Renal impairment is closely associated with plasma aldosterone concentration in patients with primary aldosteronism

Akiyuki Kawashima[1], Masakatsu Sone[1], Nobuya Inagaki[1], Yoshiyu Takeda[2], Hiroshi Itoh[3], Isao Kurihara[3], Hironobu Umakoshi[4], Takamasa Ichijo[5], Takuyuki Katabami[6], Norio Wada[7], Yoshihiro Ogawa[8], Junji Kawashima[9], Megumi Fujita[10], Shozo Miyauchi[11], Shintaro Okamura[12], Tomikazu Fukuoka[13], Toshihiko Yanase[14], Shoichiro Izawa[15], Yuichiro Yoshikawa[16], Shigeatsu Hashimoto[17], Masanobu Yamada[18], Tatsuya Kai[19], Tomoko Suzuki[20] and **Mitsuhide Naruse[4,21] on behalf of the JPAS** and **JRAS groups[†]**

[1]Department of Diabetes, Endocrinology and Nutrition, Kyoto University, Kyoto, Japan, [2]Department of Internal Medicine, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan, [3]Department of Endocrinology, Metabolism and Nephrology, Keio University School of Medicine, Tokyo, Japan, [4]Department of Endocrinology and Metabolism, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, [5]Department of Endocrinology and Metabolism, Saiseikai Yokohamashi Tobu Hospital, Yokohama, Japan, [6]Division of Metabolism and Endocrinology, Department of Internal Medicine, St. Marianna University School of Medicine, Yokohama City Seibu Hospital, Yokohama, Japan, [7]Department of Diabetes and Endocrinology, Sapporo City General Hospital, Sapporo, Japan, [8]Department of Medicine and Bioregulatory Science, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan, [9]Department of Metabolic Medicine, Faculty of Life Science, Kumamoto University, Kumamoto, Japan, [10]Division of Nephrology and Endocrinology, Faculty of Medicine, The University of Tokyo, Tokyo, Japan, [11]Department of Diabetes and Endocrinology, Ehime Prefectural Central Hospital, Matsuyama, Japan, [12]Department of Endocrinology, Tenriyorozu Hospital, Tenri, Japan, [13]Department of Internal Medicine, Matsuyama Red Cross Hospital, Matsuyama, Japan, [14]Department of Endocrinology and Diabetes Mellitus, Faculty of Medicine, Fukuoka University, Fukuoka, Japan, [15]Department of Endocrinology and Metabolism, Tottori University Hospital, Yonago, Japan, [16]Department of Endocrinology and Diabetes Mellitus,Misato Kenwa Hospital, Misato, Japan, [17]Division of Nephrology, Hypertension, Endocrinology, and Diabetology/Metabolism, Fukushima Medical University Hospital, Fukushima, Japan, [18]Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi, Japan, [19]Department of Cardiology, Saiseikai Tondabayahsi Hospital, Tondabayashi, Japan, [20]Department of Public Health, School of Medicine, International University of Health and Welfare, Narita, Japan, and [21]Center of Endocrine Diseases, Takeda General Hospital, Kyoto, Japan

[†](Details of the JPAS and JRAS groups are presented as Supplementary data)

Correspondence should be addressed to M Sone
Email
sonemasa@kuhp.kyoto-u.ac.jp

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Abstract

Objective: Several clinical studies have reported that renal impairments are sometimes observed in patients with primary aldosteronism (PA). We analyzed the prevalence of renal impairments in PA patients and identified parameters that increase the risk for them.

Design: This is a retrospective cross-sectional study. We assessed the PA database established by the multicenter Japan PA study (JPAS). Data were also collected from patients with essential hypertension (EHT).

Methods: We compared the prevalences of proteinuria and lowered estimated glomerular filtration rate (eGFR) between patients with PA and age, sex, blood pressure and duration of hypertension-matched patients with EHT. We also performed logistic regression analysis to identify parameters that increase the risk for these renal impairments.

Results: Among 2366 PA patients, the prevalences of proteinuria and lowered eGFR were 10.3 and 11.6%, respectively. The prevalence of proteinuria was significantly higher in PA patients than matched-EHT patients (16.8 vs 4.4%), whereas there was no significant difference in the prevalence of lowered eGFR (17.2 vs 15.0%). The logistic regression analysis also showed that the plasma aldosterone concentration (PAC) significantly increases the risk of proteinuria and lowered eGFR, independent of other known risk factors.

Conclusion: Plasma aldosterone levels are closely associated with renal impairment in patients with PA. This is contrast to our earlier finding that the PAC was not itself linearly associated with cardiovascular events such as stroke or ischemic heart disease. The mechanism underlying the kidney damage in patients with PA may differ from that affecting the cardiovascular system.

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Introduction

Background

Primary aldosteronism (PA) is characterized by inappropriate autonomous aldosterone secretion, which results in secondary hypertension, sodium retention, increased potassium excretion and suppression of plasma renin (1). In addition to causing secondary hypertension, evidence suggests PA is also associated with a higher risk of cardiovascular events than is essential hypertension (EHT), independent of blood pressure (2, 3, 4).

The contribution of excess aldosterone to renal impairment has been widely reported. For example, rats fed a high-salt diet along with chronically administrated aldosterone exhibit intrarenal vascular and glomerular sclerosis and proteinuria. Treatment with a mineralocorticoid blocker ameliorated these effects, irrespective of blood pressure (5, 6, 7, 8). From a clinical viewpoint, Reincke *et al*. reported that PA patients show higher serum creatinine levels and lower glomerular filtration rates (GFRs) than EHT patients. They also reported that the initial potassium concentration, plasma aldosterone concentration (PAC) and presence of hypokalemia were all independent predictors of a lower GFR (9). However, whether or not there is a difference in the GFR between patients with PA and those with EHT remains controversial. Catena (10) and Sechi (11) reported that the GFR is higher in patients with PA than in those with EHT. Ribstein *et al*. reported that the GFR does not differ between PA and EHT patients (12). Rossi *et al*. showed that 24-h urinary albumin excretion was significantly higher in PA patients than EHT patients (13). A more detailed analysis has not yet been performed due to the limited number of enrolled patients in those studies.

Objectives

Low GFR and proteinuria are reportedly independent factors in cardiovascular disease (14) and are a public health concern all over the world (15). In the present study, therefore, we examined the prevalence of renal impairment defined as the presence of proteinuria or an eGFR <60 mL/min/1.73 m^2 among patients with PA and determined the factors associated with the reduced eGFR and proteinuria indicative of renal impairment.

Patients and methods

Study design

This study was conducted as a part of the Japan Primary Aldosteronism Study (JPAS) and is a retrospective cross-sectional analysis. The nationwide PA registry in Japan was established at 29 centers, including 15 university hospitals and 14 city hospitals.

Setting and participants

PA patients who were diagnosed and underwent adrenal venous sampling (AVS) between January 2006 and October 2017 were enrolled. Patients eligible to participate in the JPAS were men and women aged 20–90 years. Patients whom the investigators deemed unsuitable were excluded.

The patients' clinical characteristics, biochemical findings and results of confirmatory testing were collected electronically using a web registry system. The present study was conducted using a data set validated in February 2018.

The diagnosis of PA was made in accordance with guidelines from the Japan Endocrine Society and the Japan Society of Hypertension (16, 17). PA was diagnosed based on positive case detection of a ratio of the PAC (measured in ng/dL) to plasma renin activity (PRA, measured in ng/mL per hour) >20 and at least one positive result from a confirmatory test, including the captopril-challenge test, saline-infusion test, furosemide-upright test or oral salt-loading test. Diagnosis of the PA subtype was based on AVS with adrenocorticotropic hormone (cosyntropin) stimulation. Adrenal vein cannulation was defined as successful if the selectivity index was >5. The selectivity index was defined as the ratio of the cortisol concentration in the adrenal vein to that in the inferior vena cava. The unilateral subtype of PA was defined as a lateralization index >4. The lateralization index was calculated by dividing the aldosterone-to-cortisol ratio on the dominant side by that on the nondominant side (18). In the diagnosis of PA subtype, patients with suspected autonomous cortisol secretion defined as serum cortisol levels ≥1.8 μg/dL after a 1 mg dexamethasone suppression test were excluded (19).

We referred to the medical history of the enrolled PA patients and those with a history of renal disease, including renal carcinoma, urinary stones, pyelonephritis, nephritis, nephrotic syndrome, polycystic kidney disease, nephrectomy and other urological disorder were excluded

from this analysis, as those conditions could distort the results of renal biochemical testing. We also excluded patients who were taking a mineralocorticoid antagonist (MRA), angiotensin-converting enzyme inhibitor (ACE-I), angiotensin II receptor blocker (ARB), diuretics or beta blocker at the time of diagnosis, as those drugs interfere with the results of PRA and PAC assays, thereby preventing accurate interpretation of the influence of aldosterone on renal impairment. The prevalences of renal impairment, proteinuria and lowered eGFR among PA patients were compared with those among EHT patients treated at the Kyoto Medical Center. Of the 274 patients with EHT treated between January 2006 and December 2013, 128 were compared after matching for age, sex, systolic blood pressure (sBP) and duration of hypertension. These parameters were matched in the range of ±1 year (age), ±3 mmHg (sBP) or ±1 year (duration of hypertension).

Variables

We collected data on age, sex, duration of hypertension, BMI, sBP, diastolic blood pressure (dBP) and blood chemistry, including basal levels of adrenocorticotropic hormone (ACTH) and cortisol, Na+, K+, Cr, BUN, eGFR, uric acid (UA), PAC, PRA, aldosterone-to-renin ratio (ARR), fasting blood sugar (FBS), HbA1c (NGSP (National Glycohemoglobin Standardization Program)), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C, calculated using Friedewald equation).

Patients were considered to have diabetes mellitus (DM) or dyslipidemia (DL) after confirming a history of DM or DL in the dataset or if they were taking medication for DM or DL. Diagnoses of DM and DL were made at each institution according to those guidelines (20, 21). Hypokalemia was considered to be present if serum potassium was lower than 3.5 mEq/L or if a patient was taking a potassium supplement. Oral potassium was supplemented if hypokalemia was present.

Study size

The number of PA patients registered was 2814.

Analyses

The clinical characteristics of PA patients with renal impairment were compared with those of PA patients without renal impairment. A diagnosis of renal impairment was made when PA patients had an eGFR <60 mL/min/1.73 m^2 or proteinuria (+, ++, +++). The eGFR was calculated using the predictive equation defined by Japanese Society of Nephrology (15): $194 \times (\text{serum creatinine})^{-1.094} \times (\text{age})^{-0.287}$ for men and $194 \times (\text{serum creatinine})^{-1.094} \times (\text{age})^{-0.287} \times 0.739$ for women. Overt proteinuria was defined as a result of +, ++ or +++ on the dip stick test, while trace proteinuria was defined as a result of ± on the dip stick test. In qualitative urine analyses, thresholds of + and ++ are unified in Japan and defined a 30 mg/dL and 100 mg/dL, respectively. The thresholds of ± and +++ are not unified, but most kits available in Japan define those thresholds a 15 mg/dL and 300 mg/dL, respectively (15). As a diagnosis of renal impairment can be made based on the presence of proteinuria or lowered eGFR, we analyzed the effect of PA on proteinuria and eGFR separately, comparing patients with and without proteinuria or lowered eGFR.

We also investigated the clinical differences between patients with sustained eGFR and those with reduced eGFR defined as eGFR <60 mL/min/1.73 m^2. We then categorized patients with/without proteinuria into five groups: −, ±, +, ++ and +++, after which we compared PACs among the five groups.

Statistical methods

We used JMP® ver. 13.2.1 developed by the SAS Institute Inc. and Stata®/SE ver. 14 developed by LightStone®. Results are presented as the median (interquartile range) and frequencies (positive/total observations) unless otherwise stated. The Wilcoxon signed-rank test was used for quantitative variables. Pearson's χ^2 test was used for categorical variables. Cuzick's non-parametric test was used to assess trends across ordered groups. To account for multiple comparisons, we defined statistical significance as $P<0.01$ (22). Spearman's rank correlation coefficients for parameters associated with renal impairment, proteinuria and lowered eGFR in the univariate analysis were deemed statistically significant when the correlation coefficient satisfied $r>0.4$ and $P<0.01$. When we found that two or more parameters showed a statistically significant correlation, we selected the most representative parameter and brought it into a logistic regression analysis. The risk of an event is expressed as the odds ratio with the 99% confidence interval (99% CI).

Assay methods

At all centers, PAC was determined using a radioimmunoassay (SPAC-S Aldosterone kits, Fuji Rebio,

Co., LTD, Tokyo, Japan). The reference range for PAC with patients in a supine position was 3.0–15.9 ng/dL. PRA was measured using a radioimmunoassay or enzyme immunoassay (EIA). The reference range for PRA with patients in a supine position was 0.3–2.9 ng/mL/h (PRA-FR RIA kits, Fuji Rebio, Co., Ltd, Tokyo, Japan) at 17 centers, 0.2–2.3 ng/mL/h (PRA EIA kits, Yamasa, Co., Ltd, Choshi, Japan) at 8 centers and 0.2–2.7 ng/mL/h (PRA RIA kits, Yamasa, Co., Ltd) at 4 centers.

In Japan, nearly all university hospitals and large city hospitals have introduced automated analyzers for urinalysis. According to the annual report of the Japan Medical Association External Quality Control Program in 2016, there are three major companies providing these automated analyzers in Japan. EIKEN CHEMICAL Co., Ltd. have the largest market share in Japan (42.5%), followed by ARKRAY, Inc. (29.6%) and Siemens Healthcare K. K. (22.4%). Regarding the +, ++ and +++ proteinuria levels, the thresholds do not differ between these three companies. For trace proteinuria (±), the threshold is 15 mg/dL with the analyzers from EIKEN CHEMICAL Co., Ltd. and ARKRAY Inc.; the analyzer from Siemens Healthcare cannot detect trace proteinuria.

Ethics

This study was conducted in accordance with the Declaration of Helsinki and the guidelines for clinical studies published by the Ministry of Health and Labor, Japan and was approved by the Ethics Committee of the National Hospital Organization Kyoto Medical Center as the project-leading center and by the institutional ethics committees of the participating centers. The present retrospective study received ethical approval for the use of the opt-out consent method according to the Ethics Guidelines for Medical Research for Humans in Japan.

Results

Participants

Of 2814 enrolled PA patients, we excluded 208 patients who had a history of renal or urological diseases, including nephritis, nephrotic syndrome or urinary stones. Also excluded were patients prescribed a MRA (n=48), ACE-I (n=14), ARB (n=108), diuretics (n=14) or beta blocker (n=56). Data from the remaining 2366 PA patients were analyzed.

Descriptive data

Baseline characteristics are summarized in Table 1. The prevalences of renal impairment, overt proteinuria and lowered eGFR were 19.7% (419/2127), 10.3% (216/2104) and 11.6% (270/2325), respectively. The prevalence of trace and overt proteinuria was 25.7% (541/2104). Patients with proteinuria were categorized into five groups. The proportions of patients with overt and trace proteinuria were 10.3 (216/2104) and 15.4% (325/2104), respectively. The proportion of patients with overt proteinuria at each severity level was as follows: +, 7.8% (164/2104); ++, 1.5% (32/2104) and +++, 1.0% (20/2104) (Table 2).

Main results

Factors associated with renal impairment, proteinuria and lowered eGFR in patients with PA.

Table 1 Baseline characteristics of all PA participants in JPAS. Data are presented as median (interquartile range).

Characteristic	All subjects
Age, year	53 (45–62)
Sex, male, %	43.6
BMI, kg/m^2	24.2 (21.8–27.1)
Duration of hypertension, year	5 (2–11)
Systolic blood pressure, mmHg	140 (129–152)
Diastolic blood pressure, mmHg	86 (78–95)
Creatinine, mg/dL	0.69 (0.59–0.82)
Blood urea nitrogen, mg/dL	13.0 (11.0–15.6)
EGFR, mL/min/1.73 m^2	78.8 (67.8–92.1)
Lowered eGFR, %	11.6
Overt proteinuria, %	10.3
Trace and overt proteinuria, %	25.7
Renal impairment, %	19.7
Uric acid, mg/dL	5.2 (4.3–6.2)
Na^+, mEqL	142 (140–143)
K^+, mEq/L	3.8 (3.4–4.1)
Hypokalemia, %	37.7
Fasting blood sugar, mg/dL	98 (91–108)
HbA1c (NGSP), %	5.6 (5.3–6.0)
Diabetes mellitus, %	16.6
Total cholesterol, mg/dL	191 (170–215)
Triglyceride, mg/dL	105 (74–148)
HDL cholesterol, mg/dL	54 (45–66)
LDL cholesterol, mg/dL	111 (93–132)
Dyslipidemia, %	27. 1
PAC, ng/dL	17.4 (12.3–27.7)
PRA, ng/mL/h	0.3 (0.2–0.5)
ARR, ng/dL per ng/mL/h	53.5 (32–105)
Laterality, unilateral, %	27.2
ACTH, pg/mL	19.3 (11.3–31.0)
Cortisol, μg/dL	11.3 (8.6–15.2)

ACTH, adrenocorticotropic hormone; ARR, aldosterone-to-renin ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

Table 2 Distribution of patients with PA according to their proteinuria level.

Result of proteinuria	Number of patients	Proportion, %
–	1563	74.3
±	325	15.4
+	164	7.8
++	32	1.5
+++	20	1.0

In a univariate logistic regression analysis, we found that age, sex (male), BMI, duration of hypertension, sBP, UA, Na, K, hypokalemia, FBS, HbA1c, DM, TG, DL, PAC, ARR and laterality (unilateral) all significantly associated with renal impairment (Table 3). We next analyzed the factors associated with overt proteinuria, which is a component of renal impairment. In a univariate logistic regression analysis, age, sex (male), BMI, duration of hypertension, sBP, dBP, UA, Na, K, hypokalemia, FBS, HbA1c, DM, HDL-C, DL, PAC, ARR and laterality (unilateral) all significantly associated with proteinuria (Table 4). We also searched for factors associated with lowered eGFR, defined as eGFR <60 mL/min/1.73 m^2. In a univariate logistic regression analysis, age, duration of hypertension, UA, Na, hypokalemia, DM, TG, DL, PAC and ARR all significantly associated with lowered eGFR (Table 5).

Given that factors such as age, sex, duration of hypertension and prevalence of DM significantly associated with each of these renal impairments, we conducted a multivariate logistic regression analysis to clarify the association between renal impairments and PA-specific factors. Before carrying out that analysis, we checked the correlation between all pairs of these parameters using Spearman's rank correlation test

Table 3 Differences in clinical characteristics between PA patients with and without renal impairment. Data are presented as median (interquartile range). Renal-associated parameters including creatinine, blood urea nitrogen and eGFR were excluded from univariate logistic regression analysis as they are apparently associated with renal impairment.

Characteristic	Patients with renal impairment *n* = 419	Patients without renal impairment *n* = 1708	Mean difference	Unadjusted OR	99% CI	*P*
Age, years	60 (51–66)	52 (44–61)	6.29	1.06	1.04–1.07	<0.001
Sex, male, %	53.5%	42.0%	–	1.59	1.20–2.11	<0.001
BMI, kg/m^2	24.8 (22.1–28.0)	24.0 (21.7–26.8)	0.89	1.05	1.02–1.09	<0.001
Duration of hypertension, years	10 (3–19.5)	5 (1–10)	4.23	1.05	1.03–1.07	<0.001
Systolic blood pressure, mmHg	142 (130–155)	140 (128–152)	3.47	1.01	1.00–1.02	<0.001
Diastolic blood pressure, mmHg	86 (77–96)	87 (78–95)	0.39	1.00	0.99–1.01	0.577
Creatinine, mg/dL	0.88 (0.76–1.10)	0.66 (0.57–0.77)	0.27	–	–	–
Blood urea nitrogen, mg/dL	15.4 (12.8–18.2)	12.7 (10.9–15.0)	2.93	–	–	–
EGFR, mL/min/1.73 m^2	57.1 (50.9–69.5)	81.9 (72.0–93.6)	22.8	–	–	–
Trace and overt proteinuria, %	65.7	16.5	–	–	–	–
Uric acid, mg/dL	5.8 (4.8–6.8)	5.1 (4.2–5.9)	0.71	1.44	1.29–1.60	<0.001
Na, mEq/L	142 (141–144)	142 (140–143)	0.52	1.11	1.04–1.19	<0.001
K, mEq/L	3.7 (3.2–4.0)	3.8 (3.5–4.1)	0.14	0.61	0.46-0.79	<0.001
Hypokalemia, %	51.0%	34.9%	–	1.94	1.46–2.58	<0.001
Fasting blood sugar, mg/dL	102 (92–117)	97 (90–107)	6.12	1.01	1.00–1.01	0.002
HbA1c (NGSP), %	5.8 (5.4–6.4)	5.6 (5.3–6.0)	0.19	1.19	1.02–1.37	0.002
Diabetes mellitus, %	25.7%	15.4%	–	1.90	1.35–2.65	<0.001
Total cholesterol, mg/dL	190 (169–217)	191 (170–214)	0.06	0.99	0.99–1.00	0.977
Triglyceride, mg/dL	120 (89–163)	103 (73–146)	13.0	1.002	1.000–1.004	0.002
HDL cholesterol, mg/dL	50 (42–63)	54 (45–66)	2.44	0.99	0.98–1.00	0.012
LDL cholesterol, mg/dL	111 (91–135)	112 (93–132)	0.46	1.00	0.99–1.01	0.810
Dyslipidemia, %	36.1%	25.5%	–	1.65	1.22–2.22	<0.001
PAC, ng/dL	21.5 (14.0–37.9)	16.8 (12.1–25.7)	8.12	1.02	1.01–1.03	<0.001
PRA, ng/mL/h	0.3 (0.2–0.5)	0.3 (0.2–0.5)	0.04	1.27	0.90–1.77	0.061
ARR, ng/dL per ng/mL/h	66 (37–139)	52 (31–100)	38.6	1.002	1.001–1.003	<0.001
Laterality, unilateral, %	38.0%	25.5%	–	1.79	1.25–2.55	<0.001
ACTH, pg/mL	21.0 (13.0–34.6)	19.0 (11.0–30.2)	3.32	1.01	0.99–1.02	0.068
Cortisol, μg/dL	11.8 (9.2–16.0)	11.1 (8.4–14.9)	0.71	1.01	0.98–1.06	0.124

ACTH, adrenocorticotropic hormone; ARR, aldosterone-to-renin ratio; BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; HbA1c, Hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

Table 4 Differences in clinical characteristics between PA patients with and without proteinuria. Data are presented as median (interquartile range). Renal-associated parameters including creatinine, blood urea nitrogen, eGFR and proteinuria were excluded from univariate logistic regression analysis since they are apparently associated with renal impairment.

Characteristic	Patients with proteinuria *n* = 216	Patients without proteinuria *n* = 1888	Mean difference	Unadjusted OR	99% CI	*P*
Age, years	56 (46–64)	53 (45–62)	2.40	1.02	1.00–1.04	0.003
Sex, male, %	66.2%	41.7%	–	2.73	1.85–4.04	<0.001
BMI, kg/m^2	25.6 (22.9–29.0)	24.0 (21.7–26.8)	1.69	1.09	1.05–1.14	<0.001
Duration of hypertension, years	10 (3.8–20)	5 (1.5–11)	4.07	1.04	1.03–1.06	<0.001
Systolic blood pressure, mmHg	146 (133–158)	140 (128–152)	5.63	1.02	1.01–1.03	<0.001
Diastolic blood pressure, mmHg	89 (78–99)	86 (78–95)	2.44	1.01	1.00–1.03	0.009
Creatinine, mg/dL	0.81 (0.66–1.03)	0.68 (0.58–0.80)	0.19	–	–	–
Blood urea nitrogen, mg/dL	14.3 (12.0–17.0)	13.0 (11.0–15.1)	1.65	–	–	–
EGFR, mL/min/1.73 m^2	68.9 (55.9-87.3)	79.5 (68.9–92.4)	10.2	–	–	–
Lowered eGFR, %	31.2	9.4	–	–	–	–
Uric acid, mg/dL	5.6 (4.7–6.8)	5.1 (4.3–6.1)	0.54	1.30	1.14–1.49	<0.001
Na, mEq/L	143 (141–144)	142 (140–143)	0.46	1.10	1.01–1.20	0.004
K, mEq/L	3.6 (3.0–3.8)	3.8 (3.5–4.1)	0.30	0.35	0.25–0.49	<0.001
Hypokalemia, %	62.3%	35.2%	–	3.04	2.07–4.46	<0.001
Fasting blood sugar, mg/dL	103 (93–127)	98 (90–107)	12.1	1.01	1.01–1.02	<0.001
HbA1c (NGSP), %	5.9 (5.3–6.7)	5.6 (5.4–6.0)	0.36	1.33	1.11–1.57	<0.001
Diabetes mellitus, %	34.9%	15.5%	–	2.92	1.95–4.36	<0.001
Total cholesterol, mg/dL	187 (166–209)	192 (170–215)	4.94	0.99	0.99–1.00	0.076
Triglyceride, mg/dL	122 (90–163)	104 (74–48)	10.8	1.00	0.99–1.00	0.051
HDL cholesterol, mg/dL	49 (41–58)	54 (45–66)	5.57	0.98	0.96–0.99	<0.001
LDL cholesterol, mg/dL	110 (92–135)	112 (93–132)	0.32	0.99	0.99–1.01	0.903
Dyslipidemia, %	37.2%	26.4%	–	1.66	1.12–2.44	0.001
PAC, ng/dL	26.4 (16.4–40.9)	17.1 (12.1–26.3)	11.1	1.02	1.01–1.03	<0.001
PRA, ng/mL/h	0.4 (0.2–0.5)	0.3 (0.2–0.5)	0.06	1.40	0.94–2.09	0.028
ARR, ng/dL per ng/mL/h	73.3 (39.5–161)	52.5 (31.6–101)	40.1	1.001	1.001–1.002	<0.001
Laterality, unilateral, %	47.9%	25.8%	–	2.64	1.67–4.18	<0.001
ACTH, pg/mL	20.0 (12.9–33.5)	19.6 (11.3–31.0)	1.81	1.00	0.99–1.01	0.439
Cortisol, μg/dL	11.7 (8.7–15.7)	11.2 (8.5–15.2)	0.24	1.01	0.96–1.05	0.701

ACTH, adrenocorticotropic hormone; ARR, aldosterone-to-renin ratio; BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

in order to avoid multicollinearity. As described earlier, we selected the most representative parameters when statistically significant correlation was observed between two or more parameters. We also excluded renal-associated parameters including Cr, BUN, eGFR, proteinuria and lowered eGFR, since they are apparently associated with renal impairment. As a result, parameters described separately in Tables 6, 7 and 8 were selected and brought into the logistic regression analysis. The logistic regression analysis showed that PAC was associated with the presence of renal impairment, proteinuria and lowered eGFR, independent of other parameters (Tables 6, 7 and 8). We also found that the presence of renal impairment and proteinuria was significantly associated with the presence of hypokalemia (Tables 6 and 7).

Other analyses

Comparison of PACs according to the severity of proteinuria

When PA patients were divided into five groups according to the severity of their proteinuria (negative for proteinuria (−), ±, +, ++, +++), Cuzick's non-parametric test for trend showed that as PAC was significantly increased so was the severity of proteinuria ($P<0.001$) (Fig. 1).

Comparison between patients with PA and those with EHT

We compared the prevalences of renal impairment, proteinuria and lowered eGFR in PA patients in the JPAS with those in EHT patients at the Kyoto Medical Center (Table 9). The prevalences of renal impairment and lowered eGFR did not statistically differ between patients

Table 5 Differences in clinical characteristics between PA patients with and without lowered eGFR. Data are presented as median (interquartile range). Renal-associated parameters including creatinine, blood urea nitrogen, eGFR and lowered eGFR were excluded from univariate logistic regression analysis since they are apparently associated with renal impairment.

Characteristic	Patients with lowered eGFR *n* = 270	Patients without lowered eGFR *n* = 2055	Mean difference	Unadjusted OR	99% CI	*P*
Age, years	62 (55–67)	52 (44–61)	9.34	1.09	1.07–1.11	<0.001
Sex, male, %	49.3%	42.9%	–	1.29	0.92–1.80	0.049
BMI, kg/m^2	24.7 (22.0–27.6)	24.1 (21.7–27.0)	0.24	1.01	0.97–1.06	0.364
Duration of hypertension, years	10 (3–20)	5 (1.5–10)	5.35	1.06	1.04–1.07	<0.001
Systolic blood pressure, mmHg	142 (130–155)	140 (129–152)	2.75	1.01	0.99–1.02	0.020
Diastolic blood pressure, mmHg	84 (76–94)	87 (78–95)	1.44	0.99	0.98–1.00	0.085
Creatinine, mg/dL	1.02 (0.82–1.19)	0.67 (0.57–0.77)	0.39	–	–	–
Blood urea nitrogen, mg/dL	16.6 (14.1–20.0)	12.9 (11.0–15.0)	4.18	–	–	–
EGFR, mL/min/1.73 m^2	53.0 (46.9–56.8)	81.8 (71.8–93.8)	33.9	–	–	–
Overt proteinuria, %	27.4	8.0	–	–	–	–
Trace and overt proteinuria, %	44.9	23.2	–	–	–	–
Uric acid, mg/dL	6.1 (5.0–7.0)	5.1 (4.2–6.0)	0.88	1.53	1.35–1.72	<0.001
Na, mEq/L	142 (141–144)	142 (140–143)	0.46	1.10	1.02–1.18	0.002
K, mEq/L	3.7 (3.3–4.1)	3.8 (3.4–4.0)	0.01	0.95	0.68–1.31	0.659
Hypokalemia, %	46.7%	36.5%	–	1.53	1.09–2.13	0.001
Fasting blood sugar, mg/dL	102 (92–115)	97 (90–107)	3.8	1.00	0.99–1.01	0.044
HbA1c (NGSP), %	5.8 (5.5–6.3)	5.6 (5.3–6.0)	0.11	1.11	0.93–1.32	0.126
Diabetes mellitus, %	22.4%	15.9%	–	1.53	1.01–2.30	0.008
Total cholesterol, mg/dL	194 (170–218)	191 (170–214)	2.47	1.00	0.99–1.01	0.316
Triglyceride, mg/dL	115 (87–163)	102 (74–146)	15.3	1.00	1.00–1.01	0.002
HDL cholesterol, mg/dL	51 (43–65)	54 (45–66)	1.01	0.996	0.98–1.01	0.366
LDL cholesterol, mg/dL	111 (91–136)	111 (93–131)	0.92	1.00	0.99–1.01	0.685
Dyslipidemia, %	37.7%	25.9%	–	1.73	1.22–2.46	<0.001
PAC, ng/dL	20.3 (13.3–34.8)	17.0 (12.2–26.9)	7.52	1.02	1.01–1.02	<0.001
PRA, ng/mL/h	0.3 (0.2–0.5)	0.3 (0.2–0.5)	0.02	1.10	0.73–1.67	0.541
ARR, ng/dL per ng/mL/h	65 (37–130)	53 (32–102)	37.8	1.001	1.001–1.002	<0.001
Laterality, unilateral, %	31.3%	26.7%	–	1.25	0.81–1.94	0.187
ACTH, pg/mL	21.6 (13.0–37.3)	19.0 (11.0–30.1)	4.23	1.01	0.99–1.02	0.050
Cortisol, μg/dL	12.0 (9.5–16.3)	11.1 (8.4–15.0)	1.07	1.03	0.99–1.07	0.051

ACTH, adrenocorticotropic hormone; ARR, aldosterone-to-renin ratio; BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; HbA1c, Hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

with PA and those with EHT; however, the prevalences of both overt and trace proteinuria were significantly higher among PA than EHT patients.

Comparison between patients with the unilateral and bilateral subtype

From among 2366 patients with PA who underwent AVS, 755 patients were excluded due to the following reasons: AVS performed without ACTH stimulation (n=200); suspected autonomous cortisol secretion, which meant serum cortisol ≥1.8 μg/dL after a 1 mg dexamethasone suppression test mg/dL (n=351) and unsuccessful AVS, which meant a selectivity index <5 or no data on the selectivity index (n=204). Consequently, 438 unilateral and 1173 bilateral patients were ultimately included in the study. We then compared the prevalences of renal impairment, proteinuria and lowered eGFR between patients with the unilateral or bilateral subtype (Supplementary Table 1, see section on supplementary data given at the end of this article). The prevalences of renal impairment and both overt and trace proteinuria were significantly higher in patients with the unilateral subtype than the bilateral subtype (26.4 vs 16.7%, 16.7 vs 7.1%, 36.2 vs 21.2%, respectively). However, the prevalence of lowered eGFR did not statistically differ (13.1 vs 10.7%).

Discussion

Key results

Using the largest database of PA patients in the world, we conducted a multi-institutional, retrospective,

Table 6 Logistic regression analysis for renal impairment.

Parameter	Adjusted estimate	Adjusted OR	99% CI	*P*
Age, years	0.054	1.06	1.04–1.07	<0.001
Sex, male	−0.181	1.44	1.03–1.99	0.004
BMI, kg/m^2	0.064	1.07	1.02–1.11	<0.001
Duration of hypertension, years	0.015	1.02	0.99–1.03	0.031
Systolic blood pressure, mmHg	0.010	1.01	1.00–1.02	0.003
Na, mEq/L	−0.008	0.99	0.92–1.07	0.776
Hypokalemia	0.187	1.45	1.01–2.09	0.008
Diabetes mellitus	0.138	1.32	0.89–1.96	0.073
Dyslipidemia	0.057	1.12	0.79–1.59	0.400
PAC, ng/dL	0.021	1.02	1.01–1.03	<0.001

BMI, body mass index; CI, confidence interval; OR, odds ratio; PAC, plasma aldosterone concentration.

cross-sectional study to evaluate the contribution of PA to renal impairment, focusing on proteinuria and lowered eGFR.

Our study demonstrates that renal impairment in PA patients significantly correlates with the PAC level itself, which is unlike other cardiovascular complications. This finding suggests that aldosterone has a greater direct effect on the kidney than on other cardiovascular organs.

Limitations

There were several limitations to this study. First, this is a retrospective cross-sectional study. Because the prevalence of renal impairment would depend on the duration of the PA, to determine the exact incidences of renal impairment, proteinuria and lowered eGFR in patients with PA, we would need to perform a prospective study. However, it would be ethically difficult to prospectively follow patients with PA without administering specific treatment of PA. Second, the JPAS database is composed of data on PA patients and does not include data on EHT patients. To reduce the difference in the backgrounds between patients with PA and those with EHT, we used data from EHT patients who were treated as outpatients at the Kyoto Medical Center, which played a central role in the JPAS study. However, the clinical data for the EHT patients were not included in the JPAS study.

Because the EHT data were from a single center and were not registered in the same registry, we cannot exclude the possibility that they do not reflect the more generalized characteristics of patients with EHT. Third, proteinuria was examined in this study through qualitative, but not quantitative, urine analyses. Fourth, the investigators participating in the JPAS study were, in principle, expected to register all PA patients during the study period. However, those investigators were allowed to refrain from registering specific patients whom they judged to be inappropriate for our study. This could potentially cause a selection bias. Finally, renal impairment was diagnosed based on a one-time measurement. Consequently, we could not show whether the renal impairment was transient or persistent. We therefore refrained from using the term ‘chronic kidney disease’. Instead we used ‘renal impairment’.

Table 7 Logistic regression analysis for proteinuria.

Parameter	Adjusted estimate	Adjusted OR	99% CI	*P*
Age, year	0.014	1.01	0.99–1.04	0.120
Sex, male	−0.357	2.04	1.32–3.16	<0.001
BMI, kg/m^2	0.076	1.08	1.03–1.14	<0.001
Duration of hypertension, year	0.023	1.02	0.99–1.05	0.015
Systolic blood pressure, mmHg	0.017	1.02	1.00–1.03	<0.001
Na, mEq/L	−0.030	0.97	0.88–1.07	0.416
Hypokalemia	0.423	2.33	1.46–3.72	<0.001
Diabetes mellitus	0.379	2.14	1.31–3.47	<0.001
Dyslipidemia	0.062	1.13	0.72–1.78	0.483
PAC, ng/dL	0.017	1.02	1.01–1.03	<0.001

BMI, body mass index; CI, confidence interval; OR, odds ratio; PAC, plasma aldosterone concentration.

Table 8 Logistic regression analysis for lowered eGFR.

Parameter	Adjusted estimate	Adjusted OR	99% CI	*P*
Age, years	0.080	1.08	1.06–1.11	<0.001
Duration of hypertension, years	0.017	1.02	0.99–1.04	0.041
Na, mEq/L	−0.007	0.99	0.91–1.08	0.857
Hypokalemia	0.043	1.09	0.71–1.68	0.531
Diabetes mellitus	0.043	1.09	0.68–1.75	0.624
Dyslipidemia	0.059	1.13	0.75–1.69	0.430
PAC, ng/dL	0.018	1.02	1.01–1.03	<0.001

CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; PAC, plasma aldosterone concentration.

Interpretation

Renal impairment associated with PA consists of two components (23). At an early stage of PA, increased sodium reabsorption and expansion of the extracellular fluid volume cause increased renal perfusion pressure and suppression of renin-angiotensin II. As a consequence, intrarenal vascular resistance decreases, leading to hyperfiltration. This is a functional adaptation, and structural renal damage is not obvious at this stage (24). The decrease in intrarenal vascular resistance at this stage was confirmed in a clinical study in which the echo Doppler technique was used to assess the resistance index for kidneys (25). The other component of renal impairment in PA reflects structural damage to the kidney resulting from persistent hypertension and hyperaldosteronism.

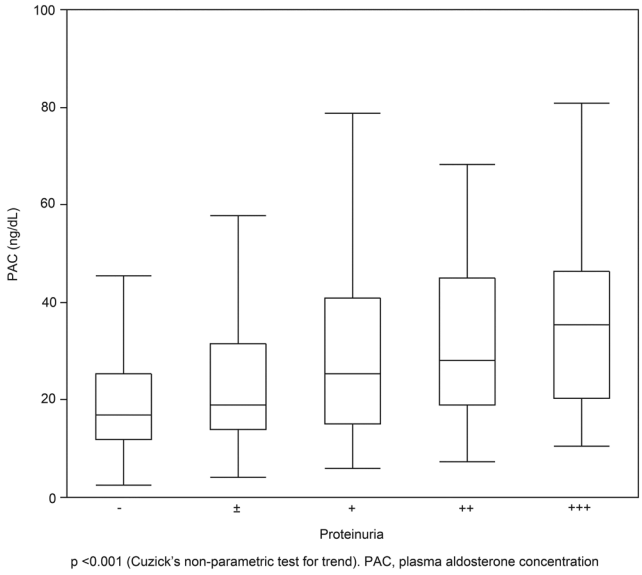

Figure 1
PACs in PA patients grouped according to their proteinuria level determined using a dip stick test. $P < 0.001$ (Cuzick's non-parametric test for trend). PAC, plasma aldosterone concentration.

Hypertension causes fibrosis within intrarenal vessels (24), and hyperaldosteronism also causes adverse structural changes. Danforth *et al*. examined renal biopsy specimens from patients with PA and reported observing moderate renal tissue damage (26).

In the present study, the prevalence of renal impairment was 19.7%. At first glance, this prevalence looks to be higher than that in the study from Iwakura *et al*., who reported the prevalence of CKD (chronic kidney disease) to be 11.7% in their cohort (27). However, their definition of renal impairment was only eGFR below 60 mL/min/1.73 m^2; proteinuria was not included. In our study, the frequency of renal impairment was increased by inclusion of patients with overt proteinuria without lowered eGFR. In our study, the prevalence of eGFR below 60 mL/min/1.73 m^2 was 11.6% or nearly the same as in the earlier study. The definition of renal insufficiency has also varied among other studies. In one case the criterion was a serum creatinine level >1.5 mg/dL (28), while in another it was 1.25 mg/dL (9), and in a third it was based on creatinine clearance (CCr) (10). This makes it difficult to interpret the differences in the prevalence of renal insufficiency between the present study and earlier ones.

In the present study, the GFR in patients with PA did not differ from that in patients with EHT. Even when we calculated eGFR by using the CKD-EPI (the Chronic Kidney Disease Epidemiology Collaboration) formula (29), the difference was not observed (PA 78.2 mL/min/1.73 m^2 vs EHT 79.9 mL/min/1.73 m^2). It was observed in some earlier studies that the GFR is higher in patients with PA than with EHT (10, 11), while others report the GFR to be lower in PA than EHT patients (9, 7). And Ribstein *et al*. reported that the GFR does not differ between PA and EHT patients (12). This inconsistency may derive in part from differences in the severity of the renal impairment in each cohort. Steichen *et al*. reported that in patients with PA, the GFR declined after adrenalectomy or administration of a mineralocorticoid receptor antagonist (30). As discussed earlier, hyperfiltration due to hyperaldosteronism occurs

Table 9 Comparison of patients with PA (JPAS Study) and EHT (Kyoto Medical Center) matched for age, sex, SBP and duration of hypertension. Data are presented as median (interquartile range).

Characteristic	Patients with PA *n* = 128	Patients with EHT *n* = 128	*P*
Age, years	59 (47–66)	59 (47–66)	0.999
Sex, male, %	49.2%	49.2%	1.000
BMI, kg/m^2	24.1 (21.5–26.3)	23.2 (21.4–26.7)	0.394
Duration of hypertension, years	3 (1–9)	3 (1–9)	0.898
Systolic blood pressure, mmHg	140 (129–152)	140 (130–151)	0.950
Diastolic blood pressure, mmHg	87 (80–92)	86 (79–92)	0.995
Creatinine, mg/dL	0.71 (0.60–0.84)	0.70 (0.60–0.90)	0.940
Blood urea nitrogen, mg/dL	13.5 (11.0–16.2)	14.0 (12.0–17.0)	0.175
EGFR, mL/min/1.73 m^2	76.1 (65.1–87.8)	75.0 (65.4–86.7)	0.970
Lowered eGFR, %	17.2%	15.0%	0.628
Overt proteinuria, %	16.8%	4.4%	0.002
Trace and overt proteinuria, %	31.1%	13.9%	0.002
Renal impairment, %	28.9%	19.1%	0.079
Uric acid, mg/dL	5.3 (4.3–6.1)	5.5 (4.4–6.5)	0.322
Na, mEq/L	142 (141–143)	140 (139–141)	<0.001
K, mEq/L	3.9 (3.4-4.1)	4.1 (3.9-4.3)	<0.001
Diabetes mellitus, %	18.8%	19.6%	0.904
Dyslipidemia, %	28.1%	32.8%	0.415
PAC, ng/dL	15.7 (11.8–25.2)	14.9 (11.2–18.0)	0.041
PRA, ng/mL/h	0.3 (0.2–0.6)	2.4 (1.4–4.6)	<0.001
ARR, ng/dL per ng/mL/h	46 (30–91)	6.5 (3.6–10.3)	<0.001

ARR, aldosterone-to-renin ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

in patients with PA. We therefore suggest the GFR is actually lower in patients with PA than with EHT, but hyperfiltration masks the true magnitude of the decrease.

There have been several studies focusing on the pathogenic role of aldosterone in renal injury. Blasi *et al*. showed that aldosterone infusion and salt intake cause hypertension, intrarenal vascular and glomerular sclerosis, fibrinoid necrosis, tubular damage and albuminuria (8). Similar results have been reported by others (5, 7, 31). Nagase *et al*. reported that podocytes, which act against urinary protein loss, are damaged by a high-salt diet with aldosterone infusion in uninephrectomized rats. As a result, these rats exhibit massive proteinuria (32). In the present study, the prevalences of overt and trace proteinuria were 10.3 and 25.7%, respectively. We also found that the prevalence of proteinuria, whether overt or trace, was significantly higher in PA than EHT patients, which is consistent with earlier results (10, 11, 12). Catena *et al*. reported that this difference was due to hyperfiltration caused by hyperaldosteronism, as the difference was normalized after adjusting for the urinary creatinine concentration (10). In the present study, however, the prevalences of overt and trace proteinuria were significantly higher in patients with PA, though eGFR in those patients did not differ from that in patients with EHT. This result suggests that the difference in the prevalence of proteinuria in our cohort is not explained by hyperfiltration. We suggest that eGFR is actually lower in patients with PA than with EHT, but hyperfiltration masks the true decline and that proteinuria is a hallmark of renal impairment in patients with PA. In our analysis, when patients were divided into five groups according to their level of proteinuria, we found that as the PAC was significantly increased, so was the level of proteinuria (Fig. 1).

This suggests a positive relationship between the PAC and the severity of proteinuria. We recently reported that the PAC is not itself associated with cardiovascular events such as stroke or ischemic heart disease (4). To the contrary, however, analyzing the same JPAS patients in the present study, the PAC was significantly associated with the odds of renal impairment, proteinuria and lowered eGFR. Although aldosterone influences both the cardiovascular system and kidney, we suggest that the mechanism by which aldosterone affects the kidney differs from the mechanism affecting the vasculature itself and that the PAC may have a more direct effect on the kidney than on other cardiovascular organs.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/EJE-19-0047.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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ORIGINAL ARTICLE

Pheochromocytoma and paraganglioma with negative results for urinary metanephrines show higher risks for metastatic diseases

Akiyuki Kawashima[1] · Masakatsu Sone[1,2] · Nobuya Inagaki[1] · Kentaro Okamoto[1] · Mika Tsuiki[3] · Shoichiro Izawa[4] · Michio Otsuki[5] · Shintaro Okamura[6] · Takamasa Ichijo[7] · Takuyuki Katabami[8] · Yoshiyu Takeda[9] · Takanobu Yoshimoto[10,11] · Mitsuhide Naruse[12] · Akiyo Tanabe[13]

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Abstract

Purpose Few studies have assessed the clinical features of pheochromocytoma and paraganglioma (PPGL) not producing excessive catecholamine. We aimed to clarify the clinical characteristics of PPGL patients with negative results for urinary metanephrines.

Methods This is a retrospective cross-sectional study. We established a database by combining datasets from the Nationwide Cohort Study on the Development of Diagnosis and Treatment of Pheochromocytoma in Japan (PHEO-J) and the Advancing Care and Pathogenesis of Intractable Adrenal diseases in Japan (ACPA-J). We compared the clinical differences between PPGL patients with negative results for urinary metanephrines and those with catecholamine-producing PPGL.

Results Five hundred PPGL patients in the combined database were analyzed. Among them, 31 were negative for metanephrines. PPGL with negative results for urinary metanephrines was significantly associated with extra-adrenal disease (Odds ratio (OR) 6.58, 95% CI (confidence interval) 3.03–14.3, $p < 0.001$), the presence of metastatic disease (OR 4.22, 95% CI 1.58–11.3, $p = 0.004$), and negativity on meta-iodobenzylguanidine (MIBG) scintigraphy (OR 0.15, 95% CI 0.03–0.77, $p = 0.023$).

Conclusions Our findings demonstrate that PPGL patients with negative results for urinary metanephrines are associated with extra-adrenal lesions, metastatic disease, and negative MIBG findings. This suggests that PPGL patients with negative results for urinary metanephrines have a greater need for systemic whole-body imaging other than MIBG scintigraphy and close follow-up to monitor for metastasis than do patients with PPGL overtly producing excessive catecholamine.

Keywords Pheochromocytoma · Paraganglioma · Catecholamine · MIBG · Metastasis

✉ Masakatsu Sone
sonemasa@kuhp.kyoto-u.ac.jp

[1] Department of Diabetes, Endocrinology and Nutrition, Kyoto University, Kyoto, Japan

[2] Division of Metabolism and Endocrinology, Department of Internal Medicine, St. Marianna University School of Medicine, Kanagawa, Japan

[3] Department of Endocrinology and Metabolism, National Hospital Organization Kyoto Medical Center, Kyoto, Japan

[4] Division of Endocrinology and Metabolism, Tottori University Faculty of Medicine, Yonago, Japan

[5] Department of Metabolic Medicine, Osaka University Graduate School of Medicine, Osaka, Japan

[6] Department of Endocrinology, Tenri Hospital, Tenri, Japan

[7] Department of Diabetes and Endocrinology, Saiseikai Yokohamashi Tobu Hospital, Yokohama, Japan

[8] Division of Metabolism and Endocrinology, Department of Internal Medicine, St. Marianna University School of Medicine Yokohama City Seibu Hospital, Yokohama, Japan

[9] Department of Internal Medicine, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

[10] Department of Diabetes and Endocrinology, Tokyo Metropolitan Hiroo Hospital, Tokyo, Japan

[11] Department of Molecular Endocrinology and Metabolism, Tokyo Medical and Dental University, Tokyo, Japan

[12] Clinical Research Institute of Endocrinology and Metabolism, NHO Kyoto Medical Center and Endocrine Center, Ijinkai Takeda General Hospital, Kyoto, Japan

[13] Division of Endocrinology, National Center for Global Health and Medicine, Tokyo, Japan

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Introduction

Pheochromocytoma and paraganglioma (PPGL) are neuroendocrine tumors that originate, respectively, from chromaffin cells in the adrenal medulla and paraganglia in the sympathetic and parasympathetic nervous systems [1]. PPGLs are rare diseases with a combined incidence of 0.57 cases per 100,000 person-years [2]. Typically, these tumors produce catecholamines, which cause patients to develop such symptoms as hypertension, glucose intolerance, panic attacks, and arrhythmias [1]. Catecholamines are converted to metanephrines by catechol-O-methyltransferase (COMT) within the cells of the catecholamine-producing tumors and are then released into the circulation [3]. Consequently, for a diagnosis of PPGL, measurements of metanephrines are a more sensitive indicator than measurements of catecholamines [4]. However, in a portion of PPGL patients, metanephrines levels are not elevated. In the recent Endocrine Society guidelines, these patients were described as being rare [4], and there have been few reports analyzing their clinical features [5, 6]. The aim of the present study, therefore, was to clarify the characteristics of PPGL patients with negative results for urinary metanephrines. To do so, we compared the clinical characteristics of patients with catecholamine-producing PPGL and those with negative results for urinary metanephrines by an analysis of data from two large databases: the Advancing Care and Pathogenesis of Intractable Adrenal Diseases in Japan (ACPA-J) and the Nationwide Cohort Study on the Development of Diagnosis and Treatment of Pheochromocytoma in Japan (PHEO-J) registries.

Materials and methods

Study population

This is a retrospective, cross-sectional study. We obtained the clinical data on PPGL patients from the PHEO-J and ACPA-J databases. The PHEO-J was built to establish a disease registry for elucidation of prognoses and the effects of treatments in PPGL patients and to establish a method for early diagnosis of PPGL based on histopathological markers. The purpose of the ACPA-J is to build a registration system and cohort for patients with adrenal tumors and to produce new evidence to apply to the management of adrenal tumors and contribute to clinical guidelines. The ACPA-J focuses not only on patients with PPGL but also on those with other adrenal diseases, such as Cushing syndrome, subclinical Cushing syndrome, and adrenocortical cancer.

In the PHEO-J study, PPGL patients who visited outpatient clinics and hospitals between April 1, 2008, and March 31, 2012, were registered, regardless of their age. There were 178 participating institutions. In the ACPA-J study, patients aged 20 to 90 who were diagnosed with PPGL between January 2006 to December 2015 were enrolled. The ACPA-J study was established at 10 centers, including the National Center for Global Health and Medicine, National Hospital Organization Kyoto Medical Center, St. Marianna University Yokohama City Seibu Hospital, Tottori University Hospital, Tokyo Medical and Dental University, Osaka University Graduate School of Medicine, Kyoto University, Kanazawa University Graduate School of Medical Science, Tenri Hospital, and Saiseikai Yokohama-shi Tobu Hospital.

Patients enrolled in both studies were mainly treated with surgery; however, a few patients diagnosed with PPGL at each institution did not undergo surgery, based upon the assessment of data that included the levels of catecholamine metabolites and the results of ^{123}I/^{131}I-meta-iodobenzylguanidine (MIBG) scintigraphy or magnetic resonance imaging. Some patients were registered in both the PHEO-J and ACPA-J studies. For those patients, we analyzed the clinical data in the ACPA-J study.

The PHEO-J study collected the patients' clinical characteristics, results of biochemical examinations, and radiological and pathological findings at the time of their initial diagnosis, at the time of enrollment, and six months after enrollment. These data were obtained throughout a web registry system. The ACPA-J study gathered these data at the time of enrollment, and no follow-up data is available at present. Collected data were registered to another web registry system. The present study was conducted using a dataset validated in March 2013 for the PHEO-J study and March 2019 for the ACPA-J study.

Of 188 PPGL patients enrolled in the ACPA-J study, we excluded 16 patients for whom there were no data on urinary metanephrine or normetanephrine. Forty PPGL patients were not categorized as having a catecholamine-producing PPGL or a PPGL with negative results for urinary metanephrines. Hence, they were not included in the analysis. Twenty-six patients who had been previously diagnosed at another institution were also excluded. A total of 939 PPGL patients were registered in the PHEO-J study. In the same way, we excluded 451 patients whose data on urinary metanephrine or normetanephrine were unavailable. Sixty-one patients were not categorized as having a catecholamine-producing PPGL or a PPGL with negative results for urinary metanephrines. Nine patients were removed due to double registration, and 24 were eliminated because they were also registered in the ACPA-J study.

Analysis

We collected data on age, sex, family history, and blood chemistry, including urinary adrenaline, urinary noradrenaline, urinary dopamine, urinary metanephrine, and

urinary normetanephrine. We also gathered radiological findings, including the location of the tumor, the presence of metastasis, and the results of MIBG scintigraphy. Metastatic PPGL was diagnosed when there were tumors in non-chromaffin cells, such as lymph nodes, liver, lung, or bones. Patients who had already been diagnosed with PPGL and received surgery in other hospitals were excluded from the analysis. The information from MIBG scintigraphy was only available for patients registered in the ACPA-J.

For the present study, PPGL with negative results for urinary metanephrines was defined when both urinary metanephrine and normetanephrine did not exceed their upper reference limits. PPGL was defined as catecholamine-producing when either urinary metanephrine or normetanephrine exceeded a level three times higher than its upper reference limit.

We also collected data on metabolic parameters, including body mass index (BMI), fasting blood sugar, HbA1c [NGSP, National Glycohemoglobin Standardization Program], total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (calculated using Friedewald equation), and triglyceride from the ACPA-J database.

Patients were considered to have hypertension or dyslipidemia after confirming a history of hypertension or dyslipidemia in the dataset. A diagnosis of glucose intolerance was made when PPGL patients had FPG ≥ 110 mg/dL, a 75 g oral glucose tolerance test with a two-hour plasma glucose level of 140 mg/dL or greater, HbA1c ≥ 6.2%, or were taking medication for diabetes mellitus.

Assay methods

Urinary adrenaline, noradrenaline, dopamine, metanephrine, and normetanephrine were measured using high-performance liquid chromatography (HPLC). We defined the upper reference limit for metanephrine as 0.18 mg/day and that for normetanephrine as 0.28 mg/day.

Statistical methods

We used JMP® ver. 13.2.1 developed by the SAS Institute Inc. and Stata®/SE ver. 14 developed by LightStone®. Results are presented as the median (interquartile range) and frequencies (positive/total observations) unless otherwise stated. The Wilcoxon signed-rank test was used for quantitative variables. Pearson's χ^2 test was used for categorical variables. The odds of an event occurring are expressed as the odds ratio (OR) with the 95% confidence interval (CI).

Ethics

The PHEO-J study was conducted in accordance with the Declaration of Helsinki and the guidelines for clinical studies published by the Ministry of Health and Labour, Japan and was approved by the Ethics Committee of the National Hospital Organization Kyoto Medical Center as the project-leading center and by the institutional ethics committees of the participating centers. The present retrospective study received ethical approval for the use of the opt-out consent method according to the Ethics Guidelines for Medical Research for Humans in Japan.

The ACPA-J study was conducted in accordance with the Declaration of Helsinki and the guidelines for clinical studies published by the Ministry of Health, Labour, and Welfare, Japan and was approved by the Ethics Committee of the National Center for Global Health and Medicine as the project-leading center and by the institutional ethics committees of the participating centers.

Results

A total of 500 PPGL patients were analyzed in the present study: 106 from the ACPA-J study and 394 from the PHEO-J study. Their baseline characteristics are summarized in Table 1. The prevalence of PPGL patients with negative results for urinary metanephrines was 6.2%. The prevalence of patients with a family history of PPGL was 6.4%. Extra-adrenal PPGLs were observed in 16.2% of patients. The prevalence of patients with metastatic PPGL was 6.7%.

In a univariate logistic regression analysis, we found that extra-adrenal PPGL was positively associated with negative results for urinary metanephrines (OR 6.58, 95% CI 3.03–14.3, $p < 0.001$). The presence of metastasis was also

Table 1 Baseline characteristics of patients with PPGL in the ACPA-J and PHEO-J studies

Characteristic	Patients in the present study
Number of patients analyzed	500
Family history, %	6.4%
Sex, male, %	42.9%
Age, year	53 (39–64)
Urinary adrenaline, μg/day	41.6 (12.3–196)
Urinary noradrenaline, μg/day	445 (194–1510)
Urinary dopamine, μg/day	841 (618–1472)
Urinary metanephrine, mg/day	0.70 (0.14–2.80)
Urinary normetanephrine, mg/day	1.92 (0.86–5.00)
PPGL with negative results for urinary metanephrines, %	6.2%
Extra-adrenal tumor, %	16.2%
Metastasis, %	6.7%
MIBG positivity[a], %	90.3%

[a]Information on MIBG positivity was available for 93 patients enrolled in the ACPA-J study. *PPGL* pheochromocytoma and paraganglioma, *MIBG* meta-iodobenzylguanidine

Table 2 Comparison of the clinical characteristics between PPGL with negative results for urinary metanephrines and catecholamine-producing PPGL

Characteristic	PPGL with negative results for urinary metanephrines $n = 31$	Catecholamine-producing PPGL $n = 469$	OR	95% CI	p
Family history, %	7.4%	6.4%	1.17	0.26–5.22	0.834
Sex, male, %	58.1%	41.9%	1.92	0.94–4.00	0.083
Age, year	54 (42–67)	53 (38–64)	1.00	0.98–1.03	0.710
Extra-adrenal, %	51.7%	14.0%	6.58	3.03–14.3	< 0.001*
Metastasis, %	20.7%	5.8%	4.22	1.58–11.3	0.004*
MIBG positivity[a], %	66.7%	92.9%	0.15	0.03–0.77	0.023*

[a]Information of MIBG positivity was available from 93 patients enrolled in ACPA-J; asterisks (*) indicate significant differences ($p < 0.05$). *PPGL* pheochromocytoma and paraganglioma, *OR* odds ratio, *CI* confidence interval, *MIBG* meta-iodobenzylguanidine

positively associated with negative results for urinary metanephrines (OR 4.22, 95% CI 1.58–11.3, $p = 0.004$). The prevalence of MIBG positivity was negatively associated with negative results for urinary metanephrines (OR 0.15, 95% CI 0.03–0.77, $p = 0.023$). Other parameters, including sex, age at the time of the first diagnosis, and family history did not differ significantly between PPGL patients with negative results for urinary metanephrines and those with a catecholamine-producing PPGL. These results are summarized in Table 2.

We next assessed the metabolic parameters in patients with PPGL. The baseline metabolic parameters in PPGL patients in the ACPA-J study are shown in Table 3. The percentages of patients with hypertension, glucose intolerance, and dyslipidemia were 64.8%, 42.3%, and 39.4%, respectively. A comparison between the metabolic parameters in patients with negative results for urinary metanephrines and catecholamine-producing PPGL patients in the ACPA-J cohort is summarized in Table 4. The prevalence of hypertension and glucose intolerance were significantly lower in PPGL patients with negative results for urinary metanephrines (35.7% vs. 69.2%, $p = 0.015$, 15.4% vs. 46.2%, $p = 0.036$). Fasting blood sugar and HbA1c were also significantly lower in PPGL patients with negative results for urinary metanephrines (89 mg/dL vs. 115 mg/dL, $p < 0.001$, 5.6% vs. 6.1%, $p = 0.007$). BMI did not significantly differ between patients with negative results for urinary metanephrines and catecholamine-producing PPGL patients.

Table 3 Baseline metabolic parameters in patients in the ACPA-J study

Characteristic	ACPA-J
Number of patients analyzed	106
Sex, male, %	39.6%
Age, year	58 (43–67)
BMI, kg/m^2	21.6 (19.2–23.7)
Hypertension, %	64.8%
Fasting blood sugar, mg/dL	111 (94–133)
HbA1c (NGSP), %	6.0 (5.7–6.7)
Glucose intolerance, %	42.3%
Triglyceride, mg/dl	83 (63–123)
HDL cholesterol, mg/dL	69 (58–84)
LDL cholesterol, mg/dL	112 (96–137)
Dyslipidemia, %	39.4%
PPGL with negative results for urinary metanephrines, %	13.2%

BMI body mass index, *HbA1c* hemoglobin A1c, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *PPGL* pheochromocytoma and paraganglioma

Discussion

Our study demonstrated that extra-adrenal PPGL and metastasis are positively associated with negative results for urinary metanephrines, while MIBG positivity is negatively associated with negative results for urinary metanephrines

We found that the prevalence of PPGL patients with negative results for urinary metanephrines was 6.2% in our cohort. Based on earlier spectrophotometry, Lenders et al. reported that 26 of 114 (22.8%) patients were negative for urinary total metanephrines. Based on an HPLC analysis, they also reported that only 3 of 105 (2.9%) patients showed negative results for urinary fractionated metanephrines [7]. A more recent study showed that the prevalence of PPGL patients with negative results for urinary deconjugated metanephrines was 16 of 226 patients (7.1%) [8]. In that study, the prevalence of patients with negative results for plasma-free metanephrines was lower than urinary levels (3.4% vs. 7.1%). If plasma-free metanephrines had been measured instead of urinary fractionated metanephrines in our study, the prevalence of patients with negative results for metanephrines in our cohort may have been lower than the present result. Heavner et al. reported that 7 of 78 (8.9%) PPGL patients were negative for serum biomarkers

Table 4 Comparison of metabolic parameters between PPGL with negative results for urinary metanephrines and catecholamine-producing PPGL in the ACPA-J study

Characteristic	PPGL with negative results for urinary metanephrines $n = 14$	Catecholamine- producing PPGL $n = 92$	p
Sex, male, %	42.9%	39.1%	0.791
Age, year	57 (44–73)	58 (43–67)	0.592
BMI, kg/m^2	22.1 (18.6–25.0)	21.6 (19.2–23.7)	0.742
Hypertension, %	35.7%	69.2%	0.015*
Fasting blood sugar, mg/dL	89 (77–92)	115 (98–133)	< 0.001*
HbA1c (NGSP), %	5.6 (5.3–5.9)	6.1 (5.7–7.1)	0.007*
Glucose intolerance, %	15.4%	46.2%	0.036*
Triglyceride, mg/dl	94 (74–227)	81 (62–121)	0.069
HDL cholesterol, mg/dL	66 (48–81)	70 (59–84)	0.402
LDL cholesterol, mg/dL	123 (87–137)	112 (97–136)	0.695
Dyslipidemia, %	38.5%	39.6%	0.940

Asterisks (*) indicate significant differences ($p < 0.05$). *BMI* body mass index, *HbA1c* hemoglobin A1c, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *PPGL* pheochromocytoma and paraganglioma

(4 patients were negative for urinary metanephrine, 2 were negative for plasma metanephrine, and 1 was negative for plasma catecholamine). In contrast to our results, there were no extra-adrenal or metastatic tumors in PPGL patients negative for catecholamines and their metabolites, whereas there were 6 extra-adrenal and 9 metastatic cases in patients with catecholamine-producing PPGL [5].

In a retrospective study from the Mayo Clinic, plasma or urinary metanephrines/catecholamines levels within the reference ranges were found in 51 of 248 (20.6%) patients with metastatic PPGL patients [9]. Similarly, PPGL patients with negative results for urinary metanephrines accounted for 18.8% (6/32) of PPGL patients with metastasis in our cohort. The proportion of patients with negative results for urinary metanephrines was higher among those with metastatic PPGL than among all PPGL patients. This implies an association between negative results for metanephrines and metastatic PPGL. In catecholamine-producing PPGL, catecholamines and their metabolites are higher in patients with metastatic PPGL than in those with non-metastatic PPGL. For example, Feng compared the clinical characteristics of 136 PPGL patients and showed that urinary metanephrine and normetanephrine were significantly higher in patients with metastatic than non-metastatic PPGL [10]. In their study, all PPGL patients exhibited elevation of catecholamines or their metabolites, whereas our study included PPGL patients with negative results for urinary metanephrines and catecholamines. We presume this discrepancy is attributable to differences in the characteristics of the enrolled patients between our study and theirs.

The clinical characteristics of PPGL patients with *SDHx* mutations were recently reported. The frequency of metastatic PPGL is reportedly higher among patients with *SDHB* mutations than among those without them [11, 12]. In addition, PPGL with *SDHB* mutations sometimes lacks tyrosine hydroxylase, which results in biochemically silent PPGL [13]. More recently, Dreijerink et al. similarly showed that *SDHD* mutations are associated with biochemically silent PPGL [14]. Neumann et al. found that the prevalence of extra-adrenal PPGL was significantly higher among patients with *SDHB*/*SDHD* mutations than among those without these mutations [15], while Timmers et al. showed that the sensitivity of MIBG scintigraphy was lower in patients with *SDHB*/*SDHD* mutations [16]. In the present study, PPGLs with negative results for urinary metanephrines were significantly associated with extra-adrenal PPGL, negative MIBG scintigraphy findings, and the presence of metastatic lesions. Considering the characteristics of *SDHx*-related PPGL, patients with *SDHx* mutations may account for a significant portion of the patients with negative results for urinary metanephrines in our cohort.

Park et al. assessed urinary excretion of catecholamines and their metabolites per tumor diameter and found that the amount of excreted vanillylmandelic acid per tumor diameter was significantly lower in patients who developed metastatic PPGL than in those who did not develop metastatic PPGL [17]. In addition, Grouzmann showed that tumoral catecholamines and metanephrine levels were lower in patients with extra-adrenal than adrenal PPGL [18]. Eisenhofer reported that PPGL patients with *SDHB* mutations showed lower tumoral catecholamines levels than did other tumors [19]. Eisenhofer et al. also reported that tumoral catecholamine content had a positive relationship with tumor-derived increases in metanephrine [20]. We suggest the higher proportion of negative results for urinary metanephrines in patients with metastatic PPGL may reflect the lower tumoral catecholamine content in patients with *SDHB* mutations or extra-adrenal PPGL, which are more prone to metastasis than other tumors.

The sensitivity of MIBG scintigraphy ranged between 85 and 88% in adrenal PPGL [4]. However, the sensitivity of MIBG scintigraphy is reportedly lower in other settings. It ranged between 56 and 75% in extra-adrenal PPGL [4], 50-59% in metastatic PPGL [16, 21, 22], and 45% in PPGL arising from *SDHx* mutations [16]. In the present study, we found that MIBG positivity was negatively associated with negative results for urinary metanephrines. This may be associated with a higher proportion of metastatic PPGL and extra-adrenal PPGL in patients with negative results for urinary metanephrines.

In the present study, the prevalence of metastatic PPGL was 6.7%, which is lower than in previous reports. For example, Eisenhofer et al. reported 35 of 365 (9.6%) PPGL patients were diagnosed with metastatic PPGL at the time of initial presentation. They also reported that another 70 patients were diagnosed with metastatic PPGL during their follow-up [19]. Another report conducted in South Korea showed that while 94 of 1048 (9.0%) patients had metastatic PPGL at the time of diagnosis, an additional 91 patients were diagnosed with metastatic PPGL during their follow-up period [23]. Considering that metastatic lesions often become apparent several years after the initial diagnosis, the lower prevalence of metastatic PPGL in our cohort is likely due in part to the lack of follow-up data in the present study. It may also partly derive from the lower sensitivity of MIBG scintigraphy for detecting metastatic lesions.

Among metabolic parameters, those associated with blood pressure and glucose intolerance were significantly worse in patients with catecholamine-producing PPGL. This is to be expected, considering the effects of catecholamines on blood pressure and glucose metabolism [24, 25]. A previous study showed the effects of catecholamines on body weight throughout a hypermetabolic and proinflammatory state [26]. Heavner et al. compared the clinical characteristics between patients with negative results for catecholamines and their metabolites and catecholamine-producing PPGLs [5]. They reported that BMIs were significantly higher in patients who were negative for catecholamines or their metabolites, though the prevalence of glucose intolerance did not significantly differ between the two groups. Our results are inconsistent with these results. This may reflect differences in the prevalence of obesity and glucose intolerance between Japan and the United States of America.

Limitations

There are several limitations to this study. The most important limitation is that genetic testing was not performed in our cohort. Second, the degree to which consanguinity was investigated was not uniform among the participating institutions. Third, no MIBG scintigraphy data were available in the PHEO-J registry. Fourth, according to the 4th edition of the WHO classification of endocrine tumors, all PPGLs may have metastatic potential. In the present study, however, a diagnosis of metastatic PPGL was based on radiological findings at the time of initial diagnosis [27, 28]. Fifth, this is a retrospective study. Sixth, we defined PPGL patients as negative for urinary metanephrines when both urinary metanephrine and normetanephrine did not exceed the upper reference limits. However, the definition of PPGL with negative results for metanephrines has not been unified in previous reports. Seventh, we did not have an adequate method for detecting dopamine-producing tumors. Although data on urinary dopamine were collected in the study, nearly all urinary dopamine is synthesized within the renal tubules from circulating DOPA through tubular DOPA-decarboxylase activity. Consequently, urinary dopamine is not an adequate indicator of dopamine-producing tumors. Eighth, the methods for measuring tumor size differed in the ACPA-J and PHEO-J studies. In the ACPA-J study, tumor size was determined by radiological imaging, whereas it was determined by measuring pathological specimens in the PHEO-J study. This made it impossible to include tumor size in the analysis. Finally, it is impossible to distinguish PPGL that did not produce catecholamines from tumors classified as PPGL with negative results for urinary metanephrines.

Conclusion

Our study revealed that PPGL patients without excessive catecholamines were more likely to have extra-adrenal lesions and metastatic disease. Because PPGL with negative results for urinary metanephrines was associated with negative MIBG scintigraphy findings, whole-body imaging other than MIBG scintigraphy is important for accurate localization PPGLs and for diagnosis of metastasis. In addition, these patients should be closely followed up and monitored for the emergence of metastatic lesions, even when there was no metastasis at the time of the first diagnosis.

Data availability

The data sets used or analyzed during the study are available from the corresponding author on reasonable request.

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Authors' contributions Akiyuki Kawashima analyzed the data and drafted the manuscript. Masakatsu Sone devised the concept and design of this study and revised the manuscript. Nobuya Inagaki, Mitsuhide Naruse, and Akiyo Tanabe contributed to the interpretation

of data and revised the manuscript. Kentaro Okamoto, Mika Tsuiki, Shoichiro Izawa, Michio Otsuki, Shintaro Okamura, Takamasa Ichijo, Takuyuki Katabami, Yoshiyu Takeda, Takanobu Yoshimoto contributed to the acquisition of data.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval All studies analyzed in the present study were in accordance with the Declaration of Helsinki and the guidelines for clinical studies published by the Ministry of Health and Labour, Japan and were approved by the Ethics Committee of the National Hospital Organization Kyoto Medical Center as the project-leading center and by the institutional ethics committees of the participating centers.

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