless, abdominal CT and MRI are unable to detect abnormalities in the size and shape of adrenal glands in 60% of patients with APA.<sup>14</sup> Therefore, the screening tests as described in the next section are important for identifying PA.

### Mechanisms Underlying the Development of PA

While variations at the locus encoding the aldosterone synthase gene *CYP11B2* affects aldosterone concentrations,<sup>15</sup> the molecular mechanisms underlying the development of PA remain unknown.<sup>16</sup> Adrenal glomerulosa cells highly express KCNJ5 (Kir 3.4), a type of potassium channel, which exists both as a homotetramer and heterotetramer with KCNJ3 to produce a highly negative membrane potential. Closure of K<sup>+</sup> channels by either angiotensin II (AngII) or increases in extracellular K<sup>+</sup> depolarizes the membranes to activate the voltage-gated calcium channel. Recently, APA was shown to possess somatic mutations (G151R and L168R) in KCNJ5,<sup>17</sup> even in the absence of increased AngII or high blood K<sup>+</sup>, which cause alterations in channel selectivity, leading to nonselective Na<sup>+</sup> entry and chronic depolarization. These mutations lead to increases in intracellular Ca<sup>++</sup> levels, resulting in constitutive aldosterone synthesis and cellular proliferation. Our recent studies demonstrated that these mutations were observed in 65% of Japanese patients with APA,<sup>18</sup> a considerably higher frequency than that reported in Western countries (34–36%).<sup>17,19</sup> In contrast, these mutations were not observed in adrenal tumors that produced cortisol. Moreover, KCNJ5 mutations were associated with high levels of KCNJ5 protein and gene expression that correlated with increased concentrations of blood aldosterone and low levels of serum potassium. Conversely, it still remains

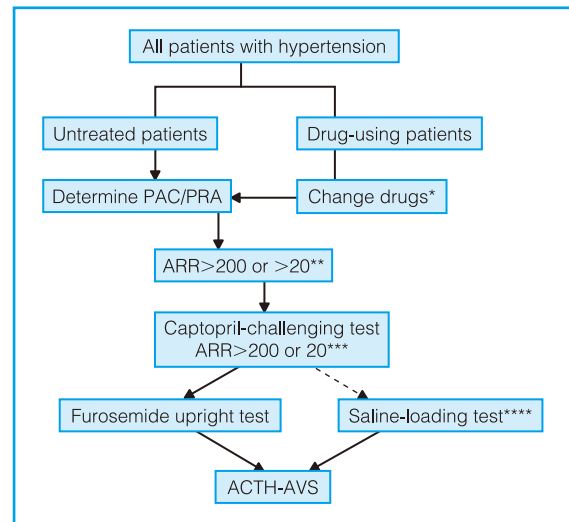
	PAC	PRA	ARR
B-Blocker	↓	↓↓	↑
Thiazide	↑	⇒	↑
Ca-blocker	⇒ ↓	↑	↓
ARB	↓	↑ ↑	↓
ACE-I	↓	↑ ↑	↓
MR antagonist	↑	⇒	↑

**Fig. 2 Impact of different classes of antihypertensive agents on renin activity and aldosterone secretion**

unknown how IHA develops, although blood pressure oscillates with the circadian rhythm and mice deficient in the clock component Cryptochromes exhibit salt-sensitive hypertension due to the abnormal production of aldosterone from the adrenal gland.<sup>20</sup>

### Characteristics of Aldosterone Secretion

To gain an insight into aldosterone secretion in PA, it is necessary to evaluate the characteristics underlying adrenal secretion of aldosterone. Under normal conditions, aldosterone secretion is regulated by the renin-AngII system and K<sup>+</sup> concentration, whereas its secretion in APA cases is regulated mainly by adrenocorticotrophic hormone (ACTH). A number of antihypertensive drugs<sup>5</sup> may also affect aldosterone levels (Fig. 2). For instance, MR antagonists and diuretics stimulate aldosterone secretions, whereas β-adrenergic blockers decrease renin activity and aldosterone secretion. Angiotensin-converting enzyme (ACE)-inhibitors inhibit aldosterone secretion and stimulates renin activity. Calcium channel antagonists, the vasodilator hydralazine and alpha-blocking agents have minimal impacts on aldosterone secretions and renin activity, whereas AngII receptor blockers (ARBs) do not have a major influence on aldosterone secretion in the early phase of treatment. Sodium



**Fig. 3 The diagnostic procedure for PA in accordance with the guideline for the diagnosis and treatment of primary aldosteronism recommended by the Japan Endocrine Society**

\* Antihypertensive drugs such as hydralazine, α-blocker and calcium channel antagonist, which possess the minimum influences on PAC and PRA, should be changed. \*\* ARR, the aldosterone (PAC) to renin (PRA) ratio. Upon expression as pg/mL or ng/dL of PAC, the ARR is 200 or 20, respectively. \*\*\* Judged to be positive with the ARR showing over 200 (20) or with a PAC of >120 pg/mL (>12 ng/dL) after captopril administration. \*\*\*\*Less sensitivity of saline-loading test to predict PA in Japan.

restriction has a grater stimulatory effect on renin activity than aldosterone secretion, resulting in a decreased aldosterone to renin ratio (ARR). Renal impairment also decreases renin activity, causing an increase in ARR.

### Screening to Identify PA

Valuable clinical practice guidelines for diagnosing PA have recently become available from the task force committee of the Japan Endocrine Society.<sup>21</sup>

Because the parameters of aldosterone secretion and renin activity may be influenced by various antihypertensive drugs, the regime should be changed to drugs, such as alpha-blocking agents, hydralazine or calcium channel antagonists that have minimal influences on these parameters. Addition of an ARB may be considered in subjects, who still have poor blood pressure control after this change in treatment.

After changing drugs for six weeks or longer, blood samples are collected, according to the guidelines for diagnosing PA shown in **Fig. 3**.<sup>21</sup>

Plasma aldosterone concentration (PAC) and plasma renin activity (PRA) are measured after the patient has rested recumbent for 20–30 minutes. The aldosterone concentration should be expressed as pg/mL or ng/dL. An ARR of >200 (pg/mL in display) or 20 (ng/dL in display) indicates the possible existence of PA. In order to confirm PA, the following loading examinations are performed.

The challenge of captopril, an ACE-inhibitor, is easy to perform in outpatients and involves collection of blood samples 60–90 min after oral administration of 50 mg captopril for measuring PAC (pg/mL or ng/mL) and PRA. Hypertensive patients without PA have an ARR of <200 or <20, because aldosterone secretions is inhibited, and renin activity increases after the captopril challenge. In contrast, patients with PA have an ARR of >200 or >20, because aldosterone secretions from PA is independent of renin. A PAC concentration of >120 pg/mL or >12 ng/dL also raises the possibility of PA. When this test is positive, doctors should initiate further tests at a specialized medical institution.

### Confirmation of PA

The examinations performed at specialized medical institutions include a furosemide upright test and saline (salt)-loading test (see the guideline for PA in detail<sup>21</sup>). Although the furosemide test is sensitive (approximately 87%) for determining PA, the sensitivity of the saline-loading test is considerably lower in Japanese patients at approximately 63%.<sup>22</sup> This is markedly more different from the high sensitivity (approximately 88%) reported in Western countries.<sup>23</sup> The saline-loading test should also not be used in patients with a history of cerebral-cardiovascular events.

In order to confirm laterality of hypersecretion of aldosterone, adrenal vein sampling (AVS) is used to measure PAC and cortisol before and after ACTH administration (ACTH-AVS). Because it is somewhat difficult to perform a right-sided AVS, it is important to be aware of cortisol concentrations of >200 µg/dL after the ACTH injection. The ACTH-AVS test is the gold standard method for determining the laterality of aldosterone hypersecretion in PA.<sup>5,21</sup>

APA (unilateral disorder) should be considered, when a unilateral aldosterone concentration is >2,000 pg/mL at baseline and/or >14,000 pg/mL after the ACTH injection. IHA (bilateral disorder) is diagnosed, when there is no laterality in aldosterone concentrations, instead of increased PAC. However, the ACTH-mediated PAC, but not the basal PAC, is reliable to distinguish the two subtypes.<sup>24</sup> Alternatively, an aldosterone to cortisol ratio (ACR) after the ACTH injection may also be calculated in the ACTH-AVS test. When the right and left difference ratio of ACR is over 2.6, this suggests APA. The sensitivity of this ratio has been reported to be 98%,<sup>25</sup> but the other group reported that this was 67% (personal communication by Omura M). A recent interesting observation<sup>26</sup> showed that aldosterone concentrations in peripheral blood were higher in APA (cut-off value of 380 pg/mL) than in IHA 90 min after the ACTH injection under 1 mg-dexamethasone suppression. Further studies are required to validate this finding.

### Treatment of PA

In principle, abnormal unilateral formation of APA can be removed under laparoscopy.<sup>5,21</sup> Prior to this surgery, oral administration of an MR antagonist and/or potassium tablet is required to normalize hypokalemia. Approximately 89% of patients with APA who have undergone a unilateral adrenalectomy achieve resolution or improvement in hypertension.<sup>27</sup> Patients with IHA are administered an oral MR antagonist, although the drug may have the side effects of alterations in libido, gynecomastia and hyperkalemia.<sup>6</sup> When the MR antagonist cannot achieve sufficient blood pressure control, combined therapy of ACE-inhibitor and ARB should be prescribed.

### Concluding Remarks

In this paper, we have discussed recent advances in the diagnosis and molecular characteristics of PA hypertension from view points of Endocrinology and Metabolism. The sensitivity of saline challenge, the frequency of APA compared with IHA and the prevalence of the KCNJ5 mutation in APA are significantly different between Japanese and Western countries. However, the reasons for these differences remain

unknown. We consider that our review may lead to improvements in the clinical managements of hypertensive patients with the potential to

reduce cerebral-cardiovascular events that occur frequently in PA patients.

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