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ARTICLE

# Interpreting stimulated plasma renin and aldosterone to select physiologically individualized therapy for resistant hypertension: importance of the class of stimulating drugs

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## Abstract

In the treatment of resistant hypertension, physiologically individualized therapy based on phenotyping with plasma renin activity (PRA) and plasma aldosterone significantly improves blood pressure control. Patients with a low-renin/low aldosterone (Liddle) phenotype respond best to amiloride, while those with low-renin/high aldosterone respond best to aldosterone antagonists, and those with high renin/high aldosterone (renal phenotype) respond best to angiotensin receptor blockers (ARB). However, it is important to measure PRA in a stimulated condition to distinguish between low levels due to high salt intake, licorice or nonsteroidal inflammatory drugs and low levels due to suppression by excess aldosterone secretion or renal tubular genetic variants causing retention of salt and water (Liddle phenotype). In the past, both diuretics and angiotensin converting inhibitors (ACEi) have been used for this purpose, and it has been assumed that these classes of drugs are equivalent. In this study of 2896 patients with hypertension, we evaluated that assumption. We found important differences among diuretics alone, ACEi/ARB alone, and ACEi/ARB + diuretics, which all stimulated PRA. However, ACEi/ARB lowers plasma aldosterone, and beta blockers lower PRA. Among patients with systolic pressure ≥ 180 mmHg ± diastolic pressure ≥ 100 mmHg stimulated only by diuretics, the phenotypes were 25% Liddle, 38% IA, 8.7% renal, and 28.3% mixed. In choosing physiologically individualized therapy based on PRA and aldosterone, it is important to consider the classes of stimulating drugs. Phenotypes are best distinguished by taking into account the aldosterone/PRA ratio in addition to the levels of PRA and aldosterone.

**Keywords** Aldosterone · Antihypertensive drug class · Personalized medicine · Renin · Stimulation

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## Introduction

Using plasma renin activity (PRA) to individualize therapy for hypertension was first proposed by Laragh et al. [1]. Despite the logic of this approach, it was not widely adopted, perhaps for two reasons: First, this approach is not particularly useful in patients with easily controlled hypertension, and second, to be informative, it is important that the PRA be assessed in a stimulated condition. In 1975, Dawson's group [2] suggested that it was important to give a dose of furosemide before assessing PRA, to make it possible to distinguish between low PRA due to high salt intake from low PRA due to suppression by pathophysiological causes, such as primary aldosteronism. Laragh recommended using captopril to stimulate PRA for the diagnosis of renal artery stenosis [3]. It should be noted that fluid retention from causes such as licorice or nonsteroidal anti-inflammatory drugs (with the possible exception of sulindac, which does not raise blood pressure (BP) [4]), will also suppress plasma renin levels.

Spence has used furosemide stimulation of PRA for the management of very severe hypertensive patients since 1977, when the treatment of hypertension was much less prevalent and effective than it has been in recent years [5]. Many patients had extraordinarily high BPs seldom seen now in practice in North America: two walked into his clinic with systolic pressure > 300 mmHg and diastolic pressure of 170 mmHg. In such patients, assessing stimulated PRA made all the difference to their treatment [5]. In the days before angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) were available, Spence routinely measured PRA at 4 h after an oral dose of furosemide (~0.5 mg/kg) or for patients coming to the clinic from a long distance away, at 30 min after an intravenous dose of ~0.5 mg/kg, (to avoid the inconvenience of frequent stops for urination on the way to clinic). In 1999, Spence reported on his experience with this approach for over 20 years and noted that patients of African descent seemed much more likely to have primary aldosteronism [5]. However, since the advent of ACEi and ARB, he has assumed that patients already taking a diuretic and/or ACEi or ARB, which represents most patients with resistant hypertension, do not need additional stimulation with furosemide. The assumption that stimulation with ACEi or ARB is equivalent to stimulation with a diuretic was tested in the present study.

Nagasawa et al. assessed the effects of different antihypertensive drug classes on the diagnosis of primary aldosteronism [6], but did not assess the effects of these drugs on the diagnosis of a Liddle phenotype.

In 2009, Egan et al. reported a randomized trial of PRA for the management of resistant hypertension [7]. Although the proportion of patients achieving BP control was not significantly improved, there was a greater reduction in systolic pressure and some reduction in the medication requirement. However, measuring PRA in the absence of plasma aldosterone (ALD) does not permit distinction between the two major categories of low-renin hypertension: a high aldosterone/low-renin phenotype suggesting primary aldosteronism or the inappropriate secretion of ALD versus a low-renin/low ALD (Liddle) phenotype, suggesting salt and water retention due to the increased renal tubular absorption of sodium.

The physiology of renin/ALD phenotyping for the physiologically individualized therapy (PhysRx) of hypertension was recently reviewed [8]. Patients with a renal/renovascular phenotype (high renin/high ALD) are best treated with ARBs; those with inappropriate ALD secretion are best treated with ALD antagonists, whereas amiloride, an antagonist of the renal epithelial sodium channel (ENaC), is the specific therapy for a Liddle phenotype (low-renin/low ALD).

In 2017, Akintunde et al. reported that compared with usual care, PhysRx significantly improved BP control among patients with uncontrolled hypertension in Africa [9]. As a result of that report, a randomized trial of this strategy is planned in China by investigators of the China Stroke Primary Prevention Trial (CSPPT) [10], as a follow-up to that trial.

This paper reports the results of a study carried out in preparation for CSPPT2 to determine the prevalence of hypertensive phenotypes in China by the measurement of PRA and plasma ALD and to assess the effect of different antihypertensive regimens on stimulated PRA, ALD, and the ALD/renin ratio.

## Methods

### Patient population

A total of 2986 hypertensive inpatients at the Second Affiliated Hospital of Nanchang University were recruited for analysis between April 2011 and October 2016. Hypertension was defined as [1] a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg [2], the use of antihypertensive medication, or [3] a physician diagnosis of hypertension as per clinical history. A physical examination and medical history were completed on each patient, and sitting PRA, angiotensin II (AII), and ALD were measured. Antihypertensive medications were classified into a single category according to the Seventh Report of the Joint National Committee on Hypertension. Each medication identified was assigned its generic equivalent. Single-pill combinations were separated into their generic components.

### Measurement of blood pressure, PRA, and ALD

BP was measured using an electronic sphygmomanometer by an inpatient nurse. The BP of the participants was measured for ~10 min while the participant was resting and sitting in an upright position with legs uncrossed and feet flat on the floor. In addition, no vigorous exercise was allowed during the 30 min before each measurement. Two BP readings were taken, with 30-s intervals between each measurement. The mean of the two measurements was used as the BP for each participant. Admission day BP data were used for analysis.

PRA and ALD were measured at the Nuclear Medicine Department of the Second Affiliated Hospital of Nanchang University by using a chemiluminescent immunoassay (New Industrial, Shenzhen, China). Following an overnight fast, blood samples were obtained from the antecubital vein of patients in a sitting position at 6–7 A.M. The blood sample was collected in a tube that contained EDTA. The blood tubes were then centrifuged in a refrigerated centrifuge, and the plasma was separated from the cells immediately after centrifugation.

PRA was measured as previously described [11]. Briefly, before the start of the assay, a protease inhibitor and generation buffer were added to the plasma sample to prevent the degradation of angiotensin-I (Ang-I) in plasma. The plasma sample was divided into two fractions and incubated at 0–4 °C (on an ice bath) and 37 °C for 60 min. The results of sitting PRA were expressed in terms of the mass of angiotensin-I (Ang-I) generated per volume of human plasma in unit time (ng/mL/h). The reference range for PRA was 0.15–2.33 ng/mL/h. For the kit (angiotensin I in vitro diagnostic kits, Chemiluminescent Immunoassay, New Industry, Shenzhen, China), the intra- and inter-assay coefficients of variation were 10–15%.

Plasma ALD was measured by a competitive immunoassay ALD kit which uses innovative chemistry and a specific antiALD antibody that binds quantitatively to all isomers of ALD. The reference range for ALD was 3–16 ng/dL. The intra- and inter-assay coefficients of variation were 10–15%.

### Phenotypes

Because some patients were unstimulated, and in those with stimulation, various different classes of drugs were involved in stimulation, it was not appropriate to use the fixed cutoffs for "normal" levels of PRA and ALD or the ALD/renin ratio that are often used, such as a PRA or ALD below or above the 95% confidence interval. The phenotypes were defined by tertiles and median levels of PRA and plasma ALD. A Liddle phenotype (low-renin/low ALD) was defined as both PRA and ALD in the lowest tertile; a renal phenotype was defined as both PRA and ALD in the highest tertile. As in the PATHWAY 2 trial [12], inappropriate ALD secretion was defined as PRA in the lowest tertile with ALD above the median level. Patients who did not fall into one of the other 3 phenotypes were designated as a "mixed" phenotype.

### Groupings for analyses of stimulation

We did not find significant differences between ALD antagonist (spironolactone) and diuretics, so these classes were combined as "diuretics"; similarly, there were no significant differences between ACEi and angiotensin receptor antagonists (ARB), so these classes were combined as ACEi/ARB.

We analyzed the stimulation of PRA and ALD as unstimulated versus stimulated by diuretics only, ACEi/ARB alone, and diuretics and ACEi or ARB. To assess the frequency of phenotypes among patients who would be regarded as having resistant hypertension, we analyzed the phenotypes by various groupings of stimulating drugs in patients with systolic pressure ≥ 180 mmHg and/or diastolic ≥ 100 mmHg, despite taking medications.

### Statistical methods

Statistical tests were performed using SPSS version 25 (IBM, Armonk, N.J.) For continuous variables, analysis of variance was used to compare means across groups; a Chi-Square test was used to compare categorical variables across groups. Quantities are shown as *n*, %.

## Results

Data on medication were available for 2896 patients; BP data were available for 2895 patients. The patient ages ranged from 15–95 years, with a mean age of 56.60 ± 14.04 years; 1594 (55.0%) patients were men.

Of these patients, 875 (30.24%) had systolic pressures ≥ 180 mmHg and/or diastolic pressures ≥ 100 mmHg, identified as a subgroup equivalent to resistant hypertension. The patient ages ranged from 15 to 94 years, with a mean age of 51.68 ± 14.63 years; 559 (63.9%) patients were men. Table 1 shows the characteristics of all patients by stimulation status (unstimulated vs. stimulated by diuretic alone, ACEi/ARB alone, or by ACEi/ARB ± diuretic).

The distribution of BPs in the population is shown in Fig. 1; some patients had very severe hypertension. The highest systolic pressure was 260 mmHg, and the highest diastolic pressure was 170 mmHg. Most of the patients were taking many antihypertensive medications: 1534 took a diuretic or spironolactone; of these patients, 88% also took

**Table 1** Characteristics of all patients with unstimulated plasma renin activity (PRA) versus those whose PRA was stimulated with diuretic alone, ACEi inhibitor or angiotensin blocker alone, or ACEi inhibitor/angiotensin receptor blocker + diuretic

| Variable | Unstimulated $n = 648$ (22.4%) | Diuretic $n = 247$ (8.5%) | ACEi/ARB $n = 1013$ (35%) | ACEi/ARB/ Diuretic $n = 987$ (34.1%) | $p$ |
|---|---|---|---|---|---|
| Continuous variables mean ± SD | | | | | ANOVA |
| Age (years) | 55.57 ± 14.14 | 56.83 ± 13.59 | 56.60 ± 13.61 | 57.22 ± 14.51 | 0.14 |
| Systolic pressure (mmHg) | 145.97 ± 24.49 | 154.47 ± 28.52 | 147.76 ± 23.95 | 157.93 ± 28.28 | <0.0001 |
| Diastolic pressure (mmHg) | 86.67 ± 16.06 | 89.01 ± 19.26 | 86.35 ± 16.03 | 90.22 ± 18.80 | <0.0001 |
| PRA (ng/mL/h) | 0.53 ± 1.47 | 0.83 ± 1.85 | 1.40 ± 2.67 | 1.76 ± 3.24 | <0.0001 |
| ALD (ng/dL) | 16.94 ± 11.85 | 17.50 ± 10.66 | 15.15 ± 12.12 | 16.93 ± 10.34 | <0.0001 |
| Aldo/renin ratio | 51.81 ± 151.31 | 63.72 ± 167.08 | 63.72 ± 167.08 | 52.24 ± 188.35 | 0.08 |
| Categorical variable % | | | | | Chi-Square |
| Male | 47.5% | 61.9% | 55.4% | 58.0% | <0.0001 |

*ACEi* angiotensin converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *Aldo* aldosterone

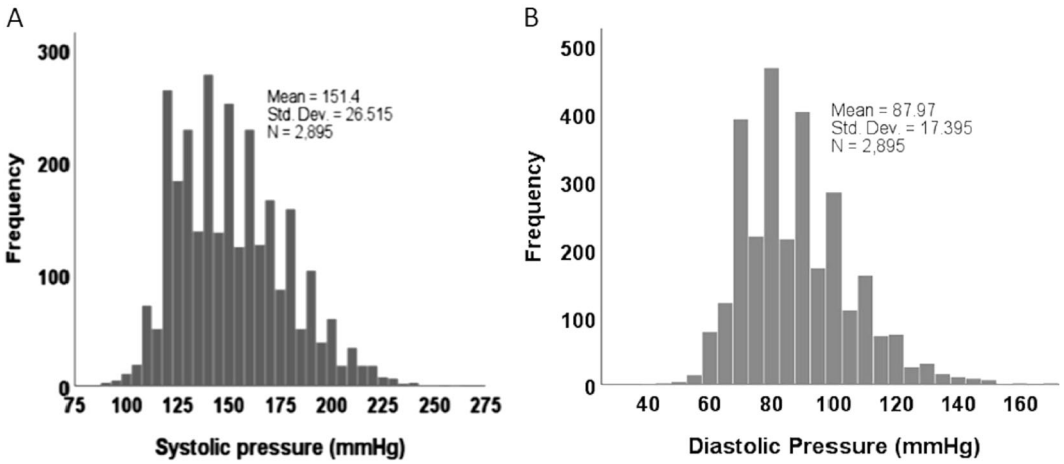


**Fig. 1** Distribution of blood pressures (all patients). **a** Systolic blood pressure. **b** Diastolic blood pressure. Many of the patients had very severe hypertension

ACEi/ARB, 89% took a CCB, 29% took a β-blocker, and 8.5% took an α-blocker. ACEi or ARB was taken by 2108 patients (1398 ACEi, 909 ARB); of these patients, 84.3% took a CCB, 52% took a diuretic, 23.4% a β-blocker, and 4.2% an α-blocker. There were 648 (22.4%) patients who were unstimulated by either a diuretic or ACEi/ARB and 2247 (77.6%) who received ACEi/ARB and/or a diuretic. A diuretic without ACEi/ARB was taken by 247 (8.5%) patients, and ACEi/ARB without a diuretic was taken by 1013 (35.0%) patients. Among the 1574 patients who took a diuretic or spironolactone, 787 (50%) took hydrochlorothiazide, 220 (14%) took indapamide, 170 (11%) took furosemide, 1 (0.06%) took bumetanide, and 396 (25.16%) took spironolactone.

As shown in Fig. 2, there were significant differences in PRA and in plasma ALD by the class of stimulating drug. The PRA level with diuretic was higher than the unstimulated level and higher with both ACEi/ARB and ACEi/ARB/diuretic than that with diuretic alone ($p < 0.0001$). The plasma ALD level with ACEi/ARB alone was significantly lower than the unstimulated level or that with diuretic or with ACEi/ARB/diuretic. This finding suggests that ACEi/ARB may mask the presence of primary aldosteronism.

However, Fig. 3 shows that the phenotypes—Liddle, inappropriate ALD secretion, renal and mixed—can be distinguished by analyzing PRA, ALD, and the ALD/PRA ratio during stimulation with ACEi/ARB and/or a diuretic.

Table 2 shows that the proportion of patients designated with each phenotype differs by the method of stimulation. Stimulation with diuretic alone seems to categorize the most patients into physiologically distinct phenotypes that would respond best to different treatments.

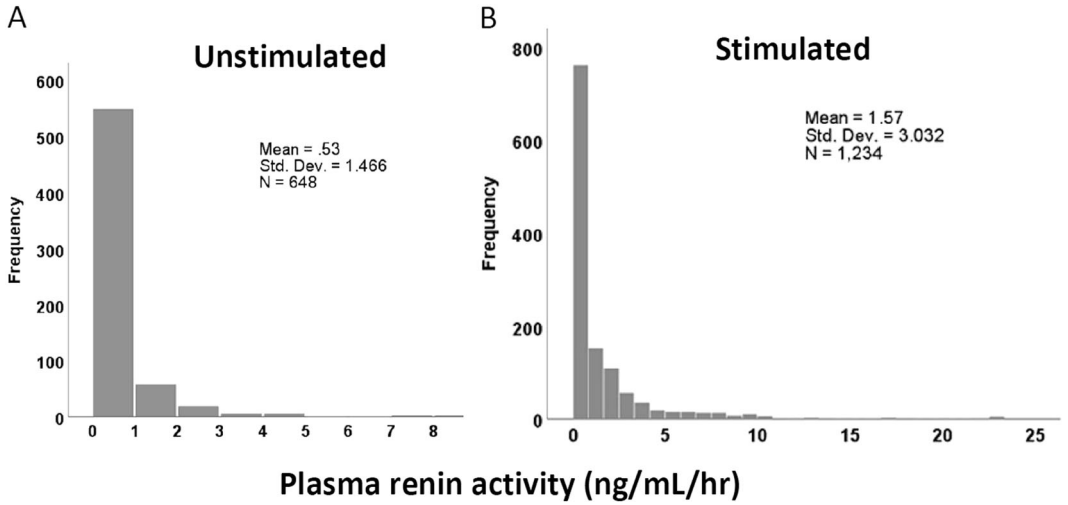


**Fig. 2** Differences in plasma renin activity (**a**) and plasma aldosterone (**b**) by the class of stimulating drug among patients with systolic pressure ≥ 180 mmHg and/or diastolic pressure ≥ 100 mmHg. Both diuretic alone and ACEi/ARB increased plasma renin activity and the increase by ACEi/ARB was more than that by diuretic. However, ACEi/ARB actually lowered plasma aldosterone, suggesting that ACEi/ARB will mask primary aldosteronism/inappropriate secretion of aldosterone

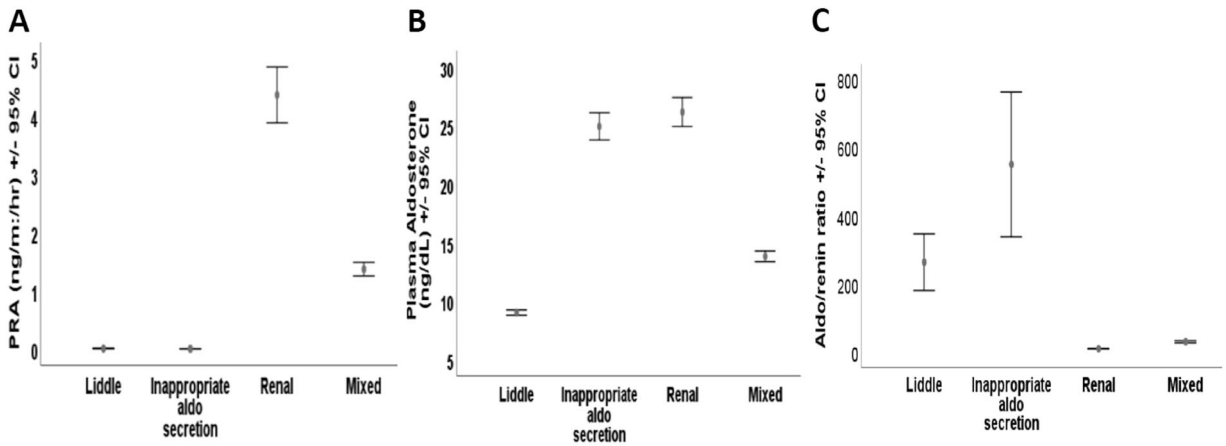


**Fig. 3** Plasma renin activity, plasma aldosterone, and aldosterone/renin ratio by phenotype, among patients with systolic pressure ≥ 180 mmHg and/or diastolic pressure ≥ 100 mmHg, stimulated by ACEi/ARB and/or diuretic. Plasma renin activity (**a**), aldosterone (**b**), and aldosterone/renin ratio (**c**). Patients with the four phenotypes can be distinguished by examining their stimulated PRA, aldosterone, and aldosterone/renin ratio during stimulation with ACEi/ARB and/or diuretic

**Table 2** Phenotype by method of stimulation, and primary medical treatment, for patients with systolic ≥ 180 and/or diastolic ≥ 100 mm/Hg

| Phenotype | Unstimulated $n = 153$ 17.5% | Diuretic only $n = 92$ 10.5% | ACEi/ ARB only $n = 252$ 28.8% | Diuretic + ACEi/ARB $n = 472$ 43.2% | Primary treatment |
|---|---|---|---|---|---|
| Liddle | 34.6% | 25.0% | 9.9% | 9.0% | Amiloride |
| Inappropriate aldo secretion | 28.8% | 38.0% | 12.7% | 18.0% | Aldo antagonist |
| Renal | 11.1% | 8.7% | 12.7% | 14.6% | ACEi/ARB |
| Mixed | 25.5% | 28.3% | 64.7% | 58.3% | Combinations[a] |

*ACEi* angiotensin converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *Aldo* aldosterone

[a]Amiloride with low aldosterone, aldosterone antagonist with high aldosterone, plus CCB and other classes

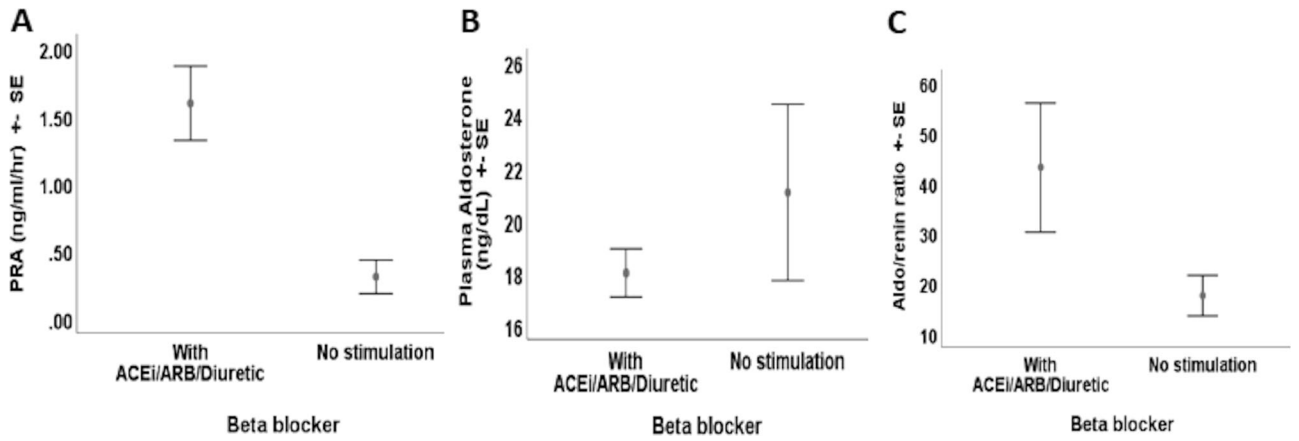


**Fig. 4** Effect of beta blocker, with and without stimulation, on plasma renin activity and plasma aldosterone among patients with systolic pressure ≥ 180 mmHg and/or diastolic pressure ≥ 100 mmHg. **a** Plasma renin activity. **b** Plasma aldosterone. **c** Aldosterone/renin ratio. Beta blockers without any stimulation lowered plasma renin activity and the aldosterone/renin ratio but not plasma aldosterone

The effect of beta blockers was mixed: among patients with systolic pressure ≥ 180 ± diastolic pressure ≥ 100, only 39 (4.5%) took a beta blocker with no stimulating drug, and only 194 (22.2%) took a beta blocker with ACEi/ARB ± diuretic. Among these patients, beta blockers in the absence of any stimulation lowered PRA (0.31 ± 0.78 ng/ml/h with no stimulation vs. 1.60 ± 3.83 ng/ml/h with ACEi/ARB ± diuretic, $p = 0.038$) but not ALD (21.06 ± 20.97 ng/L with no stimulation vs. 17.98 ± 12.76 ng/L with ACEi/ARB + diuretic, $p = 0.23$). The ALD/PRA ratio tended to be higher with beta blockers and no stimulation (84.99 ± 312.87 vs. 47.10 ± 144.87, $p = 0.17$) (Fig. 4).

## Discussion

We found that there were important differences in PRA, ALD, and the ALD/renin ratio depending on what drugs were used to stimulate the renin/angiotensin/ALD (RAA) axis. ACEi or ARB lowered the levels of plasma ALD, thereby potentially masking the presence of primary aldosteronism; for this reason, it may be more appropriate, for the purpose of selecting the best medical therapy, to consider "inappropriate secretion of ALD" (as it was called in the PATHWAY 2 study) rather than focusing on primary aldosteronism. Some patients fell into a "mixed" phenotype, with no characteristics of either a renal, Liddle or inappropriate secretion of ALD phenotype. It is likely that this mixed phenotype may occur not only in patients with ACEi/ARB but also in patients with a combination of genetic variants that predispose both to primary aldosteronism or the inappropriate secretion of ALD (CYP11B2 [13], KCNJ5, ATP1A1, ATP2B3, CACNA1D, and ARMC5 [14, 15] and perhaps others) and variants of genes that predispose patients to the Liddle phenotype (GRK, NEDD4L, CYP4A11, NPPA, UMOD, and perhaps others) [13, 16]. This hypothesis was proposed by Spence in a lecture at the 2018 International Hypertension Society Congress in Beijing, based on observations in the present study. An example of this problem is that some patients whose severe hypertension was originally due to inappropriate ALD secretion or a Liddle phenotype will develop hypertensive nephrosclerosis (which might be regarded as a form of microvascular renovascular hypertension), which will increase the levels of PRA and ALD. That mechanism perhaps accounted for what was called "malignant hypertension" in the era before the treatment of hypertension became common.

Many studies have shown that adding spironolactone to the current therapy improves the control of resistant hypertension. However, it is not optimal to simply add spironolactone to the treatment of patients with resistant hypertension; it is important to know whether the low-renin hypertension of the patient is driven by inappropriate ALD secretion or by salt and water retention due to ENaC overactivity. In the PATHWAY 2 trial [17], spironolactone was more efficacious in patients with a high ALD/renin ratio, and overall, amiloride was as efficacious as spironolactone for resistant hypertension [17]. However, for patients with a Liddle phenotype, amiloride, a specific antagonist of ENaC, is more efficacious than spironolactone, as shown by Laffer et al. in patients with a variant of CYP4A11 [16].

Although true Liddle syndrome due to variants of ENaC (SCNN1B) is relatively rare, there are many other causes of the Liddle phenotype due to the variants of other genes that affect the function of ENaC. These variants include GRK, NEDD4L, CYP4A11, NPPA, and UMOD [13, 16]. Many of these variants are common in African patients with uncontrolled hypertension, and some patients have

combinations of several different variants [13]. This effect may partly explain why hypertension in Black patients is more likely to be due to salt and water retention [8, 18].

One variant of SCNN1B was described in 5% of Blacks in London, UK (mostly of Caribbean origin) [19], while another variant of this gene was described in South African individuals [20]. Jones et al. [21] found that 20% of the Khoi San people of the Kalahari had that gene variant, while it was present in 9% of Nguni-Zulu and 6% of hypertensive patients overall in Cape Town. In a Veterans Administration clinic in Louisiana, 6% of patients had a Liddle phenotype [22]. True Liddle syndrome (variants of SCNN1B) is not common in China (1.7% of hypertensive patients), but variants of candidate genes that cause the Liddle phenotype are very common. Eighty-one nonrare variants, including SCNN1B, SCNN1G, WNK1, WNK4, KLHL3, CUL3, nuclear receptor subfamily 3, group C (NR3C)1, NR3C2, and HSD11B2, were detected in 85% of patients, and 41 rare variants were detected in 15% [23].

In the aforementioned trial in Africa, in which PhysRx significantly improved BP control [9], the most important difference in the medication used in PhysRx was that at the end of the study, amiloride was used in only 2.8% of usual care versus 19.2% of PhysRx patients. That finding is in line with the present study, in which 25% of patients stimulated by diuretic alone had a Liddle phenotype.

Beta blockers cloud the picture; in some cases, if therapy that is adjusted according to the phenotyping described here is not successful, then it may be worth tapering the patient slowly and carefully off beta blocker, substituting other classes of antihypertensives, and repeating the measurement of PRA and plasma ALD levels; in patients with angina, this approach could be hazardous. Patients not controlled by this approach should be further investigated for underlying causes of hypertension. Occasionally licorice consumption may not be obvious; for example, in a tonic preparation that the patient may not realize contains licorice.

It should be noted that the purist approach, that patients should be withdrawn from all antihypertensive drugs before testing for primary aldosteronism, is not safe in patients with severe hypertension. One of Spence's patients, a young man, died of intracerebral hemorrhage when that was done at another center. It is therefore important to understand how to interpret plasma ALD and renin in the light of the antihypertensive drugs that the patient is taking. It is also important to recognize that because many patients with primary aldosteronism have bilateral adrenocortical hyperplasia, many will not be cured by unilateral adrenalectomy, so it is important to identify the appropriate medical therapy.

An important limitation of our study is that patients were not randomized to the different drug classes. The ideal would be a random-sequence crossover study to various classes of antihypertensive drugs; however, it would be impractical to do so in such a large sample. The strength of our study is in the large number of patients examined.

## Conclusion

Diuretics, ACEi/ARB and beta blockers have different effects on plasma renin and ALD, so to identify the physiologically most appropriate therapy for a patient with resistant hypertension, it is important to take into account which class of drugs was used to stimulate the RAA axis. Nevertheless, by studying PRA, ALD, and the ALD/renin ratio, it is possible to identify the best medical therapy for most patients with resistant hypertension. Patients with a mixed phenotype may have more than one cause of hypertension, so these patients may require more thoughtful interpretation of their results.

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## Compliance with ethical standards

**Conflict of interest** All authors have completed the ICMJE uniform disclosure form and have declared the following: XH reports grants from the National Natural Science Foundation of China and Major Projects of the Science and Technology Department, Jiangxi, China; and XC reports a grant from the Major Projects of the Science and Technology Department, Jiangxi, China. The other authors declare that they have no conflict of interest.

**Ethics** This study was approved by the ethics committee of the Second Affiliated Hospital of Nanchang University, China; the protocol number was *2017.



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