学　位　論　文

Altered arginine vasopressin-cyclic AMP-aquaporin 2 pathway and prognostic impact of urine cyclic AMP levels in patients with chronic kidney disease

（慢性腎臓病患者における
バソプレシン-cyclic AMP-アクアポリン 2 経路の変化と
尿中 cyclic AMP 濃度の予後への影響）

氏　名　　掛 下　幸 太

Abstract

Background:

In the renal collecting ducts, 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. Furthermore, prognostic implication of urine cAMP levels in patients with CKD remains unknown.

Methods:

I 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. In addition, the impact of urine cAMP levels on the composite of dialysis administration, cardiovascular death, and doubling of serum creatinine concentration was investigated.

Results:

A total of 121 patients were included (median age 71 [61–78] years old, 89 men, estimated glomerular filtration rate 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. In this cohort, a urine cAMP level was an independent predictor of the primary endpoint with a hazard ratio of 0.41 (95% confidence interval 0.18–0.91, p = 0.029) adjusted for 5 potential confounders with a cutoff of 1.55 nmol/mg of creatinine.

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. A lower urine cAMP is an independent predictor of renal deterioration in patients with CKD.

Introduction

The capacity of the kidneys to concentrate and dilute urine is an important mechanism to maintain serum osmolality in human. For this purpose, the arginine vasopressin (AVP) - cyclic adenosine monophosphate (cAMP) - aquaporin 2 (AQP2) pathway plays a crucial role. In general, a slight increase in serum osmolality triggers AVP secretion from the pituitary gland. AVP subsequently binds to the vasopressin type-2 receptor located on the renal collecting duct, 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. Activated AQP2 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.

I hypothesized that cAMP might have a key role to pathophysiologically explain these findings in the CKD cohort. In addition, the clinical implication of cAMP levels in CKD has rarely been investigated [5]. Lower urine osmolality was an independent risk factor for the progression of CKD in several studies [6, 7]. Given that cAMP is located on the more upstream compared with other biomarkers including urine osmolality, I hypothesized that urine cAMP might be a more specific marker of the residual function of the collecting duct and a novel predictor of deterioration of whole kidney function. In this study, I investigated the association among AVP-cAMP-AQP2 pathway parameters, and prognostic impact of urine cyclic AMP levels 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 rate (eGFR) <60 mL/min/1.73 m^2 and satisfied the definition of CKD. Patients dependent on hemodialysis or those receiving vasopressin type-2 receptor antagonists or antidepressants were excluded. I included also those with eGFR ≧60 mL/min/1.73 m^2 as a control group. In the prognostic study, I also excluded patients receiving immunosuppressive therapy including corticosteroids or those with polycystic kidney disease.

Clinical management:

Patients received guideline-directed medical therapy, including blood pressure control with maximal tolerated dose of angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker as possible, dietary therapy with salt and protein restriction, and treatment of anemia in patients with renal anemia, as appropriate.

Study protocol:

Day 0 was defined as the day when the below-described blood and urine samples were obtained. Patients were followed until January 2022 from day 0 unless being expired or transferred to other institutes.

Data Collection:

On day 0, baseline characteristics, including demographics, results of laboratory investigations, and medication data were obtained.

Blood and urine samples were obtained from all patients in fasting condition before taking

any medications. Blood samples were centrifuged immediately for 20 minutes and stored at minus 80 °C before the assay. eGFR was calculated using the following formula: 194 × (serum creatinine [mg/dL]) - 1.094 × (age [years]) - 0.287 (× 0.739 only for women) [8]. All urine samples were stored immediately at minus 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).

Primary and secondary outcomes:

The independent variable was defined as urine cAMP at baseline. The primary outcome was a composite of end-stage renal disease (ESRD) requiring dialysis therapy, cardiovascular death, and doubling of serum creatinine concentration. The secondary outcome was an eGFR slope.

Statistical Analyses:

Continuous variables were stated as median and interquartile and compared between the groups using Mann-Whitney U test. Categorical variables were stated as number and percentage and compared between the groups using Fischer's exact test.

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.

Cox proportional hazard ratio regression analysis was performed to investigate the impact of urine cAMP on the primary outcome. Its impact was adjusted for 5 clinically potential confounders: age, gender, diabetes mellitus, use of renin-angiotensin system inhibitors, and eGFR, considering their prognostic impact upon renal function. By using receiver operating characteristics analysis, a cutoff of urine cAMP to predict the primary outcome was investigated. The cohort was stratified into two groups using the cutoff.

All statistics were performed using JMP Pro (Ver 16.2.0; SAS Institute Inc., Cary, USA) and ESR (Ver 1.55; Jichi Medical University Saitama Medical Center, Saitama, Japan). Statistical significance was defined as two-tailed $p < 0.05$.

Results

Altered AVP-cAMP-AQP2 pathway in patients with CKD

Baseline characteristics in the total cohort:

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･H2O. 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; Figure 1ab). Serum sodium level remained unchanged irrespective of the eGFR levels (Figure 1c), whereas blood urea nitrogen gradually increased at incremental deterioration of renal function (Figure 1d). Serum osmolality corrected for urea remained unchanged irrespective of the eGFR levels (Figure 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; Figure 2ab), whereas there were no such correlations in non-CKD patients.

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 (Figure 3a–c).

Association among urine parameters:

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

There was no significant correlation between plasma AVP and urine cAMP irrespective of the CKD stages (Figure 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 (Figure 5bc).

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 5 potential clinical parameters (adjusted R-squared 0.22, $p < 0.005$; Table 4).

Prognostic impact of urine cAMP levels in patients with CKD

Baseline characteristics in the prognostic study:

A total of 106 patients were included (Table 5). Median age was 72 [64–78] years old and 80 were men. Median eGFR was 28.4 [16.5–45.2] mL/min/1.73 m^2. Of them, 51 patients were assigned to G3 (eGFR 30–59 mL/min/1.73 m^2), 32 were assigned to G4 (eGFR 15–29 mL/min/1.73 m^2), and 23 were assigned to G5 (eGFR <15 mL/min/1.73 m^2). Fifty-seven (54%) patients received diuretics. Urine cAMP distributed widely between 0.35 and 4.08 nmol/mg of creatinine with a median value of 1.99 nmol/mg of creatinine (Supplementary Figure 1).

Association between urine cAMP level and other clinical variables:

Of all, 32 out of 106 total cohort had a lower urine cAMP <1.55 nmol/mg of creatinine, which was statistically calculated as below. A lower urine cAMP was associated with a higher prevalence of diabetes mellitus and more impaired renal function (Table 5). Urine cAMP had a moderate collinearity with eGFR ($r = 0.66$, $p < 0.005$; Figure 6). Of note, all patients with CKD stage G3 (eGFR >30 mL/min/1.73 m^2) had higher cAMP >1.55 nmol/mg of creatinine, whereas those with CKD stage G4 or G5 had a variety of cAMP levels (some patients had preserved cAMP levels and others had lower cAMP levels in this cohort).

Impact of urine cAMP on the primary outcome:

During an observational period for a median 2.8 [0.7–5.0] years, 40 patients encountered the primary outcome (22 patients with ESRD requiring dialysis, 1 patient with cardiovascular death due to acute aortic dissection, and 17 patients with doubling creatinine).

The level of urine cAMP had a significant prognostic impact on the primary outcome with an unadjusted hazard ratio of 0.14 (95% confidence interval 0.08–0.25, $p < 0.001$; model 1), an adjusted hazard ratio of 0.13 (95% confidence interval 0.07–0.23, $p < 0.001$) using age and gender (model 2), and an adjusted hazard ratio of 0.41 (95% confidence interval 0.18–0.91, $p = 0.029$) using 5 clinically important variables (model 3), including eGFR (Table 6), with a cutoff of 1.55 nmol/mg of creatinine (sensitivity 0.68, specificity 0.92, and area under the curve 0.84; Figure 7). C-statistics of model 2 and model 3 were 0.84 (95% confidence interval 0.76–0.92, $p < 0.001$) and 0.91 (95% confidence interval 0.85–0.96, $p < 0.001$), respectively. A low urine cAMP, which was defined below the cutoff, was associated with a higher cumulative incidence of the primary outcomes (100% versus 23%, $p < 0.005$; Figure 8). Proportional hazard assumption was confirmed by log-log plot.

Primary Outcome in CKD stage G4–5 patients

As a sub-group analysis, we performed a similar investigation for those with CKD stage G4–5 (n = 55). A similar cutoff of urine cAMP 1.55 nmol/mg of creatinine significantly stratified the cumulative incidence of the primary outcomes also among this sub-group with more progressed CKD stages ($p < 0.005$; Supplementary Figure 2).

Impact of urine cAMP on the secondary outcomes

An eGFR slope was calculated from 89 patients who were followed for over 6 months. A lower urine cAMP was associated with steeper eGFR slope (i.e., a more rapid decrease in eGFR) (Figure 9). Representative cases of lower urine cAMP and higher urine cAMP, showing trends in eGFR, are displayed in Supplementary Figure 3.

Discussion

I 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.

In addition, I investigated the prognostic impact of urine cAMP on the composite endpoint consisting of ESRD requiring dialysis, cardiovascular death, and a persistent doubling of serum

creatinine concentration among those with CKD. (5) Urine cAMP distributed widely; (6) Urine cAMP was an independent predictor of the primary outcome. Of note, its impact was independent of eGFR levels; (7) A lower urine cAMP was associated with more rapid 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. 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 AVP secretion and facilitates reabsorption of free water, resulting in hypervolemic dilutional hyponatremia [10, 11]. 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 [12, 13]. Given my 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 have higher blood urea nitrogen levels. As a result, serum osmolality was higher at incremental progression of CKD.

Reaction of kidney to the AVP stimulation:

The collecting duct in patients with advanced CKD cannot respond to the stimulation of AVP. Given my 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 [14]. In another animal experiment, cAMP did not increase against AVP stimulation in the principal cells incubated from 5/6 nephrectomy renal failure model. Of note, mRNA of the vasopressin type-2 receptor was downregulated [15]. Abnormal response of adenyl cyclase and impairment in AVP-independent pathway might also be involved [16, 17]. 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 my findings, the residual function of collecting duct would not necessarily worsen in parallel to the renal function (i.e., glomerular filtration rate). In some patients, the function of collecting duct seems to be relatively preserved despite progressed CKD. Another unique marker, independent on glomerular filtration rate, 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 [18]. 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 my findings, urine cAMP and urine AQP2 might be promising tools to assess the residual function of collecting duct independent on the glomerular filtration rate, particularly among those with CKD.

Urine cAMP in patients with CKD:

Urine cAMP was high in most of the patients with CKD stage G3, indicating a relatively preserved function of the collecting duct despite mildly impaired renal function. On the contrary,

among those with CKD stage G4 and 5, the range of urine cAMP levels was wide. Some patients had relatively preserved collecting ducts and others had impaired collecting ducts as advanced renal impairment.

The collecting duct requires less oxygenation and is relatively tolerant to ischemic stress compared with the proximal tubule [19]. On the other hand, given its anatomical feature, the collecting duct is vulnerable to damage via urinary tract infection and obstruction [20–22]. Deterioration of the collecting duct would result in end-stage renal disease involving the whole kidney.

Other markers including urine AQP2 and urine osmolality might also indicate collecting duct function. However, pathways between cAMP and AQP2 and pathway between AQP2 and urine osmolality were both well preserved despite the deterioration of the pathway between AVP and cAMP. I believe that urine cAMP would be the most sensitive marker to estimate collecting duct function.

Prognostic implication of urine cAMP

Given the above discussion, it would be plausible that cAMP is independently associated with the progression of renal disease. In the previous study involving heart failure cohort, which utilized AQP2 instead of cAMP to estimate collecting duct function, patients with impaired collecting duct accompanying lower urine AQP2 had higher mortality and heart failure readmissions compared with those with preserved collecting duct [23].

In patients with low urine cAMP despite relatively preserved eGFR, progression of collecting duct impairment due to urinary abnormality might further impair the whole renal function. In patients with preserved urine cAMP despite decreased eGFR, the collecting duct would be relatively preserved against ischemic stress. Preserved function to concentrate/dilute urine would attribute to the homeostasis of body water adjustment, delaying the progression of CKD and preventing cardiovascular death.

Further clinical implications of urine cAMP level:

Detailed assessment of residual function of collecting duct is quite useful to predict response to the vasopressin type-2 receptor antagonist tolvaptan. Pre-treatment prediction of response to tolvaptan would be of importance particularly for clinically unstable patients. Clinical utility of urine AQP2 to predict responders to tolvaptan is reported previously in patients with heart failure [23]. 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 my 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. I am now conducting another study investigating the impact of urine cAMP level on response to tolvaptan.

Limitations:

I included a moderate-size cohort. I measured baseline data just one time point. Response to AVP might change during long-term observational period. This is just an observational study, and I cannot conclude any causalities from my findings.

Compliance with Ethical Standards

Disclosure: All the authors have declared no competing interest.

Ethical approval: This study was approved by our institutional review board (IRB approval number R2015162) 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 Invest. 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. Sholokh A, Klussmann E. Local cyclic adenosine monophosphate signalling cascades-Roles and targets in chronic kidney disease. Acta Physiol (Oxf). 2021; 232: e13641.
6. Lee MJ, Chang TI, Lee J, Kim YH, Oh KH, Lee SW, et al. Urine osmolality and renal outcome in patients with chronic kidney disease: Results from the KNOW-CKD. Kidney Blood Press Res. 2019; 44: 1089-1100.
7. Tabibzadeh N, Wagner S, Metzger M, Flamant M, Houillier P, Boffa JJ, et al. Fasting urinary osmolality, CKD Progression, and mortality: A prospective observational study. Am J Kidney Dis. 2019; 73: 596-604.
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 Invest. 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. Pro Natl Acad Sci U S A. 1997; 94: 5450-5.
12. Shimamoto K, Watarai I, Miyahara M. A study of plasma vasopressin in patients undergoing chronic hemodialysis. J Clin Endocrinol Metab. 1977; 45: 714-20.
13. 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.
14. Tannen RL, Regal EM, Dunn MJ, Schrier RW. Vasopressin-resistant hyposthenuria in advanced chronic renal disease. N Engl J Med. 1969; 280: 1135-41.
15. Teitelbaum I, McGuinness S. Vasopressin resistance in chronic renal failure. Evidence for the role of decreased V2 receptor mRNA. J Clin Invest. 1995; 96: 378-85.

16. 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.
17. 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.
18. 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 study. BMC nephrol. 2010; 11: 26.
19. Olsen TS, Hansen HE. Ultrastructure of medullary tubules in ischemic acute tubular necrosis and acute interstitial nephritis in man. APMIS. 1990; 98: 1139-48.
20. Rodionova EA, Kuznetsova AA, Shakhmatova EI, Prutskova N, Nielsen S, Holtbäck U, et al. Urinary aquaporin-2 in children with acute pyelonephritis. Pediatr Nephrol. 2006; 21: 361-7.
21. Kakeshita K, Koike T, Imamura T, Fujioka H, Yamazaki H, Kinugawa K. Expression of aquaporin-2 in the collecting duct and responses to tolvaptan. CEN Case Rep. 2021; 10: 69-73.
22. Wilson DR. Pathophysiology of obstructive nephropathy. Kidney Int. 1980; 18: 281-92.
23. 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.

Tables

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

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; cAMP, cyclic adenosine monophosphate; ACE-I, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor antagonists. Variables are expressed as the median [interquartile range] or number and percentage. Comparison in continuous variables were performed by using Mann-Whitney's U test. Comparison in categorical variables were performed by using chi-square test. *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

eGFR, estimated glomerular filtration rate; ACE-I, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor antagonists. Variables are expressed as the median [interquartile range] or number and percentage. Comparison in continuous variables among the three groups were performed by using Kruskal-Wallis test. Comparison in categorical variables among the three groups were performed by using chi-square for independence test. *p <0.05.

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*

cAMP, cyclic adenosine monophosphate. Variables are expressed as the median [interquartile range]. Comparison in continuous variables among the three groups were performed by using Kruskal-Wallis test. *p <0.05.

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.

Table 5. Baseline characteristics in the prognostic study

	Total (n = 106)	Low urine cAMP (n = 32)	High urine cAMP (n = 74)	p value
Demographics				
Age, years	72 [64–78]	72 [63–79]	73 [65–78]	0.61
Male sex	80 (75)	26 (87)	54 (73)	0.46
Diabetes mellitus	28 (26)	16 (50)	12 (16)	<0.005*
Body mass index, kg/m^2	24.4 [22.1–27.4]	24.9 [23.1–27.5]	24.1 [21.8–27.1]	0.33
Systolic blood pressure, mmHg	135 [124–143]	135 [122–145]	135 [125–143]	0.85
Diastolic blood pressure, mmHg	73 [63–83]	69 [63–77]	75 [65–85]	0.067
Pulse rate, beats/min	71 [63–80]	75 [63–83]	70 [62–77]	0.088
CKD stage G3, eGFR 30–59 mL/min/1.73 m^2	51 (48)	0 (0)	51 (69)	<0.005*
CKD stage G4, eGFR 15–29 mL/min/1.73 m^2	32 (30)	13 (41)	19 (26)	0.17
CKD stage G5, eGFR <15 mL/min/1.73 m^2	23 (22)	19 (59)	4 (6)	<0.005*
Laboratory data				
Hemoglobin, g/dL	11.6 [10.1–13.8]	10.0 [9.2–10.7]	12.9 [11.1–14.3]	<0.005*
Serum creatinine, mg/dL	1.77 [1.20–3.00]	3.79 [2.43–4.99]	1.35 [1.00–1.91]	<0.005*
eGFR, mL/min/1.73 m^2	28.4 [16.5–45.2]	12.9 [9.5–20.0]	40.4 [25.7–51.8]	<0.005*
Blood urea nitrogen, mg/dL	27 [20–45]	50 [31–63]	22 [18–32]	<0.005*
Serum albumin, g/dL	3.9 [3.3–4.2]	3.3 [2.8–3.6]	4.0 [3.8–4.3]	<0.005*
Serum sodium, mEq/L	139 [138–141]	139 [138–141]	139 [138–141]	0.75
Serum potassium, mEq/L	4.5 [4.2–4.8]	4.4 [4.1–4.9]	4.5 [4.2–4.7]	0.51
Serum chloride, mEq/L	105 [103–108]	106 [103–108]	105 [103–107]	0.32
Serum calcium corrected for albumin, mg/dL	9.2 [8.9–9.5]	9.2 [8.8–9.6]	9.2 [9.0–9.4]	0.69
Serum osmolality, mOsm/kg·H_2O	295 [291–304]	302 [297–309]	293 [291–299]	<0.005*
Plasma arginine vasopressin, pg/mL	2.3 [1.5–3.8]	2.4 [1.8–3.7]	2.3 [1.4–4.0]	0.34
Plasma parathyroid hormone, intact, pg/mL	64 [46–104]	104 [58–263]	59 [43–75]	<0.005*
Urine cAMP, nmol/mg of creatinine	1.99 [1.48–2.62]	1.23 [0.91–1.47]	2.29 [1.96–2.76]	<0.005*
Urine aquaporin 2, ng/mg of creatinine	3.15 [1.71–6.07]	2.62 [1.36–4.37]	3.58 [2.05–6.41]	0.051
Urine osmolality, mOsm/kg·H_2O	414 [334–501]	351 [253–430]	442 [349–593]	<0.005*
Medications				
ACE-I or ARB	65 (61)	19 (59)	46 (62)	0.83
Calcium channel antagonists	63 (59)	28 (88)	35 (47)	<0.005*

β-adrenergic blockers	28 (26)	7 (22)	21 (28)	0.63
α-adrenergic blockers	11 (10)	7 (22)	4 (5)	0.017*
Aldosterone receptor antagonists	15 (14)	5 (16)	10 (14)	0.77
Loop diuretics	41 (39)	24 (75)	17 (23)	<0.005*
Thiazide diuretics	21 (20)	4 (13)	17 (23)	0.29

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; cAMP, cyclic adenosine monophosphate; ACE-I, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor antagonists. Variables are expressed as the median [interquartile range] or number and percentage. Comparison between the groups in continuous variables were performed by Mann-Whitney's U test. Comparison between the groups in categorical variables were performed by Fischer's exact test. *p <0.05.

Table 6. Impact of urine cAMP on the primary outcome

	Hazard ratio (95% confidence interval)	p value
Model 1: crude	0.14 (0.08–0.25)	<0.001†
Model 2: adjusted for age and male gender	0.13 (0.07–0.23)	<0.001†
Model 3: adjusted for age, male gender, and others*	0.41 (0.18–0.91)	0.029†

*Others included diabetes mellitus, use of renin-angiotensin system inhibitors, and estimated glomerular filtration rate as potential confounders. †p <0.05 by Cox proportional hazard ratio regression analysis.

Figures

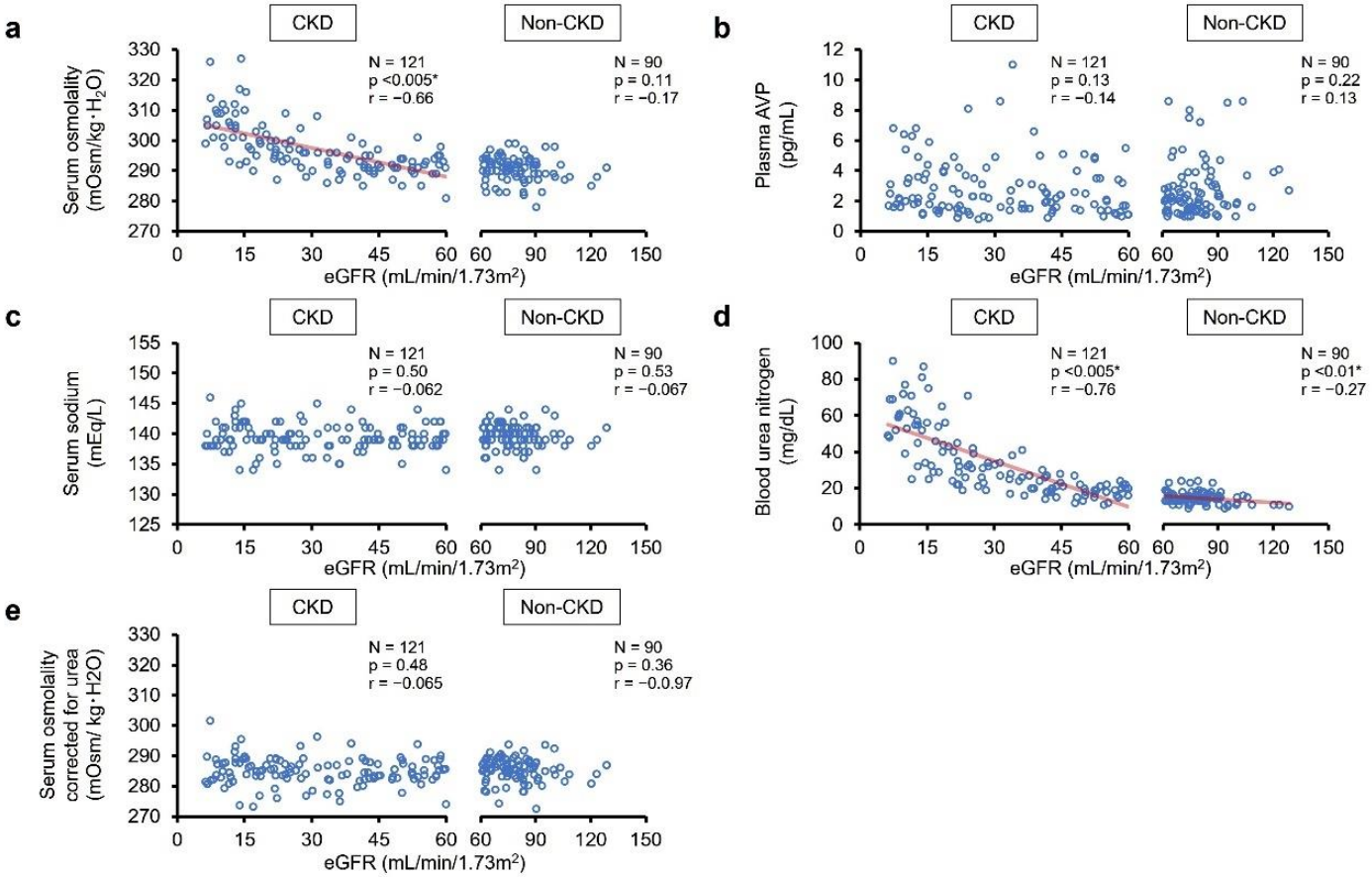


Figure 1. Correlation between eGFR and serum osmolality (a), plasma AVP (b), serum sodium (c), blood urea nitrogen (d) and serum osmolality corrected for urea (e)

*p <0.05 by Pearson's correlation coefficient.

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; AVP, arginine vasopressin.

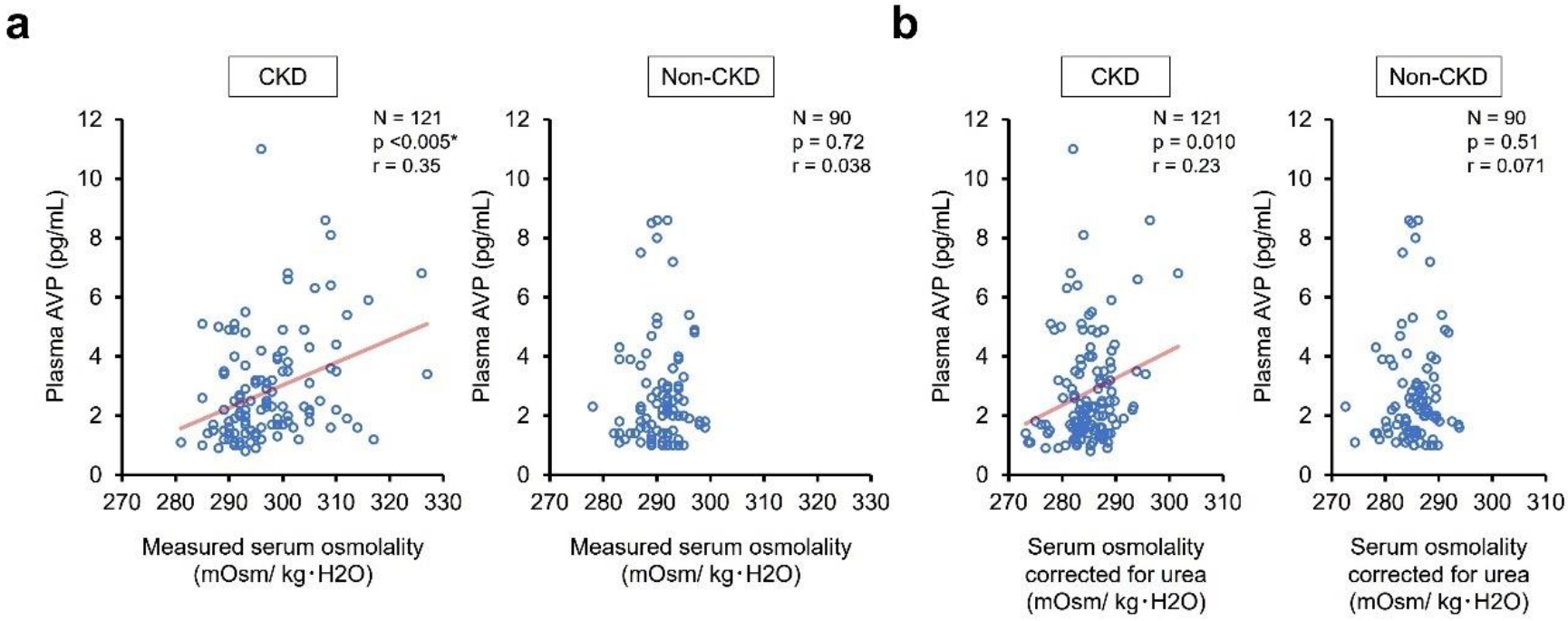


Figure 2. Correlation between measured serum osmolality and plasma AVP (a) and between serum osmolality corrected for urea and plasma AVP (b)

*p <0.05 by Pearson's correlation coefficient.

CKD, chronic kidney disease; AVP, arginine vasopressin.

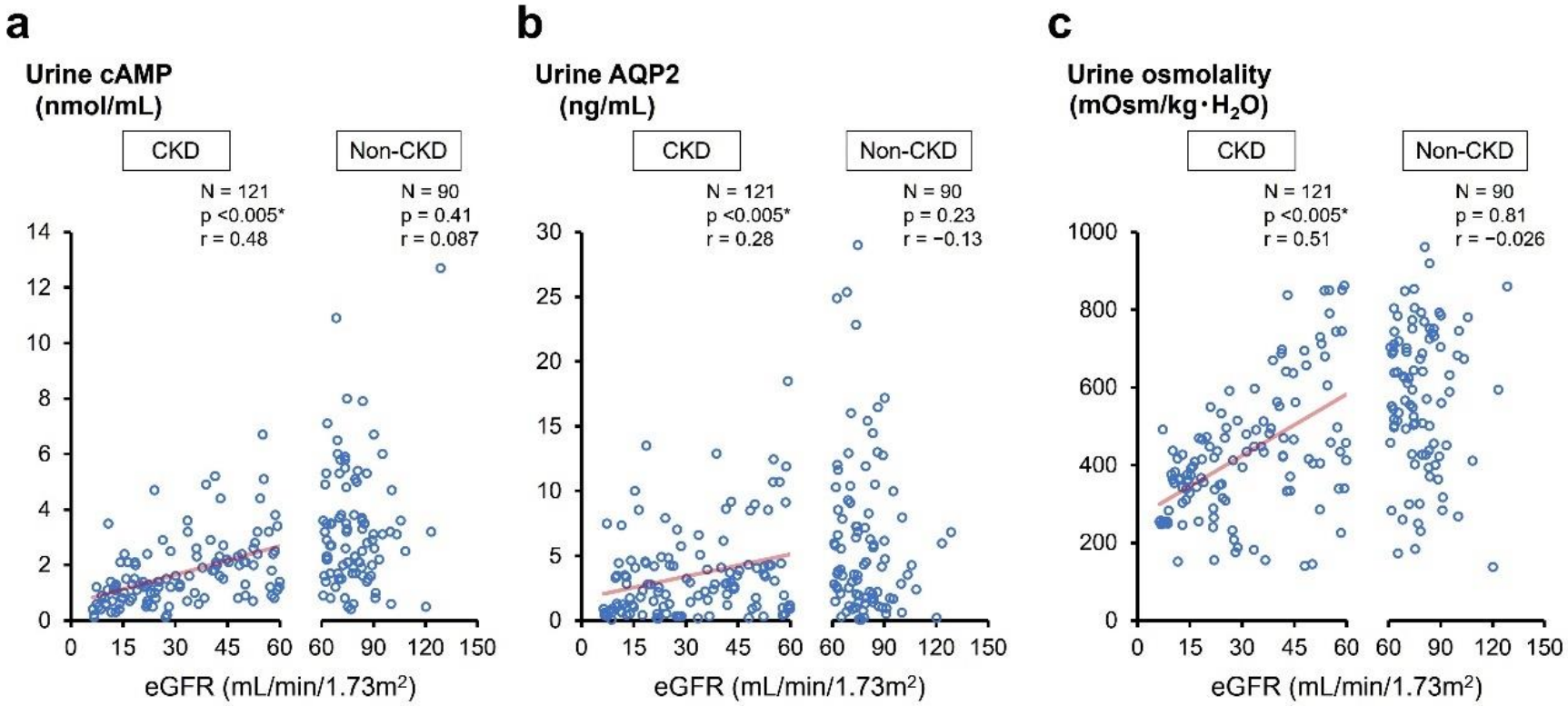


Figure 3. Correlation between eGFR and urine cAMP (a), urine AQP2 (b), and urine osmolality (c)
***p <0.05 by Pearson's correlation coefficient.**
eGFR, estimated glomerular filtration rate; cAMP, cyclic adenosine monophosphate; AQP2, aquaporin 2.

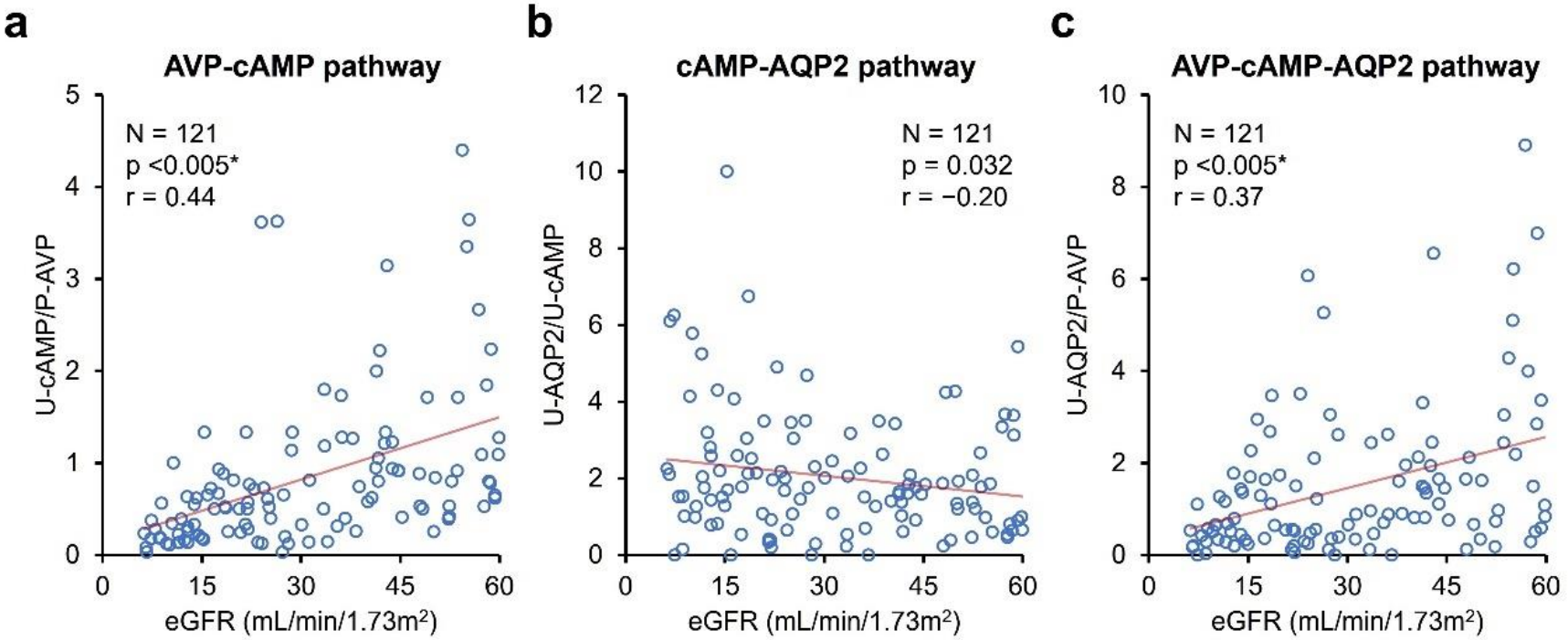


Figure 4. Correlation between eGFR and cAMP/AVP ratio (a), AQP2/cAMP ratio (b), and AQP2/AVP ratio (c)
*p <0.05 by Pearson's correlation coefficient.
eGFR, estimated glomerular filtration rate; U-cAMP, urine cyclic adenosine monophosphate; P-AVP, plasma arginine vasopressin; U-AQP2, urine aquaporin 2.

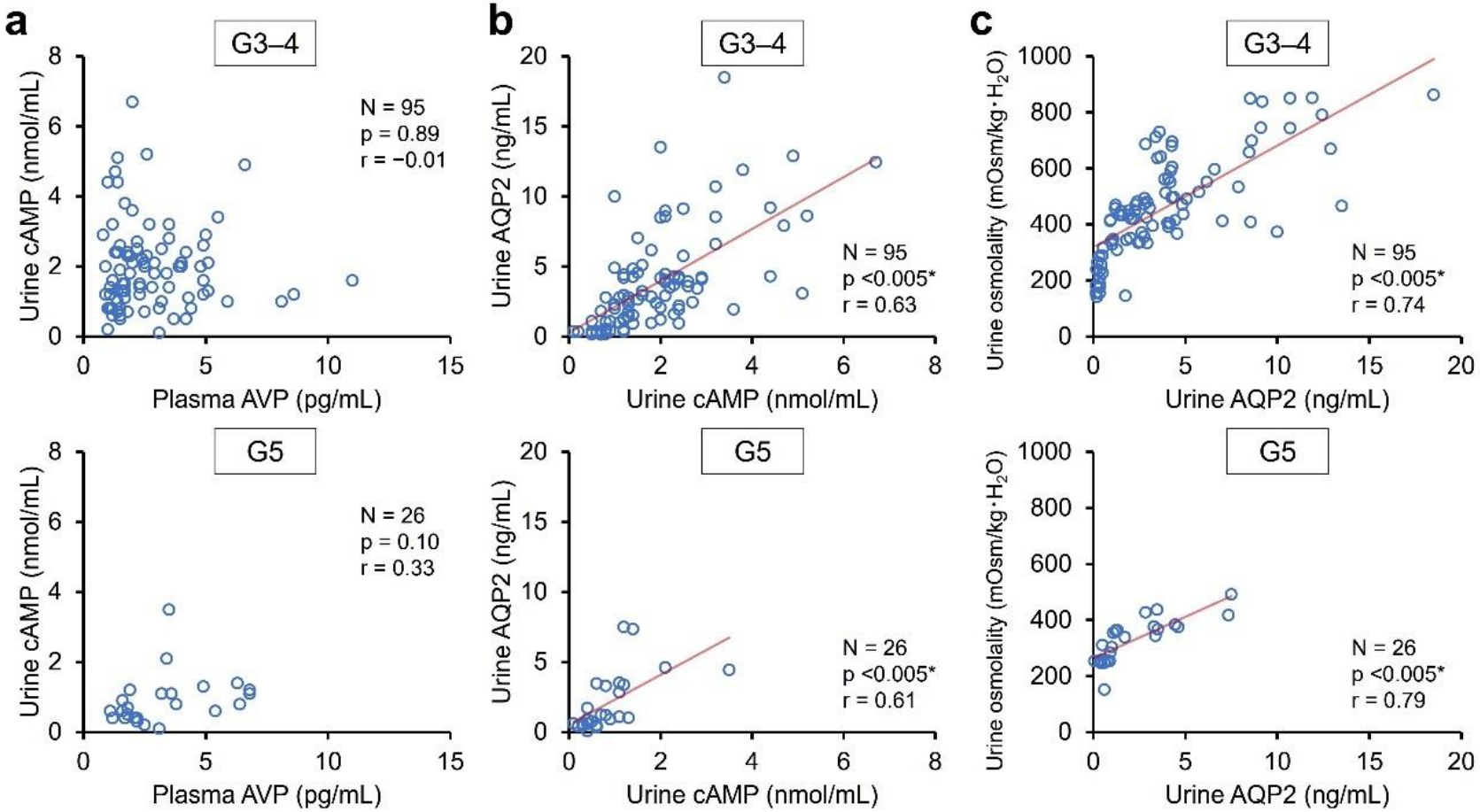


Figure 5. Correlation between plasma AVP and urine cAMP (a), cAMP and urine AQP2 (b), and urine AQP2 and urine osmolality (c) stratified by the CKD stages (G3–4 and G5)

*p <0.05 by Pearson's correlation coefficient.

AVP, arginine vasopressin; cAMP, cyclic adenosine monophosphate; AQP2, aquaporin 2.

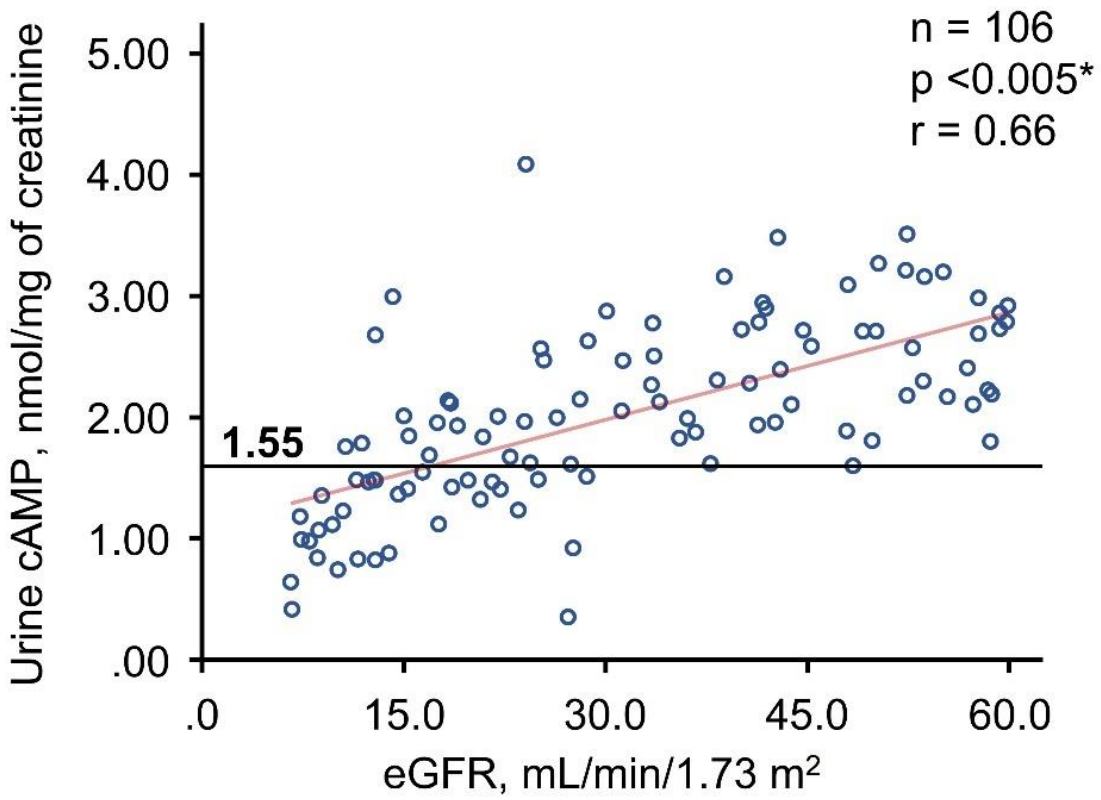


Figure 6. Correlation between eGFR and urine cAMP
*p <0.05 by Pearson's correlation coefficient.
eGFR, estimated glomerular filtration rate; cAMP, cyclic adenosine monophosphate.

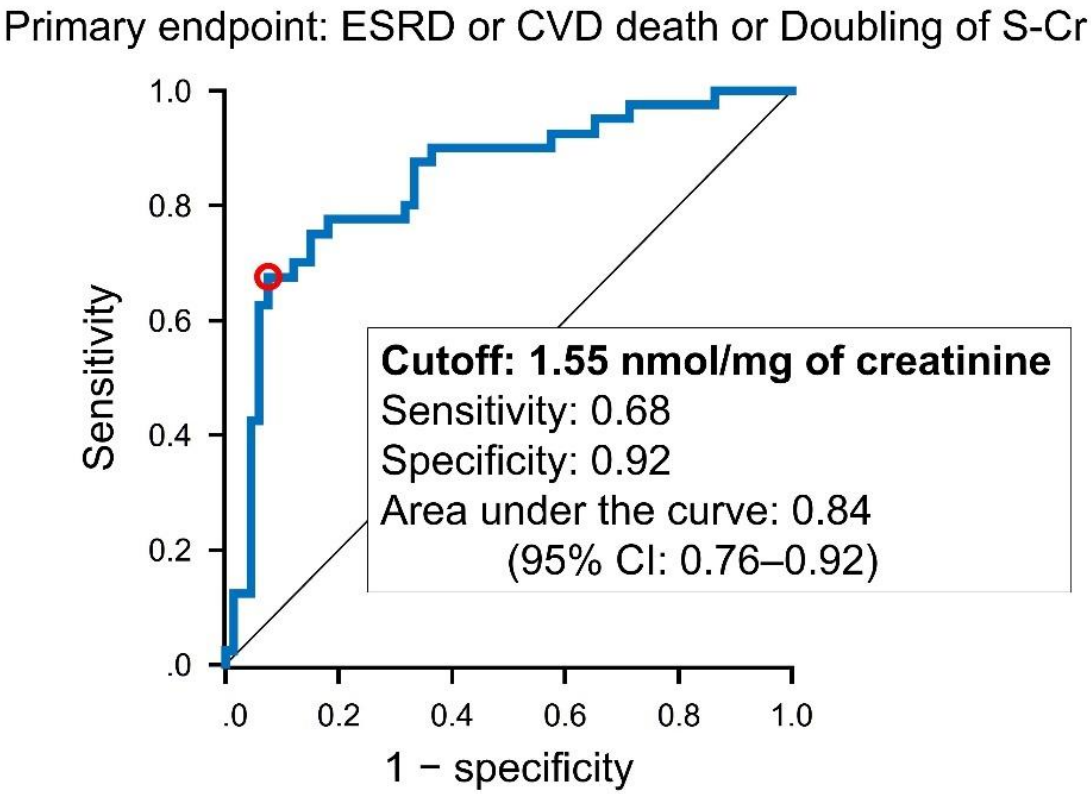


Figure 7. Receiver operating characteristic curve analysis for urine cAMP to predict the primary outcome
ESRD, end-stage renal disease; CVD, cardiovascular disease; S-Cr, serum creatinine; CI, confidence interval.

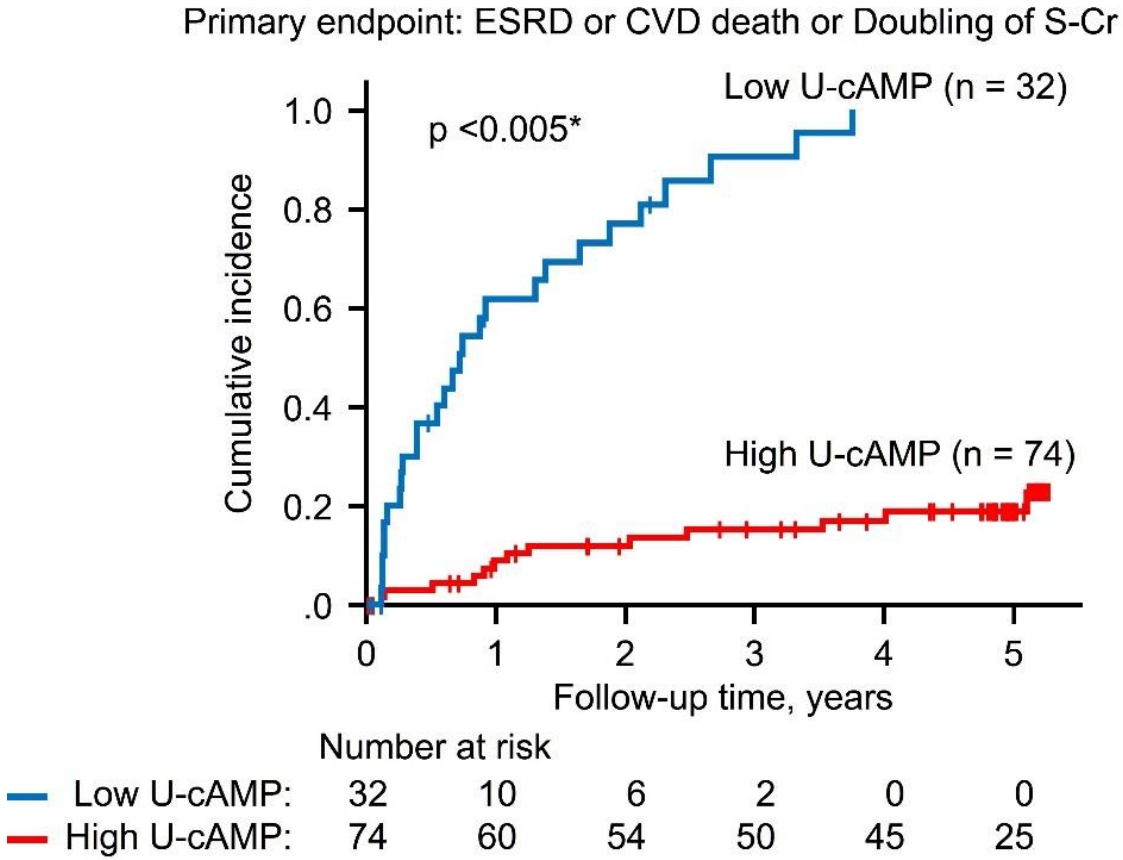


Figure 8. Cumulative incidence of the primary outcome stratified by urine cAMP level
In total CKD patients, patients were stratified by urine cAMP level, with a cutoff value of 1.55 nmol/mg of creatinine. *p <0.05 by log-rank test.
ESRD, end-stage renal disease; CVD, cardiovascular disease; S-Cr, serum creatinine; U-cAMP, urine cyclic adenosine monophosphate.

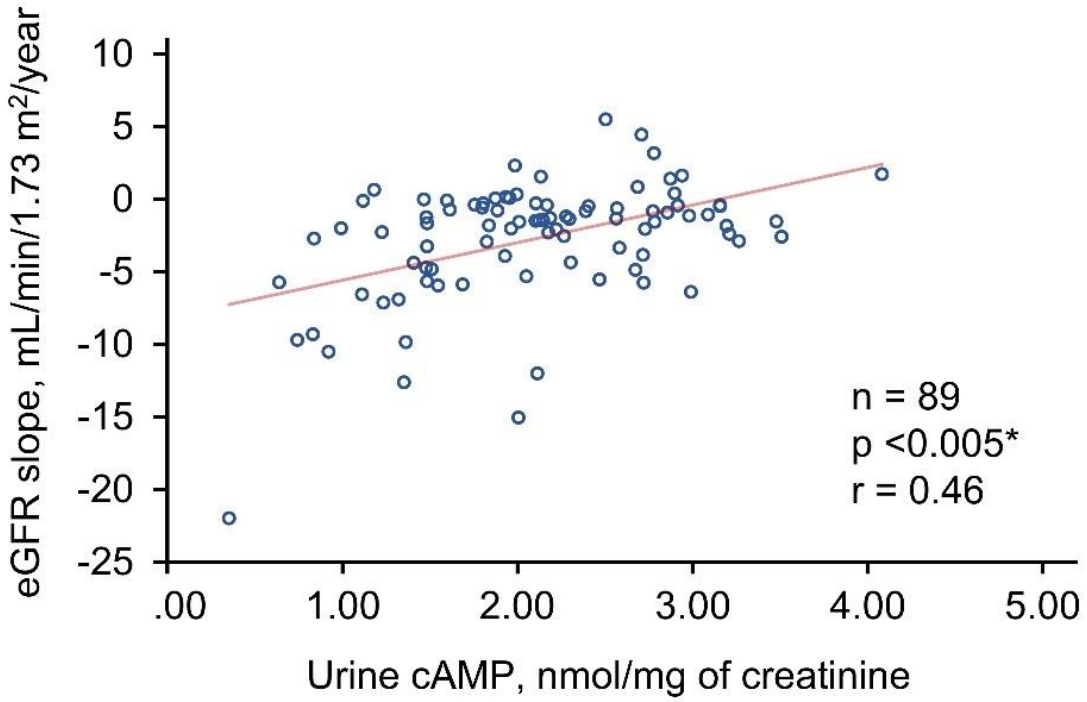
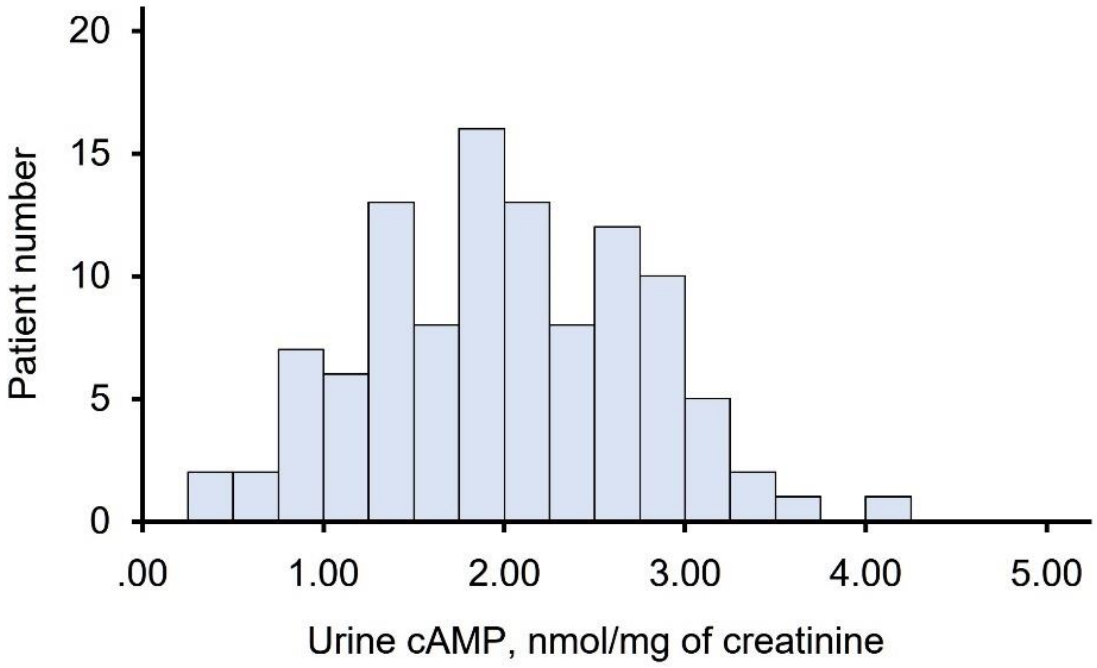
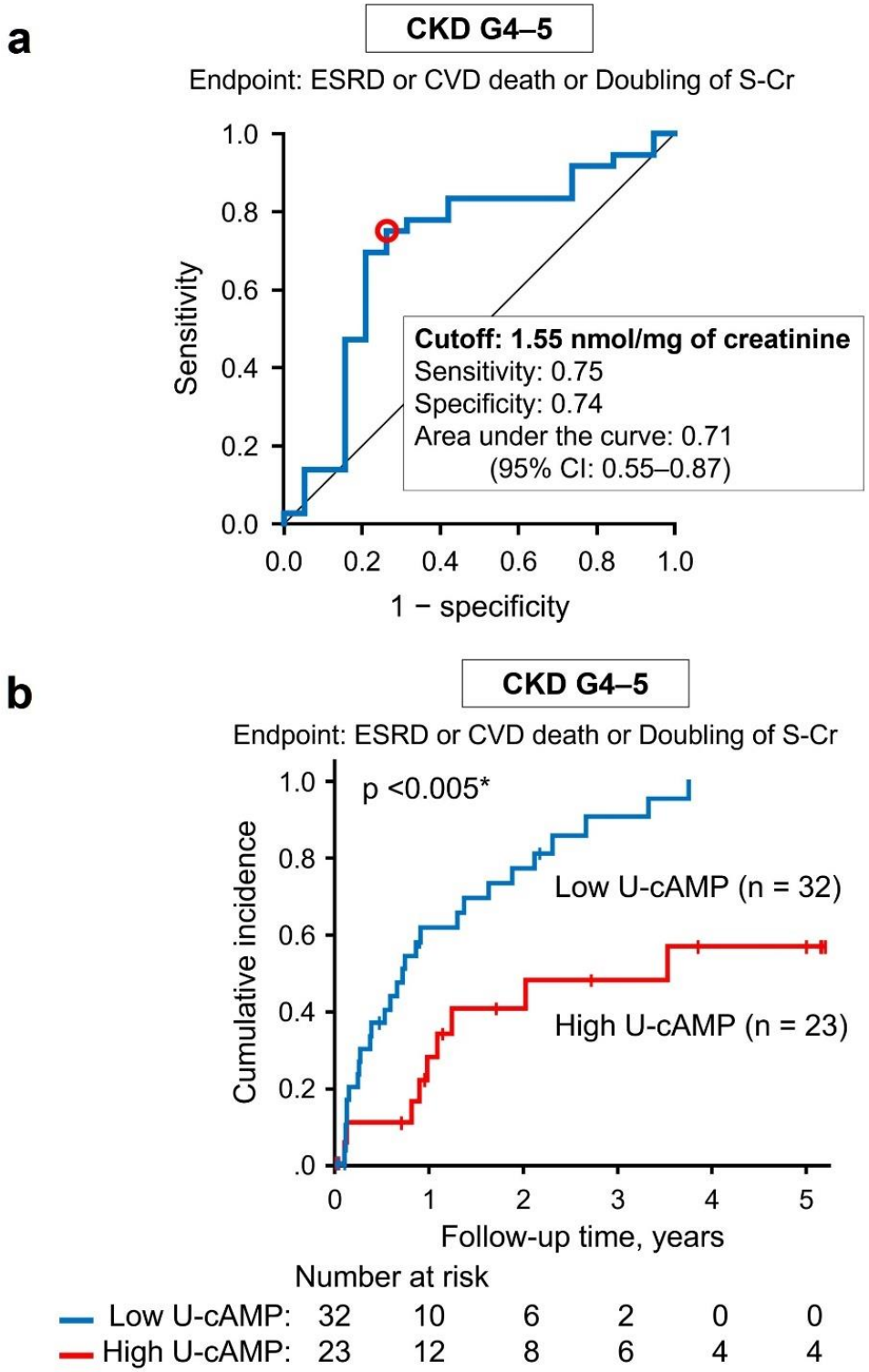


Figure 9. Correlation between urine cAMP level and eGFR slope
*p <0.05 by Pearson's correlation coefficient.
cAMP, cyclic adenosine monophosphate; eGFR, estimated glomerular filtration rate.

Supplementary Figures

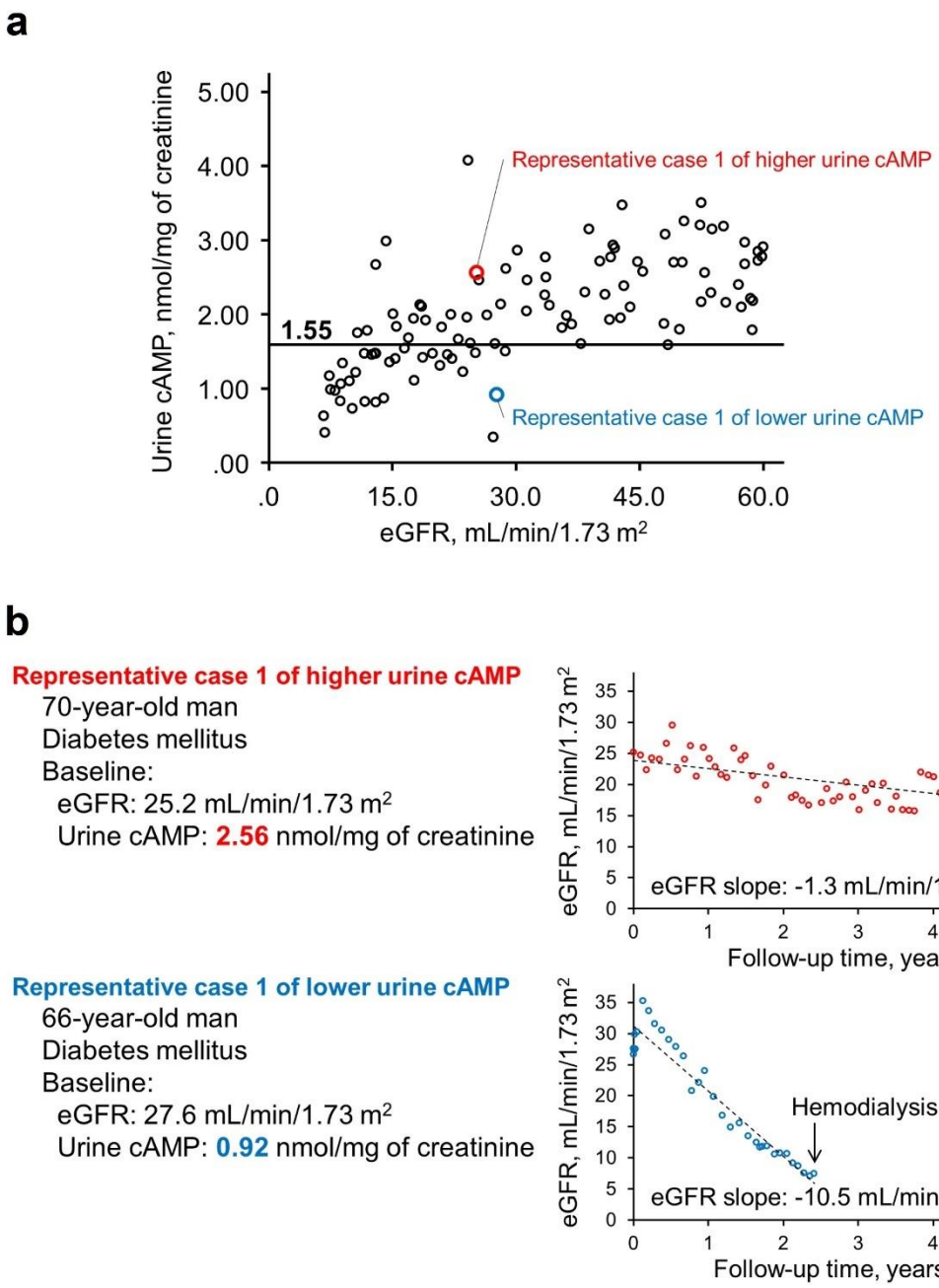


Supplementary Figure 1. Distribution of urine cAMP levels
cAMP, cyclic adenosine monophosphate.



Supplementary Figure 2. Receiver operating characteristic curve analysis for urine cAMP to predict the primary outcome (a) and cumulative incidence of the primary outcome stratified by urine cAMP level (b) in CKD stage G4–5 patients

In CKD stage G4–5 patients, patients were stratified by urine cAMP level, with a cutoff value of 1.55 nmol/mg of creatinine. *p <0.05 by log-rank test.



Supplementary Figure 3. Representative cases 1 of higher urine cAMP (red circle) and lower urine cAMP (blue circle). Correlation between eGFR and urine cAMP (a) and clinical course of eGFR (b) in each case

eGFR, estimated glomerular filtration rate; cAMP, cyclic adenosine monophosphate.