RESEARCH

Clinical impacts of endothelium-dependent flow-mediated vasodilation assessment on primary aldosteronism

Daisuke Watanabe, **Satoshi Morimoto**, **Noriko Morishima** and **Atsuhiro Ichihara**

Department of Endocrinology and Hypertension, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan

Correspondence should be addressed to S Morimoto: morimoto.satoshi@twmu.ac.jp

Abstract

Objective: Primary aldosteronism (PA) is divided into two major subtypes, aldosterone-producing adenoma (APA) and bilateral idiopathic hyperplasia (IHA) and is associated with a higher risk of cardiovascular events. However, the nature of vascular function in PA patients remains to be determined. The aim of this study was to determine the vascular function and investigate the implications of vascular function assessments in the patients.

Methods: Flow-mediated dilation (FMD), as an index of endothelial function, and cardio-ankle vascular index (CAVI), as an index of arterial stiffness, were retrospectively compared between 42 patients with APA, 37 patients with IHA, and 42 patients with essential hypertension (EH). These values were also compared with background factors, *KCNJ5* mutation and clinical outcome in terms of blood pressure reduction after adrenalectomy in the APA group.

Results: FMD was significantly lower in the APA group (4.8 ± 2.1%) and IHA group (4.1 ± 1.9%) than in the EH group (5.7 ± 2.1%). CAVI did not differ significantly among groups. Although no significant correlations were seen between FMD and background factors in the IHA group, FMD correlated negatively with BMI and plasma aldosterone concentration in the APA group (rs = −0.313, rs = −0.342, respectively). *KCNJ5* mutational status was not associated with FMD value. High FMD was associated with blood pressure normalization after adrenalectomy in the APA group.

Conclusions: Patients with PA displayed impaired endothelial function. Complete clinical success after adrenalectomy was associated with preserved endothelial function. This study provides a better understanding of FMD assessment in patients with PA.

Key Words

- hypertension
- endothelial function
- plasma aldosterone concentration

Endocrine Connections (2021) **10**, 578–587

Introduction

Primary aldosteronism (PA) is defined as autonomous aldosterone production from the adrenal glands and represents the most common and treatable cause of hypertension. Patients with PA had a significantly higher risk of developing unexplained arterial fibrillation (1). PA is characterized by two subtypes according to histological features, including adrenal aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA). Whereas appropriate aldosterone production is necessary for survival, excess aldosterone is associated with inflammation, fibrosis, vascular damage, and end-organ failure and induces vascular dysfunction and remodeling (2). The incidence of cardiovascular morbidity is higher among patients with PA than among those with essential

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0057

hypertension (EH) (3). In addition, severe arterial stiffness is associated with the absence of complete clinical success in lateralized PA after adrenalectomy (ADX) (4).

Endothelial dysfunction is recognized as a major factor in the development of atherosclerosis (5). Measurement of flow-mediated vasodilation (FMD) of the brachial artery has been widely used to assess the endothelial function in humans as a reflection of endothelial nitric oxide (NO)-mediated regulation of vascular tone and diameter. As a result, FMD could be used as a prognostic marker for the progression of atherosclerosis and future cardiovascular events. Cardio-ankle vascular index (CAVI) is an index of overall arterial stiffness of an artery from its origin at the aorta to the ankle (6). Similar to FMD, the utility of CAVI as a predictor of cardiovascular events has been demonstrated (7). Both FMD and CAVI may be useful in assessing the extent and severity of vascular dysfunction in patients with PA, but the status of vascular dysfunction as assessed by FMD and CAVI has not been elucidated in detail among these patients.

The mechanisms underlying the tumorigenesis of APA and autonomous aldosterone production remain largely unknown. Somatic mutations in the potassium channel encoded by the gene *KCNJ5* were identified by Choi *et al.* in 2011 as a candidate causally related to autonomous aldosterone secretion. Two recurrent mutations (G151R and L168R) have been reported to induce increased sodium conductance and membrane depolarization, activating voltage-gated Ca^{2+} channels, and the attendant Ca^{2+} influx then activates aldosterone synthesis and secretion (8). Accumulating evidence suggests that *KCNJ5* mutant carriers are younger, have higher levels of aldosterone, more severe hypokalemia, and better prognosis of hypertension after ADX than nonmutant carriers (9, 10, 11, 12). However, whether *KCNJ5* mutational status affects the preoperative vascular damage induced by aldosterone excess is unclear.

Although ADX is the first-line therapy for the treatment of APA, the proportion of patients achieving clinical success after ADX varies widely (13). Whether preoperative FMD or CAVI is associated with clinical outcomes after ADX remains unknown.

On the basis of these backgrounds, the present study was undertaken to: (i) compare vascular function as assessed by FMD and CAVI among patients with APA, IHA and EH; (ii) determine factors associated with vascular dysfunction, including *KCNJ5* mutations, among patients with APA and (iii) investigate whether the preoperative vascular function is associated with clinical outcomes among patients with APA after ADX.

Materials and methods

Study population

This was a retrospective study enrolling 42 patients who had been diagnosed with unilateral PA, 37 age- and BMI (BMI)-matched patients with bilateral PA and 42 patients with EH at Tokyo Women's Medical University (TWMU) Hospital between April 2012 and February 2020. The study protocol was approved by the ethics committee at TWMU Hospital (Approval Number 3480,3593). Consent was obtained from each patient or subject after a full explanation of the purpose and nature of all procedures used. At enrollment, information on sex, age, and BMI was collected.

Diagnosis of PA

All patients were diagnosed with PA according to the guidelines of the Japan Endocrine Society (14), including case detection, confirmatory tests, and subtype classification. Three confirmatory loading tests for the diagnosis of PA were performed: the captopril challenge test, the furosemide upright test, and the saline infusion test. All patients with PA showed positive results in at least two of these three tests. To avoid interfering with the renin-angiotensin-aldosterone system, these patients were treated with only calcium channel blockers or α-blockers during the workup for PA. EH was diagnosed by excluding secondary hypertension. PA was excluded according to a normal ratio of plasma aldosterone concentration ((PAC) pg/mL) to plasma renin activity ((PRA) ng/mL/h); that is, an aldosterone-to-renin ratio (ARR) <200.

Subtype classification of PA

Patients with PA were allocated to two groups, that is, APA group and IHA group, according to the results of the adrenal venous sampling (AVS). The adrenal venous blood aldosterone-to-cortisol ratio after cosyntropin stimulation was calculated bilaterally. In AVS, selectivity index (SI), lateralized ratio (LR), and contralateral ratio (CR) were used. SI was defined as $\text{cortisol}_{\text{adrenal vein}}/\text{cortisol}_{\text{inferior vena cava}}$. Adrenal vein cannulation was considered successful if the SI was >5 or cortisol in the adrenal vein was >200 μg/dL. LR was defined as $(\text{aldosterone}/\text{cortisol}_{\text{adrenal vein}})/(\text{aldosterone}/\text{cortisol}_{\text{contralateral adrenal vein}})$, and CR was defined as $(\text{aldosterone}/\text{cortisol}_{\text{contralateral adrenal vein}})/(\text{aldosterone}/\text{cortisol}_{\text{inferior vena cava}})$. Unilateral PA was diagnosed from LR > 4 after cosyntropin stimulation. All unilateral PA patients underwent laparoscopic ADX of the dominant

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0057

side based on AVS results. IHA group was defined when AVS results showed LR < 2 in patients with PA (15).

Blood pressure measurement

Office blood pressure was measured with the patient in a sitting position after resting for at least 5 min.

Blood and urinary examinations

Plasma potassium, creatinine, uric acid, cholesterol, glucose, and hemoglobin A1c levels were measured using standard methods. PAC and PRA were measured from venous blood samples obtained with the patient in a sitting position (for at least 15 min) at first admission.

Physiological tests for the diagnosis of vascular failure

Percentage changes in brachial artery diameter were calculated in response to increased FMD, as previously described using a UNEXEF38G semi-automated diagnostic ultrasound system (UNEX, Nagoya, Japan) (16, 17). CAVI of arteriosclerosis was measured using a VaSera VS-1500AN vascular screening system (Fukuda Denshi, Tokyo, Japan), as previously described (18).

RNA extraction and detection of mutations in KCNJ5 by PCR and direct sequencing

APA tissues were frozen in liquid nitrogen immediately after surgical removal and maintained at –80°C until use. Total RNA was extracted from APA using TRI REAGENT (Molecular Research Center, Cincinnati, OH) according to the instructions from the manufacturer. Synthesis of cDNA was performed using a High Capacity cDNA RT Kit (Thermo Fisher Scientific) and sequenced with specific primer sets as reported previously (19).

Clinical outcome after adrenalectomy

The primary outcome was defined as achieving complete clinical success after ADX according to the Primary Aldosteronism Surgical Outcomes (PASO) consensus (13). Hypertension was defined as 'completely cured' (C) if patients remained normotensive without taking any antihypertensive agents and as 'partially cured' (NC) if patients maintained the same blood pressure as before surgery on lower doses of antihypertensive agents.

Statistical analysis

All data are expressed as mean ± S.D. or median and interquartile range (IQR). Data were compared between groups using the Wilcoxon rank-sum test. Univariate correlations were determined by calculating Spearman rank correlation coefficients. Multiple linear regression analysis was performed to evaluate the strength of correlations between each variable. Values of $P < 0.05$ were considered significant. All analyses were performed using JMP version 14 software (SAS Institute, Cary, NC).

Results

The basic clinical characteristics of all subjects are shown in Table 1. No differences were found among the three groups (APA, IHA and EH) with regard to age, sex, duration of hypertension, BMI, blood pressure, renal function and hemoglobin A1c. In the APA group, 41 patients (97%) were hypokalemic (serum potassium < 3.5 mEq/L) and potassium level was significantly lower than that in the IHA group. In contrast, fasting glucose levels were significantly lower and low-density lipoprotein cholesterol (LDL-c) levels were significantly higher in the IHA group than in the EH group. As expected, APA and IHA groups demonstrated higher PAC values compared to the EH group. In addition, the APA group showed a higher PAC value compared to the IHA group. As shown in results from confirmatory tests of PA, ARR at 90 min of the captopril challenge test was higher in the APA group (ARR 2540 (IQR 746.5–4195)) than in the IHA group (345 (IQR 245–479.8)) and PAC at 4 h of the saline infusion test was higher in the APA group (391.5 pg/mL (IQR 283.2–540.2 pg/mL)) than in the IHA group (90.1 pg/mL (IQR 70.2–119 pg/mL)) (Table 1). The furosemide upright test was performed in 27 patients of the APA group and 34 patients of the IHA group. PRA at 120 min of the furosemide upright test was significantly lower in the APA group (0.4 ng/mL/h (IQR 0.2–0.8 ng/mL/h)) than in the IHA group (1.1 ng/mL/h (IQR 0.6–1.5 ng/mL/h)). AVS results demonstrated that median LR was 14.2 (IQR, 6.8–35.3) in the APA group and 1.4 (IQR, 1.15–1.8) in the IHA group. In particular, in the APA group, CR suppression was recognized in 46 of the 47 patients (98%) after the cosyntropin stimulation. After the diagnosis of unilateral PA, 42 patients underwent adrenalectomy. Histological findings of all 42 patients showed cortical adenoma, with hematoxylin and eosin staining revealing tumor mainly comprising clear cells with lipid-rich cytoplasm. Complete biochemical success

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0057

Table 1 Clinical characteristics of all subjects.

Characteristics	PA		EH
	APA	IHA	
n	42	37	42
Age	51 ± 10	54 ± 10	53 ± 10
Sex (male/female)	20/22	16/21	19/23
BMI (kg/m^2)	24.2 ± 3.9	25.3 ± 3.4	25.2 ± 4.6
Duration of hypertension (years)	4.6 ± 3.3	3.9 ± 4.0	4.0 ± 6.0
Blood pressure (mmHg)			
Systolic	144 ± 17	143 ± 19	147 ± 10
Diastolic	89 ± 12	88 ± 14	89 ± 12
Laboratory data			
Creatinine (mg/dL)	0.77 ± 0.18	0.77 ± 0.16	0.71 ± 0.11
eGFR (mL/min/1.73 m^2)	76.7 ± 17.9	73.2 ± 14.5	79.4 ± 17.0
Serum potassium (mEq/L)	2.9 ± 0.3†	4.0 ± 0.3	N/A
LDL-cholesterol (mg/dL)	121 ± 31	133 ± 33*	117 ± 24
HDL-cholesterol (mg/dL)	57 ± 18	61 ± 15	60 ± 16
Triglycerides (mg/dL)	105.5 (70.8–161.5)	117.0 (80.0–150.5)	99.5 (74.8–144.0)
Plasma glucose (mg/dL)	98 (91–110.2)	95 (88.5–103.5)*	98 (91–110.2)
Hemoglobin A1c (%)	5.6 (5.4–6.0)	5.7 (5.5–6.1)	5.6 (5.2–5.9)
Hormonal data			
PAC (pg/mL)	506.1 ± 269.4*,†	222.0 ± 67.0*	93.8 ± 27.1
PRA (ng/mL/h)	0.25 (0.1–0.4)*,†	0.5 (0.3–0.9)*	0.85 (0.60–1.75)
Confirmatory test			
ARR after CCT	2540 (746.5–4195)†	345 (245–479.8)	N/A
PAC after SIT (pg/mL)	391.5 (283.2–540.2)†	90 (70.2–119)	N/A
LR in AVS after cosyntropin loading	14.2 (6.8–35.3)†	1.4 (1.15–1.8)	N/A

Data are expressed as mean ± S.D. or median (25th–75th percentile).
$^{*}P < 0.05$ vs EH; $^{\dagger}P < 0.05$ vs IHA.
APA, aldosterone-producing adenoma; ARR, aldosterone-to-renin ratio; AVS, adrenal venous sampling; CCT, captopril challenge test; eGFR, estimated glomerular filtration rate; EH, essential hypertension; FUT, furosemide upright test; IHA, idiopathic hyperaldosteronism; LR, lateralization ratio; N/A, not applicable; PAC, plasma aldosterone concentration; PRA, plasma renin activity; SIT, saline infusion test.

as defined by the PASO consensus (13), such as correction of hypokalemia without potassium supplementation, was achieved in all 42 patients after ADX.

In all PA and EH patients, although sex differences in FMD were not found (male 4.7 ± 2.2% vs female 5.1 ± 2.1%), a significant correlation was found between age and CAVI (rs = 0.43, $P < 0.05$). Table 2 illustrates the FMD and CAVI in patients with PA and EH. No significant difference in FMD was seen between the APA group (4.8 ± 2.1%) and IHA group (4.1 ± 1.9%), but FMD was significantly lower in the APA and IHA groups than in the EH group (5.7 ± 2.1%).

Table 2 Comparison of FMD and CAVI in PA and EH.

	PA		EH
	APA	IHA	
FMD (%)	4.8 ± 2.1*	4.1 ± 1.9*	5.7 ± 2.1
CAVI	7.9 ± 1.3	7.8 ± 1.0	7.8 ± 1.1

Data are expressed as mean ±S.D.
$^{*}P < 0.05$ vs EH.
APA, aldosterone-producing adenoma; CAVI, cardio-ankle vascular index; EH, essential hypertension; FMD, flow-mediated dilation; IHA, idiopathic hyperaldosteronism; PA, primary aldosteronism.

Conversely, no significant difference in CAVI was apparent among the APA, IHA, and EH groups (7.9 ± 1.3%, 7.8 ± 1.0%, and 7.8 ± 1.1%, respectively).

Next, we analyzed correlations between FMD and background factors. In the IHA group, no significant correlations were seen between FMD and any background factors. However, in the APA group, FMD correlated negatively with BMI and PAC level (rs = –0.313, rs = –0.342, respectively) (Fig. 1 and Table 3). Table 4 shows the results of multiple regression analysis for the relationships of BMI, PAC and age, as potential predictors of FMD (20) in the APA group. This analysis has demonstrated BMI and PAC as significantly associated with FMD.

Among 42 patients who underwent ADX, adrenal tissues were obtained from 30 randomly selected patients and freshly frozen. Thirteen of the 30 patients with APAs displayed somatic mutations in the *KCNJ5* gene. We identified seven cases with p.G151R (two with a G-to-C (c.451G>C) substitutions and five with G-to-A (c.451G>A) substitutions) and six cases with L168R (all due to a T-to-G substitution (c.503T>G)). Clinical characteristics of the 13 patients in the *KCNJ5* mutant group and 17 patients in the

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0057

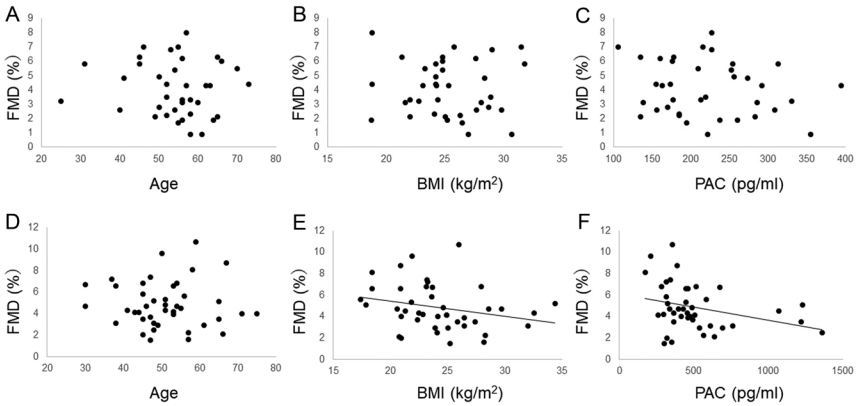

Figure 1
Correlations of FMD value with age, BMI, and PAC. (A, B and C) Patients with IHA. (D, E and F) Patients with APA. FMD, flow-mediated dilation; PAC, plasma aldosterone concentration.

wild-type group were compared. No significant differences were found between groups with regard to age, sex, BMI, duration of hypertension, blood pressure, renal function, and potassium level. PAC level tended to be higher in the *KCNJ5*-mutant group (683.6 ± 380.6 pg/mL) than in the wild-type group (437.4 ± 137.9 pg/mL), although the difference was not significant ($P=0.09$). However, the *KCNJ5*-mutant group showed higher PAC at 4 h of the saline infusion test (507 pg/mL (IQR 387–771 pg/mL)) compared to the wild-type group (354 pg/mL (IQR 285–481 pg/mL)) (Table 5). In vascular function assessment, no significant difference in FMD (4.8 ± 1.4%, 4.5 ± 2.1%, respectively) or CAVI (8.1 ± 1.8, 8.0 ± 1.1, respectively) was seen between the *KCNJ5*-mutant group and the wild-type group (Fig. 2). In addition, we found that there were no significant differences between FMD and systolic blood pressure in both *KCNJ5*-mutant and wild-type groups (rs = 0.04, rs = 0.07, respectively).

In the APA group, 20 patients were categorized as C and 22 as NC (Table 6). Male sex was significantly more frequent in the NC group than in the C group. No significant differences were seen in age, duration of hypertension, blood pressure, renal function or potassium level between NC and C groups, but BMI was significantly higher in the NC group (25.5 ± 3.3 kg/m²) than in the C group (22.8 ± 4.0 kg/m²). PAC and ARR were similar between NC and C groups, while PRA was significantly higher in the NC group (0.3 (IQR 0.1–0.525)) than in the C group (0.15 (IQR 0.1–0.3)). Among AVS results, no significant differences in LR or CR were evident between NC and C groups. FMD was significantly lower in the NC group (4.0 ± 2.1%) than in the C group (5.7 ± 1.8%) (Fig. 3A). Figure 3B shows FMD with the highest areas under the receiver operating characteristics curves. A cut-off FMD of 4.3% was selected for complete clinical success after ADX, offering 78% sensitivity and 80% specificity (AUC, 0.790).

Table 3 Univariate analysis of relationship between FMD and variables.

	APA		IHA	
	rs	*P*	rs	*P*
Age	−0.065	0.682	−0.129	0.448
BMI (kg/m²)	−0.313	**0.044**	−0.048	0.779
Blood pressure (mmHg)				
Systolic	−0.056	0.727	−0.198	0.239
Diastolic	0.030	0.853	−0.293	0.079
Laboratory data				
Creatinine (mg/dL)	−0.111	0.485	−0.206	0.220
eGFR (mL/min/1.73 m²)	0.031	0.846	0.327	0.052
Serum potassium (mEq/L)	0.216	0.170	−0.067	0.693
LDL-cholesterol (mg/dL)	−0.085	0.594	−0.099	0.561
HDL-cholesterol (mg/dL)	0.269	0.085	0.057	0.737
Triglyceride (mg/dL)	−0.161	0.310	−0.147	0.387
Plasma glucose (mg/dL)	−0.189	0.231	−0.198	0.240
Hemoglobin A1c (%)	−0.251	0.109	−0.146	0.389
Hormonal data				
PAC (pg/mL)	−0.342	**0.026**	−0.175	0.301
PRA (ng/mL/h)	0.144	0.363	0.068	0.690

APA, aldosterone-producing adenoma; eGFR, estimated glomerular filtration rate; FMD, flow-mediated vasodilation; IHA, idiopathic hyperaldosteronism; PAC, plasma aldosterone concentration; PRA, plasma renin activity. Bold indicates statistical significance, $P < 0.05$.

Table 4 Multiple regression analysis of the factors associated with FMD value in patients with APA.

	β	*t*	*P*
Age	−0.07	−0.47	0.64
BMI (kg/m²)	−0.30	−2.05	**0.04**
PAC (pg/mL)	−0.33	−2.29	**0.03**

Model-adjusted $R^2 = 0.19$.
PAC, plasma aldosterone concentration. Bold indicates statistical significance, $P < 0.05$.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0057

Table 5 Comparison of clinical characteristics between patients with and without *KCNJ5* mutations.

Characteristics	APA	
	KCNJ5 (–)	KCNJ5 (+)
n	17	13
Age	53 ± 12	50 ± 10
Sex (male/female)	7/10	5/8
BMI (kg/m^2)	23.6 ± 4.4	23.8 ± 3.9
Duration of hypertension (years)	4.5 ± 3.8	2.8 ± 2.0
Blood pressure (mmHg)		
Systolic	142 ± 22	146 ± 12
Diastolic	84 ± 12	93 ± 10
Laboratory data		
Creatinine (mg/dL)	0.8 ± 0.2	0.7 ± 0.2
eGFR (mL/min/1.73 m^2)	73.4 ± 16.1	86.0 ± 20.5
Serum potassium (mEq/L)	3.0 ± 0.3	2.8 ± 0.3
Hormonal data		
PAC (pg/mL)	437.4 ± 137.9	683.6 ± 380.6
PRA (ng/mL/h)	0.1 (0.1–0.35)	0.3 (0.1–0.4)
ARR	2826.1 ± 1884.6	4014.6 ± 4282.8
Confirmatory test		
ARR after CCT	2915 (1060–4080)	2430 (624–5825)
PAC after SIT (pg/mL)	354 (285–481)*	507 (387–771)
AVS results		
LR	13.3 (6.8–25.6)	17.6 (9.9–40.0)
CR	0.27 (0.15–0.44)	0.3 (0.14–0.41)

Data are expressed as mean ± S.D. or median (25th–75th percentile).
*P < 0.05 vs *KCNJ5*-mutant group.
APA, aldosterone-producing adenoma; ARR, aldosterone-to-renin ratio; AVS, adrenal venous sampling; CCT, captopril challenge test; CR, contralateral ratio; eGFR, estimated glomerular filtration rate; LR, lateralized ratio, PAC, plasma aldosterone concentration; PRA, plasma renin activity; SIT, saline infusion test.

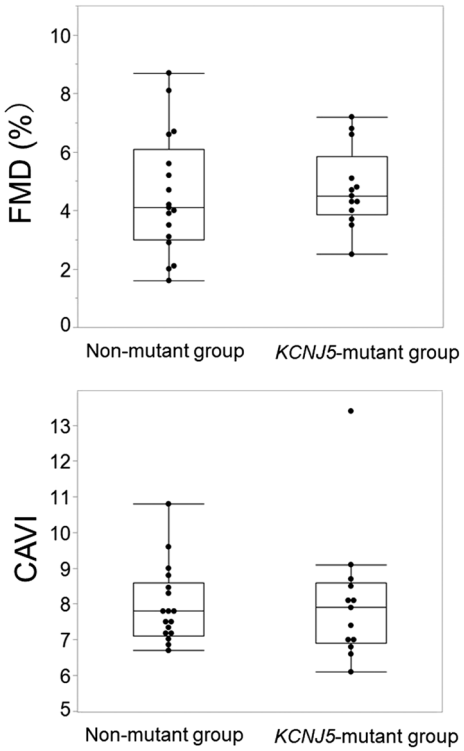

Figure 2
Flow-mediated dilation and cardio-ankle vascular index in patients with APA grouped by KCNJ5 somatic mutations. CAVI, cardio-ankle vascular index; FMD, flow-mediated dilation.

Discussion

The major findings of this study were as follows. First, vascular endothelial function, as assessed by FMD, was impaired in the PA group compared with the EH group. However, the degree of FMD attenuation was similar between IHA and APA groups. Secondly, FMD correlated significantly with BMI and PAC in the APA group. Thirdly, the impaired FMD was associated with clinically worse outcomes in the APA group after ADX. This study provides further clinical evidence for the implications of vascular endothelial function assessments in patients with PA.

Dysregulation of excess aldosterone is well known to cause patients to be at high risk of refractory hypertension, severe hypokalemia or related cardiovascular morbidity and mortality (21). A recent study reveals that aldosterone may have a pathophysiological role in microvascular remodeling in patients with PA (22). Accumulating evidence suggests that aldosterone plays important role in the initiation and progression of endothelial dysfunction. Several mechanisms have been shown to contribute to aldosterone-induced endothelial dysfunction. In particular, aldosterone itself was reported to directly limit nitrogen oxide production by increasing the production of endothelial reactive oxygen species (ROS) and decreasing ROS scavenging capacity (23, 24). Also, the previous report suggests an independent role of aldosterone in platelet activation, thereby contributing to the cardiovascular damage (25). Recent clinical studies have reported significant endothelial dysfunction in patients with high aldosterone levels by measuring FMD (26, 27, 28, 29, 30). In the present study, compared to the EH group, patients with PA displayed more severe endothelial damage as indexed by FMD. In accordance with such previous studies, our findings suggested that high levels of endogenous aldosterone were associated with deteriorated vascular function and dysfunction of the vessel endothelium. Meanwhile, arterial stiffness as assessed by CAVI showed no significant differences between patients with PA and EH. This could be because endothelial dysfunction, as evaluated by FMD, represents a fundamental component of the atherosclerotic disease process and FMD

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0057

Table 6 Comparison of clinical characteristics by complete and partial clinical success after adrenalectomy.

Characteristics	Clinical outcome after adrenalectomy (residual hypertension)	
	Complete remission	Partial remission
n	20	22
Age	48 ± 10	54 ± 10
Sex (male/female)	6/14*	14/8
BMI (kg/m^2)	22.8 ± 4.0*	25.5 ± 3.3
Duration of hypertension (years)	3.6 ± 3.1	5.1 ± 3.5
Blood pressure (mmHg)		
Systolic	139 ± 10	147 ± 21
Diastolic	86 ± 13	90 ± 12.0
Laboratory data		
Creatinine (mg/dL)	0.7 ± 0.2	0.8 ± 0.2
eGFR (mL/min/1.73 m^2)	78.5 ± 18.2	75.2 ± 17.8
Serum potassium (mEq/L)	3.0 ± 0.4	3.0 ± 0.3
Hormonal data		
PAC (pg/mL)	421.5 (315–626.5)	454 (361.8–544)
PRA (ng/mL/h)	0.15 (0.1–0.3)*	0.3 (0.1–0.525)
ARR	2702.5 (1297.5–3827.5)	1240 (806.0–3870.4)
Confirmatory test		
ARR after CCT	3247 (904–4595)	1799 (712–4195)
PAC after SIT (pg/mL)	474 (299–732)	348 (276–443)
AVS results		
LR	26.0 ± 17.1	15.8 ± 14.8
CR	0.22 (0.13–0.48)	0.36 (0.26–0.51)

Data are expressed as mean ±S.D. or median (25th–75th percentile).
*P <0 .05 vs partial group.
APA, aldosterone-producing adenoma; ARR, aldosterone-to-renin ratio; AVS, adrenal venous sampling; CCT, captopril challenge test; CR, contralateral ratio; eGFR, estimated glomerular filtration rate; LR, lateralized ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity; SIT, saline infusion test.

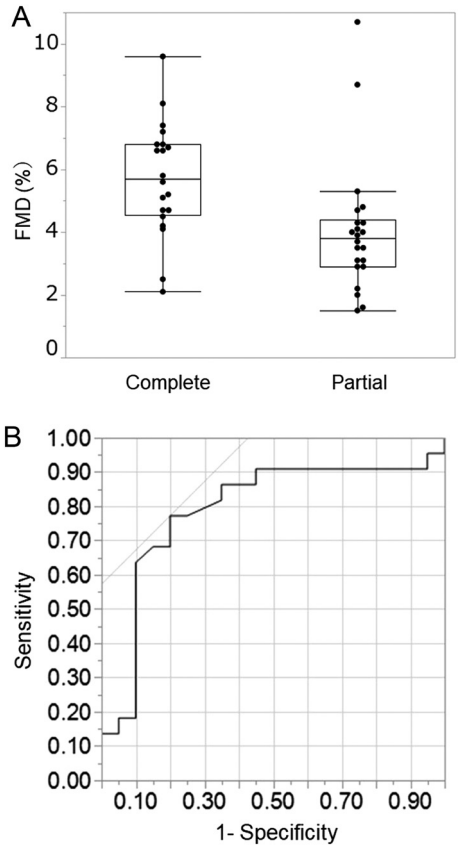

Figure 3
(A) FMD in patients with APA showing complete and partial clinical success after adrenalectomy. (B) ROC analysis of FMD in patients with APA showing complete clinical success. APA, aldosterone-producing adenoma; FMD, flow-mediated dilation; ROC, receiver operating characteristic.

evaluation is thus more sensitive than CAVI for detecting atherosclerosis (31).

We further investigated the influence of subtypes of PA on vascular function, as assessed by FMD. Unexpectedly, however, the APA and IHA groups displayed similar FMD, although the APA group demonstrated a higher PAC than patients with IHA. The reason for this phenomenon is unclear, but factors other than baseline aldosterone concentration, such as higher LDL-c level and slightly (but non-significantly) higher BMI and triglyceride and lower estimated glomerular filtration rate in the IHA group might have contributed to endothelial dysfunction in our patients with PA (Table 1). In the APA group, high BMI and PAC showed independent relationships with impaired FMD. In contrast, in the IHA group, no significant correlations existed between FMD and any vascular risk factors, such as age, BMI, or PAC. The reasons for these differences in risk factors for endothelial dysfunction between the two subtypes of PA remain unclear. One possibility is that differences exist in the diversity of physiological and hormonal circumstances (32), and further studies are needed to address this issue. Metabolic syndrome (MetS) is a frequent clinical condition in hypertensive patients especially in PA patients (33). In addition, APA and IHA may have different mechanisms to cause Mets (34). In general, aldosterone excess is often present in obesity and is closely associated with visceral adipose tissue, suggesting its involvement in the pathogenesis of obesity-related hypertension (35). On the other hand, although mouse adipocytes have been reported to release aldosterone and reduce endothelium-dependent relaxation, human conduit artery perivascular adipose tissue (PVAT) does not produce or release aldosterone (36). However, obesity-associated PVAT leads to a change in the profile of the released adipocytokines, resulting in a decreased vasorelaxing effect. It has been reported that obesity-related factors may

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0057

contribute to the pathogenesis of IHA (34). Since obesity results in an imbalance between endothelium-derived vasoactive factors favoring vasoconstriction, we expected that positive relationship between FMD and BMI is found in IHA group, as opposed to APA group. However, this relationship was not found in this study. The reason for this is unknown, however, it might be because BMIs do not reflect the degree of visceral fat accumulation precisely. The relationships among obesity, cardiovascular disorders, and IHA are complex, and thus, further research is needed to investigate the potential relationship between vascular function and IHA in more depth.

The identification of somatic mutations in patients with APA has provided new insights into the mechanisms causing the dysregulation of adrenal aldosterone production (8). Clinically, *KCNJ5* somatic gene mutations have been frequently identified in Japanese patients with APA (37). Kitamoto *et al.* demonstrated that *KCNJ5*-mutant carriers had higher levels of PAC than nonmutant carriers and may exhibit progressively advancing vascular damage compared to that seen in nonmutant carriers (38). Of note, Rossi *et al.* identified more severe echocardiographic abnormalities, including LVH and higher LVMI, in *KCNJ5*-mutant carriers compared with nonmutant carriers (39). We hypothesized that *KCNJ5* mutational status provokes functional consequences for vascular function in patients with APA. However, in the present study, although PAC in the *KCNJ5*-mutant group tended to be higher than that in the wild-type group, *KCNJ5* mutational status failed to show significant relationships with FMD or CAVI.

The present investigation represents a pivotal study, conducted in a relatively small group of patients with APA, where numerous factors such as age, blood pressure, BMI, and duration of hypertension might have affected FMD and CAVI. Our hypothesis thus needs further prospective follow-up studies in a larger number of patients.

Lower BMI has recently been identified as a predictive factor for better clinical outcomes after ADX (40). In the present study, BMI was significantly higher in the NC group. In general, obesity is associated with alterations in the production and secretion of lipids, lipoproteins and adipokines, and these changes can impact negatively on vascular endothelial function through the increased use of proinflammatory pathways (41). The present data demonstrated that incomplete clinical success after ADX is associated with endothelial dysfunction as measured by FMD. Considering the relationship between BMI and FMD, reducing increased BMI in patients with APA may be expected to prevent the impairment of FMD, in turn improving clinical outcomes after ADX.

A key strength of the present study was that all patients in this study underwent AVS, an invasive test requiring advanced techniques for the subtype classification of PA (42). Recent guidelines from the Endocrine Society advocated AVS as the gold standard to distinguish between subtypes of PA (21). The main limitation of this study was the small sample size. In addition, data regarding the duration of hypertension could not be collected precisely in our study because of its retrospective nature. Duration of hypertension may affect indices for endothelial dysfunction. The present study was performed as a retrospective investigation to collect possible cases for tissue gene analysis. This study was not population-based and may, therefore, include unknown selection biases. Future investigations in large-scale, prospective, multicenter trials are needed.

In conclusion, the present study demonstrated that patients with PA display stronger vascular endothelial dysfunction related to aldosterone excess compared with those with EH. Aldosterone induces inflammation and oxidative stress in the cardiovascular system and contributes to cardiovascular disease, so the preferential use of mineralocorticoid receptor antagonists in patients with PA may provide significant long-term benefits for the future progression of atherosclerosis. In addition, the impairment of endothelial function correlated with obesity in patients with APA, but not in patients with IHA, and clinical outcomes after ADX among patients with APA may be affected by the existing deleterious endothelial function induced by obesity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This study was supported, in part, by a Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (JSPS KAKENHI; Grant Number 19K08497, to S M).

Author contribution statement

D W, S M, N M and A I contributed to the design and implementation of the research, the analysis of the results and the writing of the manuscript.

Acknowledgements

The authors are grateful to the staff of the Department of Endocrinology and Hypertension at Tokyo Women's Medical University for their assistance.

References

1 Seccia TM, Letizia C, Muiesan ML, Lerco S, Cesari M, Bisogni V, Petramala L, Maiolino G, Volpin R & Rossi GP. Atrial fibrillation as presenting sign of primary aldosteronism: results of the Prospective Appraisal on the Prevalence of Primary Aldosteronism in Hypertensive (PAPPHY) Study. *Journal of Hypertension* 2020 **38** 332–339. (https://doi.org/10.1097/HJH.0000000000002250)

2 Ferreira NS, Tostes RC, Paradis P & Schiffrin EL. Aldosterone, inflammation, immune system and hypertension. *American Journal of Hypertension* 2021 **34** 15–27. (https://doi.org/10.1093/ajh/hpaa137)

3 Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F & Mulatero P. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet: Diabetes and Endocrinology* 2018 **6** 41–50. (https://doi.org/10.1016/S2213-8587(17)30319-4)

4 Chan CK, Yang WS, Lin YH, Huang KH, Lu CC, Hu YH, Wu VC, Chueh JS, Chu TS & Chen YM. Arterial stiffness is associated with clinical outcome and cardiorenal injury in lateralized primary aldosteronism. *Journal of Clinical Endocrinology and Metabolism* 2020 **105** dgaa566. (https://doi.org/10.1210/clinem/dgaa566)

5 Widlansky ME, Gokce N, Keaney Jr JF & Vita JA. The clinical implications of endothelial dysfunction. *Journal of the American College of Cardiology* 2003 **42** 1149–1160. (https://doi.org/10.1016/s0735-1097(03)00994-x)

6 Tanaka A, Tomiyama H, Maruhashi T, Matsuzawa Y, Miyoshi T, Kabutoya T, Kario K, Sugiyama S, Munakata M, Ito H, *et al.* Physiological diagnostic criteria for vascular failure. *Hypertension* 2018 **72** 1060–1071. (https://doi.org/10.1161/HYPERTENSIONAHA.118.11554)

7 Satoh-Asahara N, Kotani K, Yamakage H, Yamada T, Araki R, Okajima T, Adachi M, Oishi M, Shimatsu A & Japan Obesity and Metabolic Syndrome Study (JOMS) Group. Cardio-ankle vascular index predicts for the incidence of cardiovascular events in obese patients: a multicenter prospective cohort study (Japan Obesity and Metabolic Syndrome Study: JOMS). *Atherosclerosis* 2015 **242** 461–468. (https://doi.org/10.1016/j.atherosclerosis.2015.08.003)

8 Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Patel A, Men CJ, *et al.* K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science* 2011 **331** 768–772. (https://doi.org/10.1126/science.1198785)

9 Arnesen T, Glomnes N, Strømsøy S, Knappskog S, Heie A, Akslen LA, Grytaas M, Varhaug JE, Gimm O & Brauckhoff M. Outcome after surgery for primary hyperaldosteronism may depend on KCNJ5 tumor mutation status: a population-based study from Western Norway. *Langenbeck's Archives of Surgery* 2013 **398** 869–874. (https://doi.org/10.1007/s00423-013-1093-2)

10 Ip JC, Pang TC, Pon CK, Zhao JT, Sywak MS, Gill AJ, Soon PS & Sidhu SB. Mutations in KCNJ5 determines presentation and likelihood of cure in primary hyperaldosteronism. *ANZ Journal of Surgery* 2015 **85** 279–283. (https://doi.org/10.1111/ans.12470)

11 Lenzini L, Rossitto G, Maiolino G, Letizia C, Funder JW & Rossi GP. A meta-analysis of somatic KCNJ5 K(+) channel mutations in 1636 patients with an aldosterone-producing adenoma. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** E1089–E1095. (https://doi.org/10.1210/jc.2015-2149)

12 Chang CH, Hu YH, Tsai YC, Wu CH, Wang SM, Lin LY, Lin YH, Satoh F, Wu KD & Wu VC. Arterial stiffness and blood pressure improvement in aldosterone-producing adenoma harboring KCNJ5 mutations after adrenalectomy. *Oncotarget* 2017 **8** 29984–29995. (https://doi.org/10.18632/oncotarget.16269)

13 Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, Satoh F, Amar L, Quinkler M, Deinum J, *et al.* Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet: Diabetes and Endocrinology* 2017 **5** 689–699 (https://doi.org/10.1016/S2213-8587(17)30135-3).

14 Nishikawa T, Omura M, Satoh F, Shibata H, Takahashi K, Tamura N, Tanabe A & Task Force Committee on Primary Aldosteronism, The Japan Endocrine Society. Guidelines for the diagnosis and treatment of primary aldosteronism – the Japan Endocrine Society 2009. *Endocrine Journal* 2011 **58** 711–721. (https://doi.org/10.1507/endocrj.ej11-0133)

15 Rossi GP, Sacchetto A, Chiesura-Corona M, De Toni R, Gallina M, Feltrin GP & Pessina AC. Identification of the etiology of primary aldosteronism with adrenal vein sampling in patients with equivocal computed tomography and magnetic resonance findings: results in 104 consecutive cases. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1083–1090. (https://doi.org/10.1210/jcem.86.3.7287)

16 Morimoto S, Yurugi T, Aota Y, Sakuma T, Jo F, Nishikawa M, Iwasaka T & Maki K. Prognostic significance of ankle-brachial index, brachial-ankle pulse wave velocity, flow-mediated dilation, and nitroglycerin-mediated dilation in end-stage renal disease. *American Journal of Nephrology* 2009 **30** 55–63. (https://doi.org/10.1159/000201416)

17 Watanabe K, Mori T, Iwasaki A, Kimura C, Matsushita H, Shinohara K & Wakatsuki A. Increased oxygen free radical production during pregnancy may impair vascular reactivity in preeclamptic women. *Hypertension Research* 2013 **36** 356–360. (https://doi.org/10.1038/hr.2012.208)

18 Shirai K, Utino J, Otsuka K & Takata M. A novel blood pressure-independent arterial wall stiffness parameter; cardio-ankle vascular index (CAVI). *Journal of Atherosclerosis and Thrombosis* 2006 **13** 101–107. (https://doi.org/10.5551/jat.13.101)

19 Kitamoto T, Suematsu S, Yamazaki Y, Nakamura Y, Sasano H, Matsuzawa Y, Saito J, Omura M & Nishikawa T. Clinical and steroidogenic characteristics of aldosterone-producing adenomas with ATPase or CACNA1D gene mutations. *Journal of Clinical Endocrinology and Metabolism* 2016 **101** 494–503. (https://doi.org/10.1210/jc.2015-3284)

20 Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF Jr, Lehman BT, Fan S, Osypiuk E & Vita JA. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation* 2004 **109** 613–619. (https://doi.org/10.1161/01.CIR.0000112565.60887.1E)

21 Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M & Young Jr WF. The management of primary aldosteronism: case detection, diagnosis, and treatment: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* 2016 **101** 1889–1916. (https://doi.org/10.1210/jc.2015-4061)

22 Concistrè A, Petramala L, Bonvicini M, Gigante A, Collalti G, Pellicano C, Olmati F, Iannucci G, Soldini M, Rosato E, *et al.* Comparisons of skin microvascular changes in patients with primary aldosteronism and essential hypertension. *Hypertension Research* 2020 **43** 1222–1230. (https://doi.org/10.1038/s41440-020-0475-4)

23 Petramala L, Pignatelli P, Carnevale R, Zinnamosca L, Marinelli C, Settevendemmie A, Concistrè A, Tonnarini G, De Toma G, Violi F, *et al.* Oxidative stress in patients affected by primary aldosteronism. *Journal of Hypertension* 2014 **32** 2022–2029; discussion 2029. (https://doi.org/10.1097/HJH.0000000000000284)

24 Chen ZW, Tsai CH, Pan CT, Chou CH, Liao CW, Hung CS, Wu VC, Lin YH & TAIPAI Study Group. Endothelial dysfunction in primary aldosteronism. *International Journal of Molecular Sciences* 2019 **20** 5214. (https://doi.org/10.3390/ijms20205214)

25 Petramala L, Iacobellis G, Carnevale R, Marinelli C, Zinnamosca L, Concistrè A, Galassi M, Iannucci G, Lucia P, Pignatelli P, *et al.* Enhanced soluble serum CD40L and serum P-selectin levels in primary aldosteronism. *Hormone and Metabolic Research* 2016 **48** 440–445. (https://doi.org/10.1055/s-0042-103588)

26 Nishizaka MK, Zaman MA, Green SA, Renfroe KY & Calhoun DA. Impaired endothelium-dependent flow-mediated vasodilation in hypertensive subjects with hyperaldosteronism. *Circulation* 2004 **109** 2857–2861. (https://doi.org/10.1161/01.CIR.0000129307.26791.8E)

27 Tsuchiya K, Yoshimoto T & Hirata Y. Endothelial dysfunction is related to aldosterone excess and raised blood pressure. *Endocrine Journal* 2009 **56** 553–559. (https://doi.org/10.1507/endocrj.k09e-014)

28 Matsumoto T, Oki K, Kajikawa M, Nakashima A, Maruhashi T, Iwamoto Y, Iwamoto A, Oda N, Hidaka T, Kihara Y, *et al.* Effect of aldosterone-producing adenoma on endothelial function and Rho-associated kinase activity in patients with primary aldosteronism. *Hypertension* 2015 **65** 841–848. (https://doi.org/10.1161/HYPERTENSIONAHA.114.05001)

29 Kishimoto S, Matsumoto T, Oki K, Maruhashi T, Kajikawa M, Matsui S, Hashimoto H, Kihara Y, Yusoff FM & Higashi Y. Microvascular endothelial function is impaired in patients with idiopathic hyperaldosteronism. *Hypertension Research* 2018 **41** 932–938. (https://doi.org/10.1038/s41440-018-0093-6)

30 Demirkiran A, Everaars H, Elitok A, van de Ven PM, Smulders YM, Dreijerink KM, Tanakol R & Ozcan M. Hypertension with primary aldosteronism is associated with increased carotid intima-media thickness and endothelial dysfunction. *Journal of Clinical Hypertension* 2019 **21** 932–941. (https://doi.org/10.1111/jch.13585)

31 Tachibana H, Washida K, Kowa H, Kanda F & Toda T. Vascular function in Alzheimer's disease and vascular dementia. *American Journal of Alzheimer's Disease and Other Dementias* 2016 **31** 437–442. (https://doi.org/10.1177/1533317516653820)

32 Ohno Y, Sone M, Inagaki N, Yamasaki T, Ogawa O, Takeda Y, Kurihara I, Umakoshi H, Ichijo T, Katabami T, *et al.* Obesity as a key factor underlying idiopathic hyperaldosteronism. *Journal of Clinical Endocrinology and Metabolism* 2018 **103** 4456–4464. (https://doi.org/10.1210/jc.2018-00866)

33 Iacobellis G, Petramala L, Cotesta D, Pergolini M, Zinnamosca L, Cianci R, De Toma G, Sciomer S & Letizia C. Adipokines and cardiometabolic profile in primary hyperaldosteronism. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 2391–2398. (https://doi.org/10.1210/jc.2009-2204)

34 Zhang Z, Luo Q, Tuersun T, Wang G, Wu T, Zhang D, Wang M, Zhou K, Sun L, Yue N, *et al.* Higher prevalence of metabolic disorders in patients with bilateral primary aldosteronism than unilateral primary aldosteronism. *Clinical Endocrinology* 2021 **94** 3–11. (https://doi.org/10.1111/cen.14318)

35 Kawarazaki W & Fujita T. The role of aldosterone in obesity-related hypertension. *American Journal of Hypertension* 2016 **29** 415–423. (https://doi.org/10.1093/ajh/hpw003)

36 Assersen KB, Jensen PS, Briones AM, Rasmussen LM, Marcussen N, Toft A, Vanhoutte PM, Jensen BL & Hansen PBL. Periarterial fat from two human vascular beds is not a source of aldosterone to promote vasoconstriction. *American Journal of Physiology: Renal Physiology* 2018 **315** F1670–F1682. (https://doi.org/10.1152/ajprenal.00391.2018)

37 Nanba K, Yamazaki Y, Bick N, Onodera K, Tezuka Y, Omata K, Ono Y, Blinder AR, Tomlins SA, Rainey WE, *et al.* Prevalence of somatic mutations in aldosterone-producing adenomas in Japanese patients. *Journal of Clinical Endocrinology and Metabolism* 2020 **105**. (https://doi.org/10.1210/clinem/dgaa595)

38 Kitamoto T, Suematsu S, Matsuzawa Y, Saito J, Omura M & Nishikawa T. Comparison of cardiovascular complications in patients with and without KCNJ5 gene mutations harboring aldosterone-producing adenomas. *Journal of Atherosclerosis and Thrombosis* 2015 **22** 191–200. (https://doi.org/10.5551/jat.24455)

39 Rossi GP, Cesari M, Letizia C, Seccia TM, Cicala MV, Zinnamosca L, Kuppusamy M, Mareso S, Sciomer S, Iacobone M, *et al.* KCNJ5 gene somatic mutations affect cardiac remodelling but do not preclude cure of high blood pressure and regression of left ventricular hypertrophy in primary aldosteronism. *Journal of Hypertension* 2014 **32** 1514–1521; discussion 1522. (https://doi.org/10.1097/HJH.0000000000000186)

40 Naruse M, Yamamoto K, Katabami T, Nakamaru R, Sone M, Kobayashi H & Tanabe A. Age, gender, and body mass index as determinants of surgical outcome in primary aldosteronism. *Hormone and Metabolic Research* 2020 **52** 454–458. (https://doi.org/10.1055/a-1139-1783)

41 Rodríguez-Hernández H, Simental-Mendía LE, Rodríguez-Ramírez G & Reyes-Romero MA. Obesity and inflammation: epidemiology, risk factors, and markers of inflammation. *International Journal of Endocrinology* 2013 **2013** 678159. (https://doi.org/10.1155/2013/678159)

42 Rossi GP, Bisogni V, Bacca AV, Belfiore A, Cesari M, Concistrè A, Del Pinto R, Fabris B, Fallo F, Fava C, *et al.* The 2020 Italian Society of Arterial Hypertension (SIIA) practical guidelines for the management of primary aldosteronism. *International Journal of Cardiology: Hypertension* 2020 **5** 100029. (https://doi.org/10.1016/j.ijchy.2020.100029)

Received in final form 19 April 2021
Accepted 6 May 2021
Accepted Manuscript published online 6 May 2021