Clinical and Experimental Nephrology (2022) 26:788–796
https://doi.org/10.1007/s10157-022-02220-1

ORIGINAL ARTICLE

# Altered arginine vasopressin-cyclic AMP-aquaporin 2 pathway in patients with chronic kidney disease

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Received: 4 February 2022 / Accepted: 28 March 2022 / Published online: 3 May 2022


## Abstract

**Background** In the collecting ducts of the kidney, arginine vasopressin (AVP), cyclic adenosine monophosphate (cAMP), and aquaporin 2 (AQP2) play a pivotal role in maintaining fluid volume and serum osmolality in humans. However, their association among those with chronic kidney disease (CKD) remains uncertain.

**Methods** We prospectively included the out-patients with CKD and measured osmolality-related biomarkers including plasma AVP, urine cAMP, urine AQP2, and urine osmolality levels. Association among these parameters at each CKD stage was investigated.

**Results** A total of 121 patients were included (median age 71 years old [61–78], 89 men, estimated glomerular filtration ratio 28.6 [16.4–45.3] mL/min/1.73 $m^2$). Serum osmolality increased as CKD progression, accompanying incremental plasma AVP levels, whereas urine cAMP, urine AQP2, and urine osmolality decreased as CKD progression. At advanced CKD stage, urine cAMP remained low irrespective of the AVP stimulation, whereas urine cAMP levels varied according to the levels of plasma AVP at less advanced CKD stage. The associations between urine cAMP and urine AQP2 and between urine AQP2 and urine osmolality remained preserved irrespective of the CKD stages.

**Conclusions** Vasopressin type-2 receptor seems to be particularly impaired in patients with advanced CKD, whereas the signal cascade of the downstream of vasopressin type-2 receptor is relatively preserved. Urine cAMP might be a promising marker to estimate the residual function of the collecting duct.

**Keywords** Arginine vasopressin · cAMP · Aquaporin 2 · Chronic kidney disease

## Introduction

The capacity of the kidneys to concentrate and dilute urine is an important mechanism to maintain serum osmolality in human. In detail, the arginine vasopressin (AVP)-cyclic adenosine monophosphate (cAMP)-aquaporin 2 (AQP2) pathway plays a crucial role.

AVP is secreted from the pituitary gland when serum osmolality increases. AVP subsequently binds to the vasopressin type-2 receptor on the principal cells within the late distal tubule and collecting ducts, and the formation of cAMP is promoted after stimulation of adenylate cyclase. This initiates a cascade leading to an increase in cAMP levels and activation of protein kinase A-dependent phosphorylation of AQP2. This is the main pathway of AQP2 trafficking to the apical plasma membrane of the collecting duct principal cells, which increases the osmotic water permeability and facilitates free water reabsorption. As a result, urine osmolality is increased [1].

Urine concentrating/diluting ability is impaired in patients with chronic kidney disease (CKD), probably due to impairment in some parts of the above-described signal cascade [2]. In patients with CKD, plasma AVP is increased and urine AQP2 is decreased [3, 4]. However, detailed pathophysiological mechanism that links these findings remains uncertain.

We hypothesized that cAMP might have a key role to pathophysiologically explain these findings in the CKD cohort. Of note, these knowledge would be useful to consider response to tolvaptan, vasopressin type-2 receptor

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antagonist, which seems to require residual function in the AVP-cAMP-AQP2 pathway for the clinical effectiveness [5–7]. In this study, we investigated the association among these urine biomarkers in the CKD cohort.

## Materials and methods

### Patient selection

Patients who were followed at our out-patient clinic at clinically stable conditions to treat CKD between December 2015 and July 2020 were included in this prospective study. All patients had estimated glomerular filtration ratio (eGFR) < 60 mL/min/1.73 $m^2$. Patients dependent on hemodialysis or those receiving vasopressin type-2 receptor antagonist or antidepressants were excluded. We included also those with eGFR ≧ 60 mL/min/1.73 $m^2$ as a control group.

### Data collection

Blood and urine samples were obtained from all patients at fasting condition before taking any medications. Blood samples were centrifuged immediately for 20 min and stored at −80 °C before the assay. eGFR was calculated using the following formula: $194 \times (\text{serum creatinine [mg/dL]})^{-1.094} \times (\text{age [years]})^{-0.287}$ (×0.739 only for women) [8].

All urine samples were stored immediately at −80 °C until assay. Of note, urine osmolality, AQP2, and cAMP were measured. Urine and serum osmolality was measured by freezing-point depression. To assess the effective osmolality, serum osmolality was corrected for urea by subtracting the measured blood urea nitrogen from the measured serum osmolality [9]. Urine AQP2 was measured using a sandwich enzyme-linked immunosorbent assay (Otsuka Pharmaceutical Co., Ltd., Japan). Urine cAMP was measured by a radioimmunoassay in the LSI Medience Co. (Tokyo, Japan). Plasma AVP was measured using a radioimmunoassay (Yamasa Shoyu Co., Ltd., Japan).

### Statistical analyses

Categorical data were presented as numbers and percentages. Continuous data were presented as median and interquartile range. The interaction of variables associating with vasopressin type-2 receptor signal cascade, including plasma AVP, urine cAMP, urine AQP2, and urine osmolality, was investigated by Pearson's correlation coefficient. Linear regression analyses were performed to investigate clinical parameters that were associated with urine cAMP relative to plasma AVP levels. Five potential parameters including age, eGFR, serum calcium corrected with albumin, serum osmolality, and plasma parathyroid hormone were considered. Variables significant in the univariable analyses were included in the multivariable analysis. Two-tailed $p$ value < 0.05 was assumed as statistically significant. All statistics were performed using JMP pro ver14.2 (SAS Institute Inc).

## Results

### Baseline characteristics

A total of 121 CKD patients and 90 non-CKD patients were included (Table 1). In CKD patients, median age was 71 [61–78] years old and 89 were men. eGFR was 28.6 [16.4–45.3] mL/min/1.73 $m^2$ and plasma AVP was 2.2 [1.5–3.5] pg/mL. Urine cAMP was 1.4 [0.8–2.3] nmol/mL, urine AQP2 was 2.77 [0.98–4.35] ng/mL, and urine osmolality was 412 [329–496] mOsm/kg $H_2O$. Forty-six (38%) patients received loop diuretics.

### Stratification of baseline characteristics by CKD stage

Of them, there were 59 patients assigned to G3 (eGFR 30–59 mL/min/1.73 $m^2$), 36 assigned to G4 (eGFR 15–29 mL/min/1.73 $m^2$), and 26 assigned to G5 (eGFR < 15 mL/min/1.73 $m^2$) (Table 2). Patients with more progressed CKD had a higher prevalence of diabetes mellitus, more advanced anemia, and lower serum albumin ($p < 0.05$ for all). As CKD progressed, the prevalence of loop diuretics prescription increased.

### Serum osmolality at incremental deterioration of renal function

In CKD patients, serum osmolality increased at incremental CKD grades accompanying incremental trend in plasma AVP levels ($p < 0.005$ and $p = 0.13$, respectively; Fig. 1a, b). Serum sodium level remained unchanged irrespective of the eGFR levels (Fig. 1c), whereas blood urea nitrogen gradually increased at incremental deterioration of renal function (Fig. 1d). Serum osmolality corrected for urea remained unchanged irrespective of the eGFR levels (Fig. 1e).

### Plasma AVP and serum osmolality

In CKD patients, plasma AVP levels had collinearity with actual serum osmolality and those corrected for urea ($p < 0.005$ and $p = 0.010$, respectively; Fig. 2a, b), whereas there were no such correlations in non-CKD patients.

**Table 1** Baseline characteristics

| | CKD patients ($N=121$) | Non-CKD patients ($N=90$) | $p$ value |
|---|---|---|---|
| Demographics | | | |
| Age (years) | 71 [61–78] | 65 [49–71] | < 0.005* |
| Male sex | 89 (74) | 50 (56) | 0.0064* |
| Diabetes mellitus | 29 (24) | 20 (22) | 0.77 |
| Autosomal dominant polycystic kidney disease | 7 (6) | 0 (0) | 0.020* |
| Weight (kg) | 63.4 [55.6–71.6] | 63.6 [56.1–76.3] | 0.42 |
| Body mass index (kg/m$^2$) | 24.2 [21.7–27.1] | 25.2 [22.4–28.0] | 0.077 |
| Systolic blood pressure (mmHg) | 134 [124–143] | 133 [125–145] | 0.68 |
| Diastolic blood pressure (mmHg) | 73 [63–83] | 82 [72–89] | < 0.005* |
| Pulse rate (/min) | 71 [63–80] | 71 [65–80] | 0.51 |
| Laboratory data | | | |
| Hemoglobin (g/dL) | 11.4 [9.9–13.6] | 14.3 [13.1–15.2] | < 0.005* |
| Serum creatinine (mg/dL) | 1.72 [1.18–3.00] | 0.70 [0.60–0.83] | < 0.005* |
| eGFR (mL/min/1.73 m$^2$) | 28.6 [16.4–45.3] | 76.2 [69.3–86.1] | < 0.005* |
| Blood urea nitrogen (mg/dL) | 27 [20–44] | 14 [13–16] | < 0.005* |
| Serum albumin (g/dL) | 3.9 [3.3–4.1] | 4.2 [4.0–4.5] | < 0.005* |
| Serum sodium (mEq/L) | 139 [138–141] | 140 [139–141] | 0.19 |
| Serum potassium (mEq/L) | 4.4 [4.2–4.7] | 4.3 [4.0–4.5] | < 0.005* |
| Serum chloride (mEq/L) | 105 [103–107] | 103 [102–105] | < 0.005* |
| Serum calcium corrected for albumin (mg/dL) | 9.2 [8.9–9.4] | 9.2 [8.9–9.4] | 0.91 |
| Serum osmolality (mOsm/kg $H_2O$) | 295 [288–300] | 291 [288–293] | < 0.005* |
| Serum osmolality corrected for urea (mOsm/ kg $H_2O$) | 285 [283–288] | 286 [283–288] | 0.24 |
| Plasma arginine vasopressin (pg/mL) | 2.2 [1.5–3.5] | 2.2 [1.4–3.1] | 0.72 |
| Plasma parathyroid hormone, intact (pg/mL) | 64 [46–103] | Not applicable | |
| Urine data | | | |
| Urine cAMP (nmol/mL) | 1.4 [0.8–2.3] | 2.9 [1.8–3.8] | < 0.005* |
| Urine aquaporin 2 (ng/mL) | 2.77 [0.98–4.35] | 4.06 [1.74–8.19] | < 0.005* |
| Urine protein (g/g of Creatinine) | 0.71 [0.15–3.40] | 0.057 [0.035–0.10] | < 0.005* |
| Urine osmolality (mOsm/kg $H_2O$) | 412 [329–496] | 592 [441–717] | < 0.005* |
| Urine sodium (mEq/L) | 82 [64–116] | 128 [87–165] | < 0.005* |
| Urine potassium (mEq/L) | 25 [15–38] | 49 [36–61] | < 0.005* |
| Medications | | | |
| ACE-I or ARB | 73 (60) | 60 (67) | 0.35 |
| Calcium channel antagonists | 71 (59) | 52 (58) | 0.90 |
| β-Adrenergic blockers | 32 (26) | 13 (14) | 0.035* |
| α-Adrenergic blockers | 13 (11) | 7 (8) | 0.47 |
| Aldosterone receptor antagonists | 16 (13) | 9 (10) | 0.47 |
| Loop diuretics | 46 (38) | 0 (0) | < 0.005* |
| Thiazide diuretics | 23 (19) | 18 (20) | 0.86 |

Variables are expressed as the median [interquartile range] or number and percentage. Comparison in continuous variables were performed using Mann–Whitney's *U* test. Comparison in categorical variables were performed using chi-square test

*CKD* chronic kidney disease, *eGFR* estimated glomerular filtration rate, *cAMP* cyclic adenosine monophosphate, *ACE-I* angiotensin converting enzyme inhibitors, *ARB* angiotensin II receptor antagonists

*$p < 0.05$

**Table 2** Comparison in baseline characteristics

| | G3 ($N=59$) | G4 ($N=36$) | G5 ($N=26$) | $p$ value |
|---|---|---|---|---|
| Demographics | | | | |
| Age (years) | 70 [61–77] | 75 [69–80] | 69 [58–75] | 0.097 |
| Male sex | 40 (68) | 31 (86) | 18 (69) | 0.12 |
| Diabetes mellitus | 7 (12) | 9 (25) | 13 (50) | < 0.005* |
| Autosomal dominant polycystic kidney disease | 4 (7) | 3 (8) | 0 (0) | 0.34 |
| Weight (kg) | 63.2 [55.8–71.4] | 63.5 [55.3–71.0] | 63.5 [56.0–71.4] | 0.87 |
| Body mass index (kg/m$^2$) | 23.3 [21.9–26.0] | 24.2 [20.8–27.4] | 24.6 [23.4–28.4] | 0.35 |
| Systolic blood pressure (mmHg) | 136 [127–145] | 125 [115–139] | 136 [127–157] | < 0.005* |
| Diastolic blood pressure (mmHg) | 80 [69–89] | 68 [63–82] | 70 [62–76] | < 0.005* |
| Pulse rate (/min) | 71 [63–76] | 70 [63–82] | 75 [63–84] | 0.39 |
| Laboratory data | | | | |
| Hemoglobin (g/dL) | 13.4 [12.5–14.6] | 10.8 [10.1–11.5] | 9.5 [8.6–10.0] | < 0.005* |
| Serum creatinine (mg/dL) | 1.17 [0.98–1.37] | 2.28 [2.03–2.79] | 4.24 [3.85–5.36] | < 0.005* |
| eGFR (mL/min/1.73 m$^2$) | 47.9 [40.4–54.7] | 22.0 [18.5–25.1] | 10.6 [8.7–12.9] | < 0.005* |
| Blood urea nitrogen (mg/dL) | 20 [17–24] | 32 [26–43] | 58 [50–69] | < 0.005* |
| Serum albumin (g/dL) | 4.1 [3.9–4.4] | 3.6 [3.1–4.0] | 3.1 [2.7–3.5] | < 0.005* |
| Serum sodium (mEq/L) | 139 [138–141] | 139 [138–140] | 140 [138–142] | 0.56 |
| Serum potassium (mEq/L) | 4.4 [4.2–4.7] | 4.4 [4.1–4.9] | 4.6 [4.1–5.2] | 0.78 |
| Serum chloride (mEq/L) | 105 [103–106] | 105 [102–108] | 108 [104–110] | 0.016* |
| Serum calcium corrected for albumin (mg/dL) | 9.2 [9.0–9.4] | 9.3 [9.0–9.6] | 9.0 [8.5–9.4] | 0.060 |
| Serum osmolality (mOsm/kg $H_2O$) | 292 [290–294] | 297 [295–300] | 306 [301–310] | < 0.005* |
| Serum osmolality corrected for urea (mOsm/ kg $H_2O$) | 284 [282–287] | 285 [283–287] | 286 [282–289] | 0.50 |
| Plasma arginine vasopressin (pg/mL) | 2.0 [1.5–3.3] | 2.3 [1.4–3.8] | 2.4 [1.8–3.8] | 0.31 |
| Plasma parathyroid hormone, intact (pg/mL) | 51 [39–66] | 74 [54–114] | 215 [121–335] | < 0.005* |
| Medications | | | | |
| ACE-I or ARB | 38 (64) | 20 (56) | 15 (58) | 0.66 |
| Calcium channel antagonists | 22 (37) | 28 (78) | 23 (41) | < 0.005* |
| β-Adrenergic blockers | 13 (5) | 13 (36) | 8 (31) | 0.32 |
| α-Adrenergic blockers | 2 (3) | 5 (14) | 7 (27) | < 0.005* |
| Aldosterone receptor antagonists | 8 (14) | 7 (19) | 1 (4) | 0.20 |
| Loop diuretics | 4 (7) | 20 (56) | 22 (87) | < 0.005* |
| Thiazide diuretics | 16 (27) | 3 (8) | 4 (15) | 0.067 |

Variables are expressed as the median [interquartile range] or number and percentage. Comparison in continuous variables among the three groups were performed using Kruskal–Wallis test. Comparison in categorical variables among the three groups were performed using chi-square for independence test

*eGFR* estimated glomerular filtration rate, *ACE-I* angiotensin converting enzyme inhibitors, *ARB* angiotensin II receptor antagonists

*$p < 0.05$

## Urine parameters at incremental deterioration of renal function

Despite incremental trend in plasma AVP stimulation as progression of CKD stage, urine cAMP, urine AQP2, and urine osmolality rather decreased at incremental CKD stages ($p < 0.05$ for all; Table 3). These trends remained when renal function was expressed as continuous data, i.e., eGFR (Fig. 3a–c).

## Association among urine parameters

Urine cAMP levels relative to plasma AVP stimulation decreased at incremental deterioration of renal function ($p < 0.005$, $r = 0.44$; Fig. 4a). On the contrary, urine AQP2 levels relative to cAMP stimulaton remained preserved irrespective of the renal function ($p = 0.032$; $r = -0.20$; Fig. 4b). As a result, urine AQP2 levels relative to plasma AVP stimulation decreased at incremental deterioration of renal function ($p < 0.005$, $r = 0.37$; Fig. 4c).

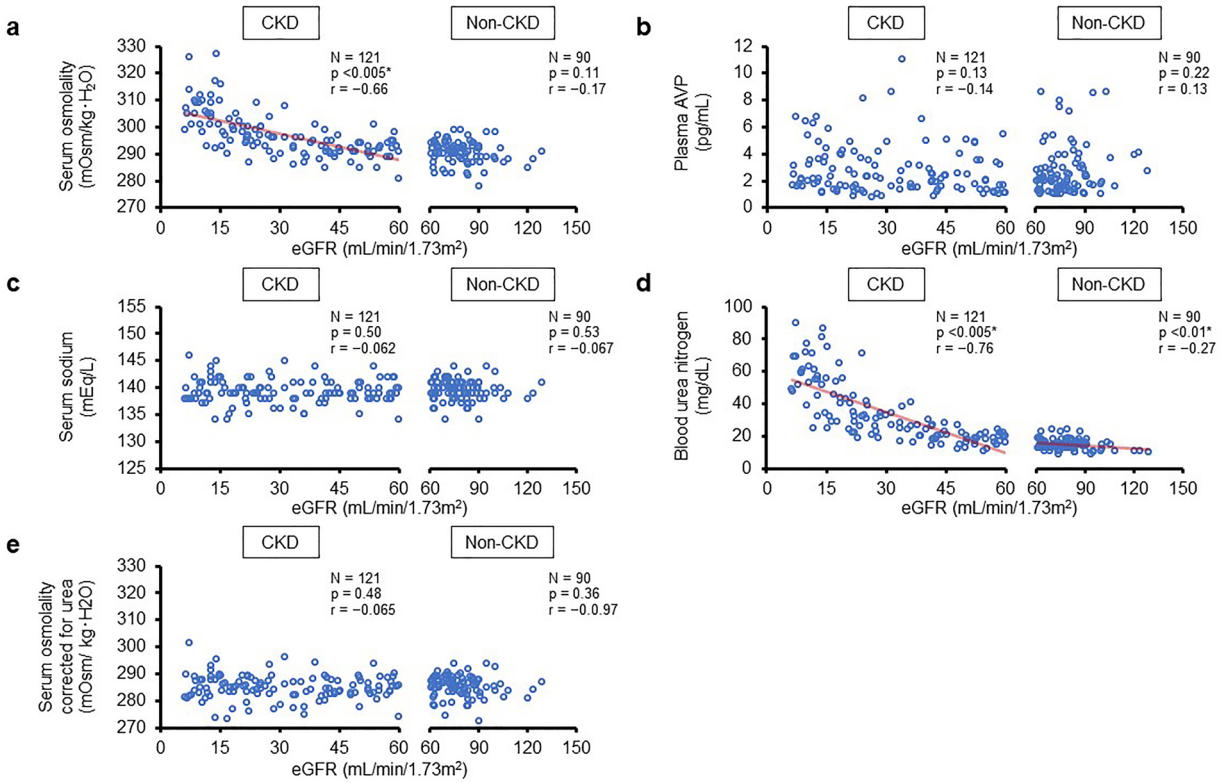


**Fig. 1** Correlation between eGFR and serum osmolality (**a**), plasma AVP (**b**), serum sodium (**c**), blood urea nitrogen (**d**) and serum osmolality corrected for urea (**e**). *$p < 0.05$ by Pearson's correlation coefficient. CKD, chronic kidney disease; eGFR, estimated glomerular filtration ratio; AVP, arginine vasopressin

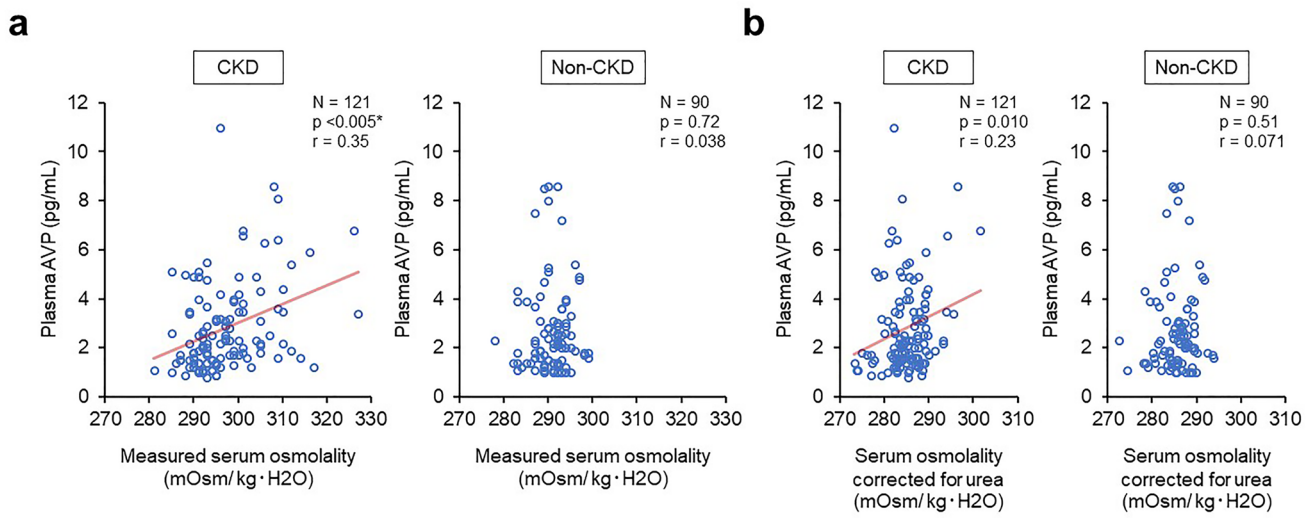


**Fig. 2** Correlation between measured serum osmolality and plasma AVP (**a**) and between serum osmolality corrected for urea and plasma AVP (**b**). *$p < 0.05$ by Pearson's correlation coefficient. CKD, chronic kidney disease; AVP, arginine vasopressin

There was no significant correlation between plasma AVP and urine cAMP irrespective of the CKD stages (Fig. 5a). Of note, urine cAMP levels remained low at any plasma AVP levels in stage G5, whereas urine cAMP showed a variety of levels at each plasma AVP level in stage G3–4. The correlation between urine cAMP and urine AQP2 and between urine AQP2 and urine osmolality remained preserved in all CKD stages including stage 5 (Fig. 5b, c).

**Table 3** Comparison in urine data

| | G3 ($N=59$) | G4 ($N=36$) | G5 ($N=26$) | $p$ value |
|---|---|---|---|---|
| Urine cAMP (nmol/mL) | 2.1 [1.3–2.7] | 1.2 [0.8–1.5] | 0.7 [0.4–1.1] | < 0.005* |
| Urine aquaporin 2 (ng/mL) | 3.14 [1.63–4.91] | 2.30 [0.51–4.51] | 1.15 [0.61–3.36] | 0.017* |
| Urine protein (g/g of Creatinine) | 0.16 [0.07–0.48] | 1.10 [0.18–4.31] | 3.51 [1.96–5.36] | 0.16 |
| Urine osmolality (mOsm/kg $H_2O$) | 481 [386–631] | 362 [290–416] | 304 [237–350] | < 0.005* |
| Urine sodium (mEq/L) | 102 [74–143] | 74 [55–96] | 70 [58–86] | < 0.005* |
| Urine potassium (mEq/L) | 38 [22–49] | 20 [16–31] | 13 [9–17] | < 0.005* |

Variables are expressed as the median [interquartile range]. Comparison in continuous variables among the three groups were performed using Kruskal–Wallis test

*cAMP* cyclic adenosine monophosphate

*$p < 0.05$

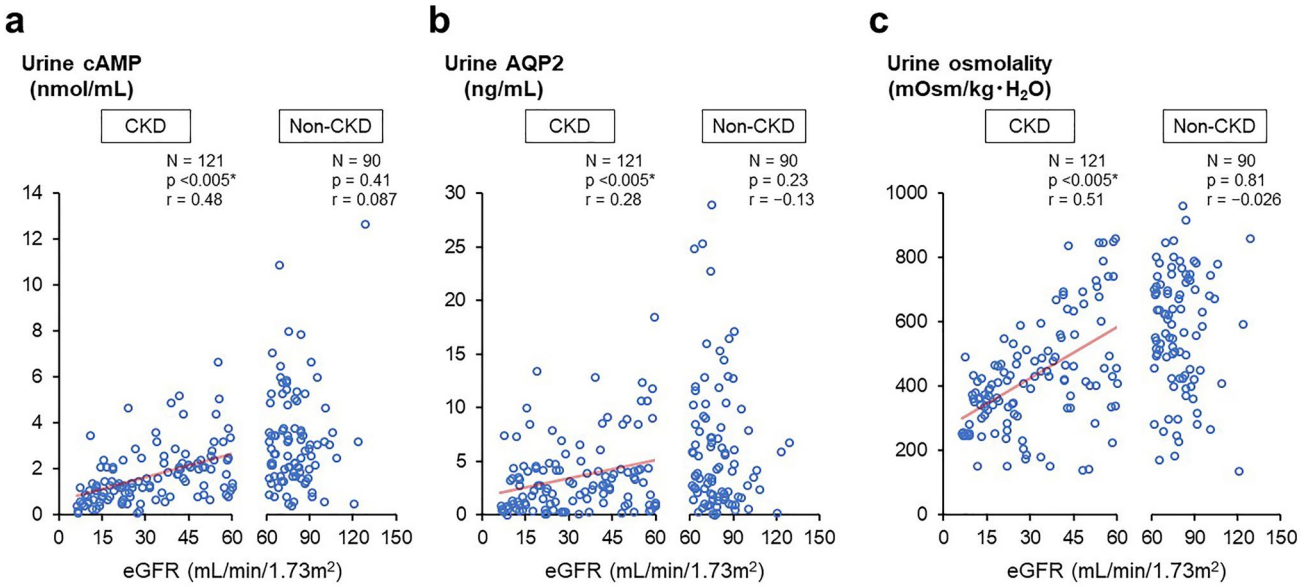


**Fig. 3** Correlation between eGFR and urine cAMP (**a**), urine AQP2 (**b**), and urine osmolality (**c**). *$p < 0.05$ by Pearson's correlation coefficient. eGFR, estimated glomerular filtration ratio; cAMP, cyclic adenosine monophosphate; AQP2, aquaporin 2

### Factors related to urine cAMP levels to plasma AVP stimulation

According to the findings of univariable and multivariable analyses, only eGFR was independently associated with the levels of urine cAMP relative to plasma AVP among five potential clinical parameters (adjusted $R^2$ 0.22, $p < 0.005$; Table 4).

## Discussion

We investigated the association of urine biomarkers at each CKD stage. (1) Serum osmolality increased as the progression of CKD, dominantly due to incremental blood urea nitrogen; (2) Despite AVP stimulation, urine cAMP, urine AQP2, and urine osmolality levels decreased as progression of CKD; (3) Urine cAMP showed a variety of levels at each plasma AVP levels at less progressed CKD stage, whereas urine cAMP levels were low irrespective of the plasma AVP levels at progressed CKD stage; (4) The downstream of cAMP (i.e., urine AQP2 relative to urine cAMP level and urine osmolality relative to urine AQP2 level) were relatively preserved irrespective of the progression of CKD.

### Regulation of serum osmolality in patients with CKD

AVP secretion is regulated dominantly by the two major pathways: non-osmotic pathway and osmotic pathway,

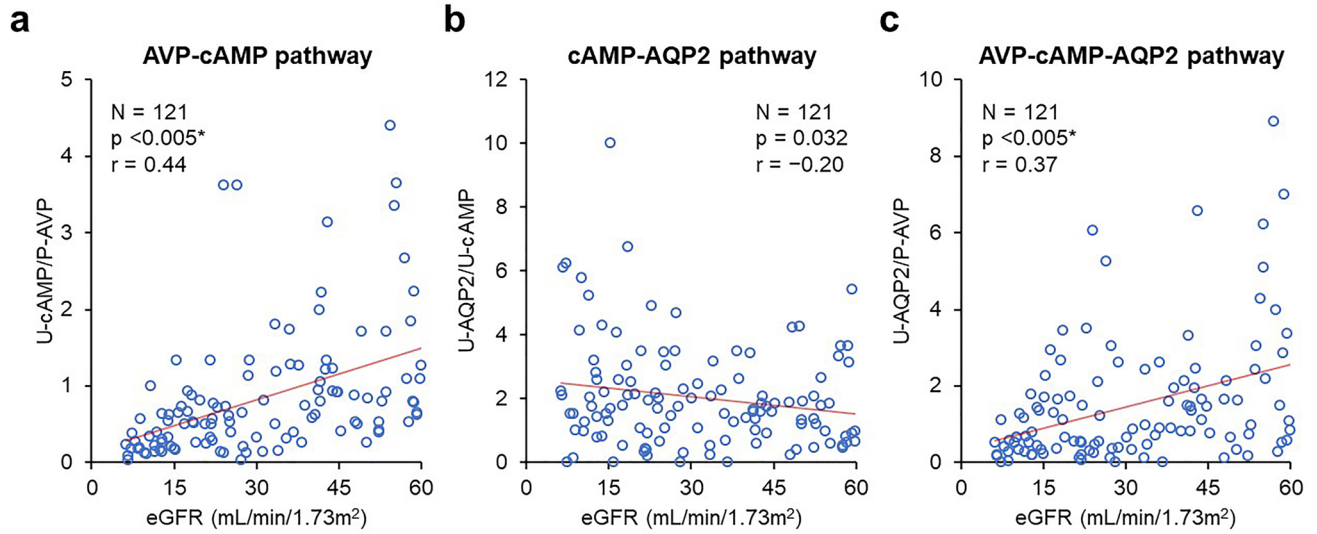


**Fig. 4** Correlation between eGFR and cAMP/AVP ratio (**a**), AQP2/cAMP ratio (**b**), and AQP2/AVP ratio (**c**). $*p < 0.05$ by Pearson's correlation coefficient. eGFR, estimated glomerular filtration ratio; U-cAMP, urine cyclic adenosine monophosphate; P-AVP, plasma arginine vasopressin; U-AQP2, urine aquaporin 2

**Table 4** Regression analysis for urine cAMP levels relative to plasma AVP stimulation in CKD patients ($N = 121$)

| Explanatory variables | Estimated regression coefficient | Standard error | *t* value | *p* value |
|---|---|---|---|---|
| *Univariable analysis* | | | | |
| Age (years) | − 0.20 | 0.0060 | − 2.2 | 0.030* |
| eGFR (mL/min/1.73 $m^2$) | 0.44 | 0.0043 | 5.2 | < 0.005* |
| Serum calcium corrected for albumin (mg/dL) | − 0.0036 | 0.15 | − 0.039 | 0.97 |
| Serum osmolality (mOsm/kg $H_2O$) | − 0.35 | 0.0091 | − 4.0 | < 0.005* |
| Serum osmolality corrected for urea (mOsm/ kg $H_2O$) | − 0.064 | 0.017 | − 0.70 | 0.48 |
| Plasma parathyroid hormone, intact (pg/mL) | −0.27 | 0.0010 | − 2.9 | < 0.005* |
| *Multivariable analysis* | | | | |
| Age (years) | − 0.14 | 0.0058 | − 1.6 | 0.11 |
| eGFR (mL/min/1.73 $m^2$) | 0.35 | 0.0062 | 2.8 | 0.0056* |
| Serum osmolality (mOsm/kg $H_2O$) | − 0.12 | 0.012 | − 0.98 | 0.33 |
| Plasma parathyroid hormone, intact (pg/mL) | 0.00 | 0.0011 | 0.016 | 0.99 |

*cAMP* cyclic adenosine monophosphate, *AVP* arginine vasopressin, *CKD* chronic kidney disease, *eGFR* estimated glomerular filtration rate
$*p < 0.05$

depending on a variety of clinical scenario. In patients with heart failure, serum osmolality is dominantly regulated by the non-osmotic pathway. A reduced systemic circulation due to low cardiac output stimulates the secretion of AVP and facilitates reabsorption of free water, resulting in hypervolemic dilutional hyponatremia [10, 11]. In patients with liver cirrhosis and pregnant women, in the same manner, systemic arterial vasodilation and arterial underfilling stimulate AVP secretion via non-osmotic pathway [12].

Few studies investigated the relationship between plasma AVP levels and serum osmolality in patients with renal impairment. Hemodialysis patients had high plasma AVP levels, but its regulation remains uncertain [13, 14]. Given our findings, AVP seems to be regulated dominantly by serum osmolality levels (i.e., osmotic pathway). A major determinant of the serum osmolality seems to be blood urea nitrogen, instead of serum sodium level. Patients with more progressed CKD in general have higher blood urea nitrogen levels. As a result, serum osmolality was higher at incremental progression of CKD.

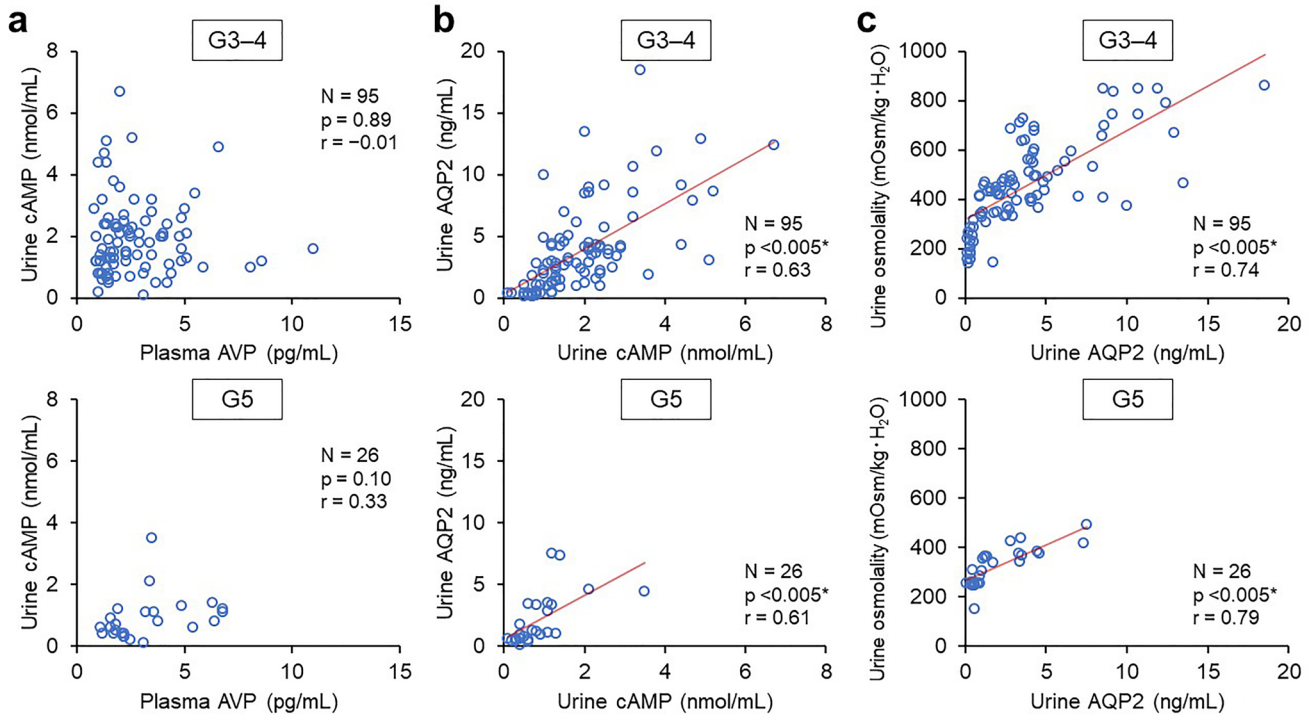


**Fig. 5** Correlation between plasma AVP and urine cAMP (**a**), cAMP and urine AQP2 (**b**), and urine AQP2 and urine osmolality (**c**) stratified by the CKD stages (G3–4 and G5) $*p < 0.05$ by Pearson's correlation coefficient. AVP, arginine vasopressin; cAMP, cyclic adenosine monophosphate; AQP2, aquaporin 2

## Reaction of kidney to the AVP stimulation

At the fasting condition in the early morning, physiological volume depletion increases serum osmolality, which triggers secretion of AVP as discussed above. However, the collecting duct in patients with advanced CKD cannot respond to the stimulation of AVP. Given our findings, a dominant cause of refractoriness to AVP would be vasopressin type-2 receptor. Vasopressin type-2 receptor seems to be refractory to AVP stimulation and cannot increase cAMP synthesis in patients with advanced CKD. On the contrary, the downstream pathway, i.e., cAMP-AQP2 pathway seems to be relatively preserved irrespective of the CKD stages.

In the advanced CKD patients, the administration of AVP could not increase urine osmolality, indicating refractoriness of kidney to AVP stimulation [15]. In another animal experiment, cAMP did not increase against AVP stimulation in the principle cells incubated from 5/6 nephrectomy renal failure model. Of note, mRNA of the vasopressin type-2 receptor was downregulated [16]. Abnormal response of adenyl cyclase and impairment in AVP-independent pathway might also be involved [17, 18]. Further studies are warranted to clarify the detailed mechanism why vasopressin type-2 receptor is relatively vulnerable to the progression of CKD compared to the other downstream pathway.

## Clinical implications

Given our findings, the residual function of collecting duct would not necessarily worsen in parallel to the renal function (i.e., glomerular filtration ratio). In some patients, the function of collecting duct seems to be relatively preserved despite progressed CKD. Another unique marker, independent on glomerular filtration ratio, would be required to assess the residual function of collecting duct.

However, in the real-world practice, there are scarcity of index to assess the function of collecting duct thus far. Water restriction test and water intake test are applied to assess urine concentration and urine dilution ability, respectively [19]. However, these tests are at risk of worsening renal function and/or volume overflow in patients with CKD. The interpretation of test results is sometimes challenging in patients receiving diuretics. According to our findings, urine cAMP and urine AQP2 might be promising tools to assess the residual function of collecting duct independent on the glomerular filtration ratio, particularly among those with CKD.

Detailed assessment of residual function of collecting duct is, for example, quite useful to predict response to tolvaptan. Pre-treatment prediction of response to tolvaptan would be of great importance particularly for clinically unstable patients, in whom delayed clinical decision leads to fatal. Clinical utility of urine AQP2 to predict responders to tolvaptan is

reported previously in patients with various clinical situations including heart failure and liver cirrhosis [6, 20]. However, urine AQP2 cannot be measured in the medical insurance. Urine cAMP might be more practical, given that it can be measured in insurance to differentiate the etiologies of calcium level abnormality. Given our findings that most of the patients with CKD stage 3–4 had a variety of urine cAMP levels per AVP stimulation, at least some of them seem to have relatively preserved reactivity to vasopressin type-2 receptor. Urine cAMP measurement would be useful to predict response to tolvaptan. Most of the patients with CKD stage G5 seem to have impaired reactivity of vasopressin type-2 receptor, indicating non-response to tolvaptan.

## Limitations

We included a moderate size cohort. We measured all data just one time point. Response to AVP might change during long-term observational period. Also, the urine cAMP level might have prognostic impacts. We are now conducting another study investigating the impact of urine cAMP level on future CKD progression and response to tolvaptan. This is just an observational study, and we cannot conclude any causalities from our findings. Copeptin is recently receiving great concern as a surrogate of AVP given that it is relatively easy to measure, but we did not measure plasma copeptin level [21].

## Declarations

**Conflict of interest** All the authors have declared no competing interest.

**Ethical approval** This study was approved by our institutional review board (IRB approval number 27-162) and carried out following the declaration of Helsinki.

**Informed consent** Written informed consent was obtained from all patients before the inclusion in this study.

## References


1. Noda Y, Sasaki S. Updates and perspectives on aquaporin-2 and water balance disorders. Int J Mol Sci. 2021;22:12950.
2. Bricker NS, Dewey RR, Lubowitz H, Stokes J, Kirkensgaard T. Observations on the concentrating and diluting mechanisms of the diseased kidney. J Clin Investig. 1959;38:516–23.
3. Ettema EM, Heida J, Casteleijn NF, Boesten L, Westerhuis R, Gaillard C, et al. The effect of renal function and hemodialysis treatment on plasma vasopressin and copeptin levels. Kidney Int Rep. 2017;2:410–9.
4. Nielsen S, Kwon TH, Christensen BM, Promeneur D, Frøkiaer J, Marples D. Physiology and pathophysiology of renal aquaporins. J Am Soc Nephrol. 1999;10:647–63.
5. Imamura T, Kinugawa K, Shiga T, Kato N, Muraoka H, Minatsuki S, et al. Novel criteria of urine osmolality effectively predict response to tolvaptan in decompensated heart failure patients—association between non-responders and chronic kidney disease. Circ J. 2013;77:397–404.
6. Imamura T, Kinugawa K, Fujino T, Inaba T, Maki H, Hatano M, et al. Increased urine aquaporin-2 relative to plasma arginine vasopressin is a novel marker of response to tolvaptan in patients with decompensated heart failure. Circ J. 2014;78:2240–9.
7. Hanatani A, Shibata A, Kitada R, Iwata S, Matsumura Y, Doi A, et al. Administration of tolvaptan with reduction of loop diuretics ameliorates congestion with improving renal dysfunction in patients with congestive heart failure and renal dysfunction. Heart Vessels. 2017;32:287–94.
8. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53:982–92.
9. Argent NB, Burrell LM, Goodship TH, Wilkinson R, Baylis PH. Osmoregulation of thirst and vasopressin release in severe chronic renal failure. Kidney Int. 1991;39:295–300.
10. Xu DL, Martin PY, Ohara M, St John J, Pattison T, Meng X, et al. Upregulation of aquaporin-2 water channel expression in chronic heart failure rat. J Clin Investig. 1997;99:1500–5.
11. Nielsen S, Terris J, Andersen D, Ecelbarger C, Frokiaer J, Jonassen T, et al. Congestive heart failure in rats is associated with increased expression and targeting of aquaporin-2 water channel in collecting duct. Proc Natl Acad Sci USA. 1997;94:5450–5.
12. Schrier RW. Vasopressin and aquaporin 2 in clinical disorders of water homeostasis. Semin Nephrol. 2008;28:289–96.
13. Shimamoto K, Watarai I, Miyahara M. A study of plasma vasopressin in patients undergoing chronic hemodialysis. J Clin Endocrinol Metab. 1977;45:714–20.
14. Horký K, Srámková J, Lachmanová J, Tomásek R, Dvoráková J. Plasma concentration of antidiuretic hormone in patients with chronic renal insufficiency on maintenance dialysis. Horm Metab Res. 1979;11:241–6.
15. Tannen RL, Regal EM, Dunn MJ, Schrier RW. Vasopressin-resistant hyposthenuria in advanced chronic renal disease. N Engl J Med. 1969;280:1135–41.
16. Teitelbaum I, McGuinness S. Vasopressin resistance in chronic renal failure evidence for the role of decreased V2 receptor mRNA. J Clini Investig. 1995;96:378–85.
17. Rieg T, Tang T, Murray F, Schroth J, Insel PA, Fenton RA, et al. Adenylate cyclase 6 determines cAMP formation and aquaporin-2 phosphorylation and trafficking in inner medulla. J Am Soc Nephrol. 2010;21:2059–68.
18. Wilke C, Sheriff S, Soleimani M, Amlal H. Vasopressin-independent regulation of collecting duct aquaporin-2 in food deprivation. Kidney Int. 2005;67:201–16.
19. Pedersen EB, Thomsen IM, Lauridsen TG. Abnormal function of the vasopressin-cyclic-AMP-aquaporin2 axis during urine concentrating and diluting in patients with reduced renal function. A Case Control Stud BMC Nephrol. 2010;11:26.
20. Nakanishi H, Kurosaki M, Hosokawa T, Takahashi Y, Itakura J, Suzuki S, et al. Urinary excretion of the water channel aquaporin 2 correlated with the pharmacological effect of tolvaptan in cirrhotic patients with ascites. J Gastroenterol. 2016;51:620–7.
21. Roussel R, Fezeu L, Marre M, Velho G, Fumeron F, Jungers P, et al. Comparison between copeptin and vasopressin in a population from the community and in people with chronic kidney disease. J Clin Endocrinol Metab. 2014;99:4656–63.