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Urinary Aquaporin 2 as a Potential Indicator Predicting Tolvaptan Response in Patients With ADPKD

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Introduction: Tolvaptan is used to treat autosomal dominant polycystic kidney disease (ADPKD) because it inhibits binding of the antidiuretic hormone vasopressin to the vasopressin V2 receptor (V2R), which suppresses the insertion of preformed water channel aquaporin 2 (AQP2) molecules in the luminal membrane of the collecting duct cells.

Methods: This single-center, prospective observational cohort study investigated whether decreased AQP2 elimination in urine affects the renal prognosis of patients who received tolvaptan. We selected 92 patients with ADPKD who were administered tolvaptan in our hospital. We evaluated correlations between changes in urinary AQP2 (U-AQP2) and clinical parameters and the annual change in total kidney volume (TKV) and estimated glomerular filtration rate (eGFR) as renal prognostic factors using univariable and multivariable multiple regression analyses.

Results: The observation period was 2.4 ± 1.5 years. U-AQP2 per milligram of urinary creatinine (U-AQP2/Cr) decreased from 67.8 ± 50.6 to 20.7 ± 15.1 fmol/mg urinary creatinine after 1 month of tolvaptan treatment. This initial change in U-AQP2/Cr was correlated with high baseline U-AQP2/Cr, low baseline eGFR, and a large initial change in eGFR (baseline to 1 month). The initial change in U-AQP2/Cr (baseline to 1 month) was strongly correlated with the annual change in TKV and eGFR in multivariable analysis.

Conclusion: Initial decrease in U-AQP2/Cr in the first month of treatment reflects the pharmacologic effect of tolvaptan and could be an indicator of renal prognosis during tolvaptan treatment.

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ADPKD, the most common genetic renal disease, causes the growth of multiple renal cysts owing to mutations in *PKD1* or *PKD2*.[1] In patients with ADPKD, the kidneys increase in size while renal function decreases. Approximately half of the patients reach end-stage renal failure by 60 years of age.[1]

A water channel, AQP2 is present in the principal cells of the renal collecting duct. Mutations in *AQP2* result in an inability to concentrate urine and, consequently, to nephrogenic diabetes insipidus.[2] AQP2 is a preformed water channel. When inserted into the cell membrane, AQP2 increases the permeability to water of the membrane and AQP2 will allow water transport if there is a favorable osmotic driving force. The antidiuretic hormone arginine vasopressin (AVP) binding to the V2R induces the insertion of preformed AQP2 molecules in the apical membrane of the collecting duct cells. A portion of the luminal AQP2 (~3%) is excreted into the urine; hence, U-AQP2 serves as an indicator of AVP action.[3]

Stimulation of V2R causes renal cysts to become enlarged in patients with ADPKD, resulting in disease aggravation. In 2003, the efficacy of V2R antagonists was revealed in an animal model of PKD.[4] Large clinical trials involving tolvaptan (a V2R antagonist) revealed a reduction in the rate of increase in kidney volume and decrease in eGFR.[5,6] Therefore, tolvaptan is now often administered to patients with ADPKD across the world. The inhibition of AVP binding to its V2 receptor suppresses the insertion of AQP2 in the luminal

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membrane of the collecting duct cells and subsequently reduces AQP2 excretion into the urine. Tamma *et al.*[7] revealed a reduced urinary excretion of AQP2 in patients receiving tolvaptan and the major role of AQP2 blockade in the aquaretic effect of tolvaptan. We, therefore, hypothesized that the initial decrease in U-AQP2 represents a direct pharmacologic effect that occurs after initiation of tolvaptan treatment. In this study, we investigated the initial decrease in AQP2 elimination into the urine as an indicator of tolvaptan efficacy.

METHODS

Protocol

The goal of the study was to clarify whether reduced U-AQP2 excretion is a predictive factor of renal prognosis. Patients were first analyzed for the short-term efficacy of tolvaptan administration (study A), followed by analysis of the long-term efficacy of tolvaptan administration (study B). We evaluated annual changes in TKV and eGFR as defined outcomes, with the main variables including baseline and initial changes in U-AQP2/Cr. The inclusion criteria were as follows: patients diagnosed with having ADPKD by Ravine's criteria.[8] Those with a TKV greater than 750 ml and more than 5% annualized TKV growth rate were indicated, whereas those with eGFRs $<$ 15 ml/min per 1.73 m^2 were contraindicated. The exclusion criteria were as follows: (i) Patients for whom the U-AQP2 level was not quantified within 2 to 6 weeks of initiating tolvaptan and (ii) for study B, patients whose observation periods were less than 6 months.

Patients

A flowchart of the study population is found in Figure 1. We enrolled 92 patients who started tolvaptan at our hospital between September 2014 and February 2019. Nevertheless, only 84 patients with available U-AQP 2 data were analyzed for the short-term efficacy of tolvaptan administration (study A). This analysis was repeated incorporating TKV as a variable in 48 patients with available baseline TKV data. Meanwhile, to determine the long-term efficacy of tolvaptan administration (study B), renal prognosis (annual change in eGFR) was only evaluated in 73 patients, because 11 patients whose observation periods were less than 6 months were excluded from the 84 patients in study A. This analysis was also performed with TKV as a variable in 43 patients with available baseline TKV data. TKV growth (annual change in TKV) was evaluated in 42 patients, whose TKV data were available both at baseline and 1 year after tolvaptan initiation to determine the long-term efficacy of tolvaptan administration.

All procedures involving human participants were conducted in accordance with the ethical standards of our Institutional Research Committee (approval number: 140807) and adhered to the Declaration of Helsinki (1964) and its later amendments. Written informed consents were obtained from all participants enrolled in the study.

Study Design

This was a single-center, prospective observational cohort study. On the day of admission, we performed blood tests and urinalysis for all patients and started a 3-day inpatient management program involving tolvaptan administration. According to the Japanese package insert, the starting dose is defined as 60 mg/d, and it is recommended to start at a lower dose for patients whose creatinine clearance is below 30 ml/min. After discharge, patients visited the outpatient department on a monthly basis for follow-up examinations. The dose was adjusted with 30 to 120 mg daily depending on renal function and tolerability. Tolvaptan was discontinued in patients with eGFR $<$ 15 ml/min per 1.73 m^2 or liver enzyme elevation during follow-up because tolvaptan is contraindicated for such patients in Japan. We followed up and collected data until tolvaptan was discontinued or November 2019.

eGFR was calculated from serum creatine using the formula applied as a standard in Japan.[9] Plasma AVP was measured by radioimmunoassay using Yamasa's AVP kit (Research Reagents; Yamasa Corporation, Tokyo, Japan). U-AQP2 measurements were performed twice using a sandwich enzyme-linked immunosorbent assay protocol according to the guidelines of the human AQP2 enzyme-linked immunosorbent assay kit (Research Reagents; Otsuka Pharmaceutical, Tokyo, Japan).[10] Evaluation was performed using the U-AQP2/Cr ratio, taking the spot urine concentration into account. Urine was collected on admission, before initial tolvaptan treatment, and at the outpatient visit 1 month after the initiation of tolvaptan. Dividing the U-AQP2 concentration by the creatinine concentration provides an information of the amount of U-AQP2 excreted, independently of possible differences in urine concentration. Three-dimensional reconstruction computed tomography was performed within 45 days before and 1 year after starting tolvaptan to measure TKV. All kidney volumes were measured using the 3-dimensional workstation of Ziostation2 (version 2.4.2.3; Ziosoft, Tokyo, Japan).

Initial changes (%) in eGFR, AVP, urine osmolarity (U-Osm), and U-AQP2/Cr were calculated using data collected on initiation of tolvaptan and 1 month after.

Annual changes in TKV (%/yr) were calculated as an accurate annualized growth rate by correcting for intervals between measurements. Annual changes in eGFR

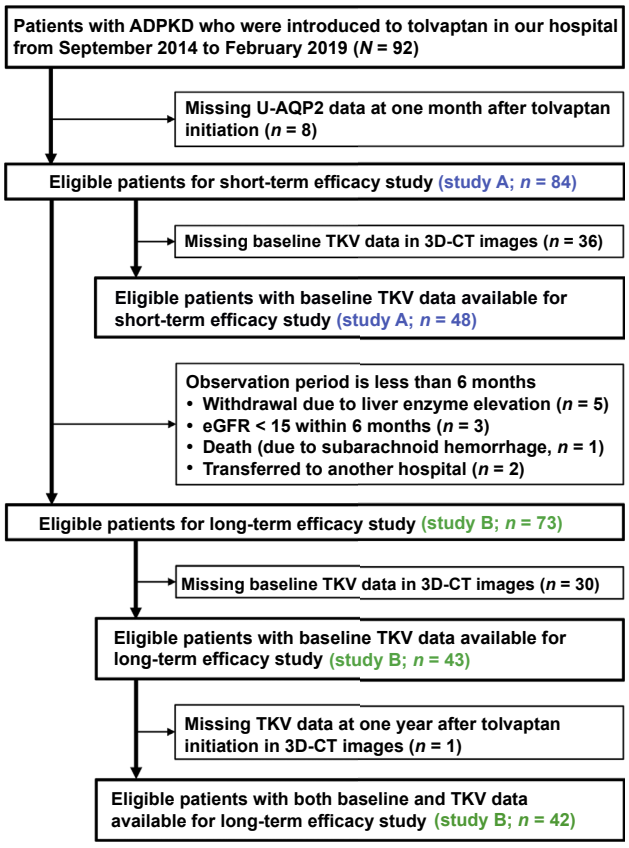

Figure 1. Flowchart of the study population. 3D-CT, 3-dimensional reconstruction computed tomography; ADPKD, autosomal dominant polycystic kidney disease; eGFR, estimated glomerular filtration rate; TKV, total kidney volume; U-AQP2, urinary aquaporin 2.

(%/yr) were calculated using eGFR values determined 1 month after tolvaptan initiation to the final observation period. The initial small decline in GFR observed on initiation of tolvaptan was not due to a decline in kidney function and was fully reversed on discontinuation of tolvaptan. In the REPRISE study, considering this initial decline, baseline eGFR was compared with eGFR approximately 1 month after termination of treatment.[6] Nevertheless, in this clinical study, tolvaptan could not be discontinued at the final observation period. Considering eGFR changes during the first month of tolvaptan treatment (initial change), the value obtained at 1 month was considered the baseline for the rate of change, which was used to calculate the slope function, as performed in TEMPO3:4 trial.[11]

Comorbidities were recorded according to the following criteria: hypertension defined as systolic blood pressure $\geq$ 140 mm Hg, diastolic blood pressure $\geq$ 90 mm Hg, or receiving antihypertensive agents; hyperuricemia defined as serum uric acid $\geq$ 7.0 mg/dl or receiving antihyperuricemic agents.

Statistical Analysis

Continuous variables are reported as the mean $\pm$ SD. Categorical variables are reported as percentages (%) unless otherwise stated. Data were evaluated using paired t test as appropriate. The Pearson's correlation coefficient (r) was used to evaluate bivariable relationships. Univariable and multivariable multiple regression analyses were performed to investigate the factors associated with baseline U-AQP2/Cr, initial changes in U-AQP2/Cr, annual changes in TKV, and annual changes in eGFR. Variables of interest and general risk factors for outcomes based on existing knowledge were included in multivariable analysis. To avoid multicollinearity, independent variables with a variance inflation factor $<$ 2.5 were selected for multivariable analysis.[12] The relative contribution of the independent variable to the dependent variable was verified using standardized partial regression coefficients, which reveals how the sum of the variances for the dependent variables can be explained by the variances of independent variables. The sample size calculation was based on the assumption that a minimum of 5 subjects was required for regression analysis.[13,14] A $P < 0.05$ was considered statistically significant. All statistical analyses were performed using JMP software (version 15.0; SAS Institute, Cary, NC).

RESULTS

Short-Term Efficacy After Tolvaptan Initiation (Study A)

The baseline characteristics of the 84 patients observed within 1 month of tolvaptan treatment are found in Table 1 (study A).

In univariable analyses, baseline eGFR, baseline AVP, and baseline U-Osm were significantly associated with baseline U-AQP2/Cr (see Supplementary Table S1). Specifically, the bivariable correlation plot revealed that baseline U-Osm correlated strongly and positively with baseline U-AQP2/Cr (Figure 2). The other bivariable correlation plots are presented in Supplementary Figure S1A–C. Multivariable analysis revealed that baseline U-Osm correlated strongly and positively with baseline U-AQP2/Cr, and baseline AVP was positively and sex (male) was negatively associated with baseline U-AQP2/Cr (Table 2).

Changes in clinical data after initiating tolvaptan are found in Figure 3a-d. Mean eGFR decreased. Meanwhile, AVP largely increased, and U-Osm and U-AQP2/Cr largely decreased. Moreover, in the measurements taken at 3-hour intervals after initiating tolvaptan in 1 patient, U-AQP2/Cr decreased sharply during the first 6 hours and became relatively constant afterward until the next morning (see Supplementary Figure S2).

Next, correlation analysis between each parameter and initial changes in U-AQP2/Cr from baseline to 1

Table 1. Baseline patient characteristics

Variables	Study A (n = 84)	Study B (n = 73)
Age (yr)	43.3 ± 9.5	43.1 ± 9.6
Sex (male; %)	49 (58.3)	40 (54.8)
Body weight (kg)	66.6 ± 13.3	66.1 ± 13.7
SBP (mm Hg)	130.0 ± 13.3	130.7 ± 13.4
DBP (mm Hg)	84.0 ± 9.7	84.5 ± 9.6
Hypertension, n (%)	69 (82.1)	59 (80.8)
Hb (g/dl)	13.2 ± 1.4	13.1 ± 1.4
Alb (g/dl)	4.4 ± 0.3	4.4 ± 0.3
UA (mg/dl)	6.1 ± 1.5	6.0 ± 1.5
Hyperuricemia, n (%)	37 (44.0)	32 (43.8)
Cr (mg/dl)	1.43 ± 0.71	1.38 ± 0.68
eGFR (ml/min per 1.73 m^2)	50.6 ± 24.7	52.2 ± 25.0
AVP (pg/ml)	1.9 ± 1.7	1.7 ± 1.5
U-Prot/Cr (g/gCr)	0.31 ± 0.47	0.28 ± 0.42
U-Osm (mOsm/kg)	351.1 ± 139.3	349.2 ± 141.4
U-AQP2 (ng/ml)	2.65 ± 2.82	2.61 ± 2.89
U-AQP2/Cr (fmol/mgCr)	67.8 ± 50.6	68.1 ± 52.7
Tolvaptan initial dose (mg/d)	55.1 ± 11.8	55.6 ± 10.7
TKV (ml)	2183 ± 1283 (n = 48)	2166 ± 1298 (n = 43)
MIC 1A, n (%)	0 (0.0) (n = 48)	0 (0.0) (n = 43)
MIC 1B, n (%)	1 (2.1) (n = 48)	0 (0.0) (n = 43)
MIC 1C, n (%)	20 (41.7) (n = 48)	18 (41.9) (n = 43)
MIC 1D, n (%)	13 (27.1) (n = 48)	13 (30.2) (n = 43)
MIC 1E, n (%)	14 (29.2) (n = 48)	12 (27.9) (n = 43)

Alb, albumin; AVP, arginine vasopressin; Cr, serum creatinine; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; gCr, g urinary creatinine; Hb, hemoglobin; mgCr, mg urinary creatinine; MIC, Mayo imaging class; mOsm, milliosmolarity; SBP, systolic blood pressure; TKV, total kidney volume; UA, uric acid; U-AQP2, urinary aquaporin 2; U-AQP2/Cr, urinary aquaporin 2 per mg urinary creatinine; U-Osm, urinary osmolality; U-Prot/Cr, urinary protein per mg urinary creatinine.

month was investigated. In univariable analyses, baseline AVP, U-Osm, and U-AQP2/Cr and initial change in eGFR and U-Osm were significantly associated with initial changes in U-AQP2/Cr (see Supplementary Table S2). Specifically, the bivariable correlation plot revealed that initial changes in U-AQP2/Cr also correlated positively with initial changes in eGFR (Figure 4). Other bivariable correlation plots are presented in Supplementary Figure S3A–F.

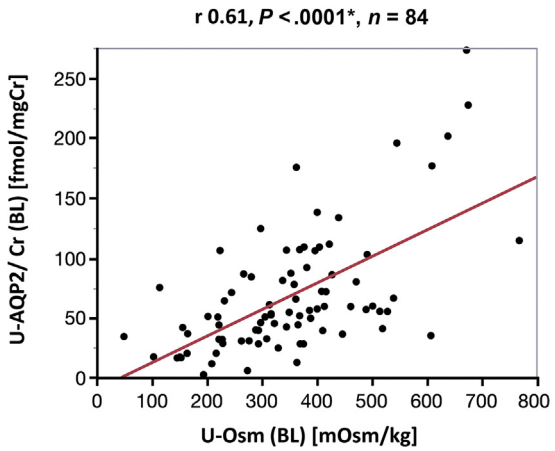

Figure 2. Bivariable correlation plot between BL U-Osm and BL U-AQP2/Cr in study A (n = 84). *P < 0.05. BL, baseline; mgCr, mg urinary creatinine; mOsm, milliosmolarity; U-AQP2/Cr, urinary aquaporin 2 per mg urinary creatinine; U-Osm, urinary osmolality.

Table 2. Multivariable analyses: factors associated with baseline U-AQP2/Cr in study A (n = 84)

Variables	Partial r	P value
Age	−0.09	0.3774
Sex (male)	−0.18	0.0458[a]
eGFR (BL)	0.13	0.218
AVP (BL)	0.22	0.0161[a]
U-Prot/Cr (BL)	−0.12	0.1615
U-Osm (BL)	0.57	<0.0001[a]

AVP, arginine vasopressin; BL, baseline; eGFR, estimated glomerular filtration rate; partial r, standardized partial regression coefficient; U-AQP2/Cr, urinary aquaporin 2 per mg urinary creatinine; U-Osm, urinary osmolality; U-Prot/Cr, urinary protein per mg urinary creatinine.
[a]P < 0.05.
Variables of interest (eGFR, U-Prot/Cr), including general associated factors for the outcome based on existing knowledge (age, sex, AVP, U-Osm), were included in the multivariable model.

Multivariable analysis revealed that high baseline U-AQP2/Cr correlated strongly and positively with initial changes in U-AQP2/Cr (Table 3). In addition, low baseline eGFR and large changes in initial eGFR correlated with initial changes in U-AQP2/Cr (Table 3).

Long-Term Efficacy of Tolvaptan Administration (Study B)

The baseline characteristics of 73 patients with favorable long-term adherence (Table 1 [study B]) and their changes in clinical data after initiation of tolvaptan (see Supplementary Figure S4A–D) were equivalent to those observed in study A. The observation period was 2.6 ± 1.4 years.

In univariable analyses of 73 patients, baseline eGFR, baseline U-AQP2/Cr, and U-AQP2/Cr 1 month after tolvaptan initiation were significantly and positively associated with annual changes in eGFR, and baseline U-Prot/Cr was significantly and negatively associated with annual changes in eGFR (see Supplementary Table S3A). The bivariable correlation plots are presented in Supplementary Figure S5A–E. In the multivariable analysis, the factors associated with an annual change in eGFR were baseline eGFR, U-Prot/Cr, and initial change in U-AQP2/Cr (Table 4). In addition, a multivariable analysis using the baseline or 1-month U-AQP2/Cr, rather than the initial change in U-AQP2/Cr, revealed that both U-AQP2/Cr values were not significantly associated with annual change in eGFR (see Supplementary Table S3B).

Univariable analysis was also performed for the 43 patients with available baseline TKV data. The results reveal that baseline eGFR and baseline U-AQP2/Cr were significantly and positively associated with annual changes in eGFR after initiating tolvaptan treatment. Baseline TKV, baseline MIC 1E, and baseline U-Prot/Cr were significantly and negatively associated with

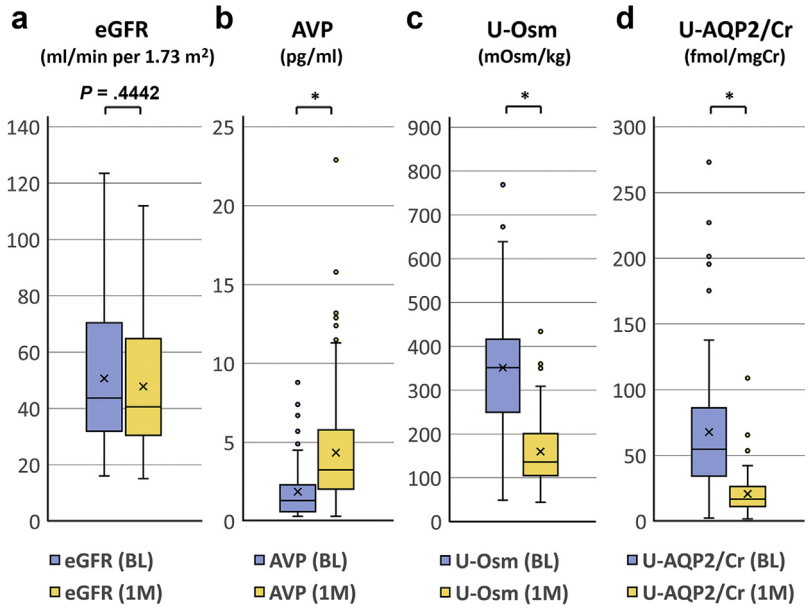

Figure 3. Changes in clinical data (BL to 1M) for study A ($n = 84$). (a) Mean eGFR was 50.6 ± 24.7 at BL and 47.8 ± 23.1 ml/min per 1.73 m^2 at the visit after 1M, revealing an initial decline of −4.9% ± 7.8%. (b) Mean AVP was 1.9 ± 1.7 at BL and 4.3 ± 3.8 pg/ml at the visit after 1M, revealing an initial increase of 193.3% ± 220.7%. (c) Mean U-Osm was 351.1 ± 139.3 mOsm/kg at BL and 159.8 ± 81.0 mOsm/kg at the visit after 1M, with initial change of −47.7% ± 32.1%. (d) Mean U-AQP2/Cr was 67.8 ± 50.6 at BL and 20.7 ± 15.1 fmol/mgCr at the visit after 1M, revealing an initial decrease of −59.2% ± 27.6%. *$P < 0.0001$, BL versus 1M. 1M, 1 month after tolvaptan initiation; AVP, arginine vasopressin; BL, baseline; eGFR, estimated glomerular filtration rate; mgCr, mg urinary creatinine; mOsm, milliosmolarity; U-AQP2/Cr, urinary aquaporin 2 per mg urinary creatinine; U-Osm, urinary osmolality.

annual changes in eGFR (see Supplementary Table S4A). The bivariable correlation plots are presented in Supplementary Figures S6A,B and S7A–E. A multivariable analysis, including baseline TKV, revealed that the factors associated with annual change in eGFR were baseline eGFR and initial change in U-AQP2/Cr (Table 5). Alternative multivariable analysis using MIC 1E, instead of TKV, revealed nearly identical results (see Supplementary Table S4B). Neither TKV nor MIC 1E was significantly associated with the annual changes in eGFR.

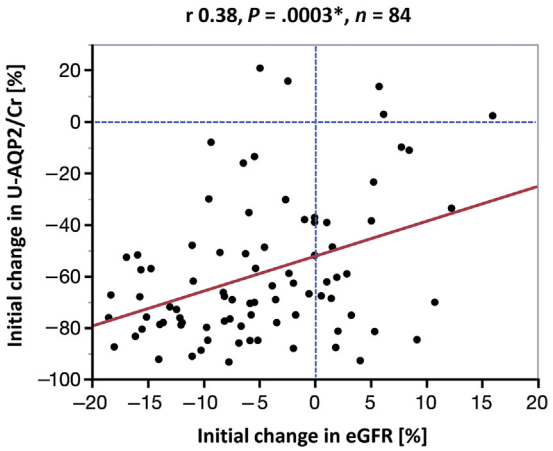

Figure 4. Bivariable correlation plot between initial changes in eGFR and initial changes in U-AQP2/Cr (baseline to 1 month) in study A ($n = 84$). *$P < 0.05$. eGFR, estimated glomerular filtration rate; U-AQP2/Cr, urinary aquaporin 2 per mg urinary creatinine.

TKV data analysis was performed for only 43 patients with TKV measured within 45 days before tolvaptan initiation. In 1 patient who was able to continue tolvaptan for 6 months, TKV was not measured 1 year after treatment. In univariable analyses of 42 patients, no factors were significantly associated with annual changes in TKV in the first year after initiation of tolvaptan treatment (see Supplementary Table S5). The bivariable correlation plots are presented in Supplementary Figure S8A–E. Bivariable analysis revealed that initial changes in U-AQP2/Cr tended to be associated with annual changes in TKV (see

Table 3. Multivariable analysis: factors associated with initial changes in U-AQP2/Cr (BL to 1M) in study A ($n = 84$)

Variables	Partial r	*P* value
Age	0	0.9625
Sex (male)	0.08	0.3692
eGFR (BL)	0.31	0.0054[a]
AVP (BL)	−0.14	0.1693
U-Osm (BL)	−0.16	0.16
U-AQP2/Cr (BL)	−0.33	0.0045[a]
Initial change in eGFR (BL to1M)	0.26	0.0053[a]
Initial change in U-Osm (BL to 1M)	0.17	0.0697

1M, 1 month after tolvaptan initiation; AVP, arginine vasopressin; BL, baseline; eGFR, estimated glomerular filtration rate; Partial r, standardized partial regression coefficient; U-AQP2/Cr, urinary aquaporin 2 per mg urinary creatinine; U-Osm, urinary osmolality.
[a]$P < 0.05$.
Variables of interest (eGFR, initial change in eGFR), including general associated factors for the outcome based on existing knowledge (age, sex, AVP, U-Osm, U-AQP2/Cr, initial change in U-Osm), were included in the multivariable model.

Table 4. Multivariable analysis: factors associated with annual changes in eGFR (1M to end of study) in study B ($n = 73$)

Variables	Partial r	*P* value
Age	0.21	0.0785
Sex (male)	0.04	0.7367
Hypertension	−0.07	0.5286
eGFR (BL)	0.52	<0.0001[a]
U-Prot/Cr (BL)	−0.32	0.0016[a]
Initial change in U-AQP2/Cr (BL to 1M)	−0.25	0.0106[a]

1M, 1 month after tolvaptan initiation; BL, baseline; eGFR, estimated glomerular filtration rate; Partial r, standardized partial regression coefficient; U-AQP2/Cr, urinary aquaporin 2 per mg urinary creatinine; U-Prot/Cr, urinary protein per mg urinary creatinine.
[a]$P < 0.05$.
Variables of interest (initial change in U-AQP2/Cr), including general risk factors for the outcome based on existing knowledge (age, sex, hypertension, eGFR, U-Prot/Cr), were included in the multivariable model.

Table 6. Multivariable analysis: factors associated with annual changes in TKV in study B ($n = 42$)

Variables	Partial r	*P* value
Age	0.04	0.8337
Sex (male)	0.23	0.2294
Hypertension	0.3	0.1
TKV (BL)	−0.2	0.3601
eGFR (BL)	0.07	0.7246
U-Prot/Cr (BL)	0.27	0.1187
Initial change in U-AQP2/Cr (BL to 1M)	0.29	0.0798

1M, 1 month after tolvaptan initiation; BL, baseline; eGFR, estimated glomerular filtration rate; Partial r, standardized partial regression coefficient; TKV, total kidney volume; U-AQP2/Cr, urinary aquaporin 2 per mg urinary creatinine; U-Prot/Cr, urinary protein per mg urinary creatinine.
Variables of interest (initial change in U-AQP2/Cr), including general risk factors for the outcome based on existing knowledge (age, sex, hypertension, TKV, eGFR, U-Prot/Cr), were included in the multivariable model.

Supplementary Figure S8E), and multivariable analysis also revealed that initial changes in U-AQP2/Cr were generally associated with annual changes in TKV (Table 6).

DISCUSSION

This study investigated indicators of renal prognosis in patients with ADPKD administered tolvaptan and focused specifically on initial changes in U-AQP2/Cr between baseline and 1 month after administration. Subjects with a larger decrease in U-AQP2/Cr after initiating tolvaptan exhibited lower annual decline in eGFR, indicating favorable renal prognosis.

Nearly all AQP2 become recycled in the collecting duct cells, with only 3% eliminated in the urine.[4] Nevertheless, when AVP is elevated, AQP2 production increases by V2R, which also increases AQP2 elimination in the urine.[15] Meanwhile, U-AQP2 elimination decreases sharply in cases of diabetes insipidus[16] and increases in patients with hepatic cirrhosis, compared with normal individuals.[17,18] Mean U-AQP2/Cr (fmol/mg urinary creatinine) in healthy people is 176.3, whereas it was 42.1 after an acute oral water load test and 685.0 in patients with syndrome of inappropriate antidiuretic hormone secretion.[19] To date, at least 3 studies have measured U-AQP2 levels in patients with ADPKD.[20–22] Graffe *et al.*[20] reported that U-AQP2/Cr is not increased in patients with ADPKD compared with that in healthy controls. Zittema *et al.*[22] also reported no difference in U-AQP2/Cr between patients with ADPKD and IgA nephropathy. This study observed a mean baseline U-AQP2/Cr of 67.8 fmol/mg urinary creatinine, which was slightly lower than that in healthy people. Patients with ADPKD had impaired capacity to concentrate urine, which is reportedly the cause of their poor response to AVP.[23] After 14 hours of restricted water consumption, urinary osmolarity in patients with ADPKD becomes equivalent to that of healthy people, whereas AVP excretion significantly increases,[24] suggesting AVP-stimulated AQP2 activation is blunted irrespective of increasing AVP excretion in patients with ADPKD. This study revealed that U-AQP2/Cr was more strongly correlated with urinary osmolarity than with AVP (Table 2), suggesting U-AQP2 is more indicative of the ability to concentrate urine than AVP in cases of ADPKD.

It is well known that eGFR declines soon after initiating tolvaptan. Specifically, a study in which tolvaptan was administered for 1 week reported 8.6% decline in eGFR,[25] whereas another study reported a 5.4% decline in eGFR after 3 weeks of tolvaptan treatment, which was recovered after stopping treatment.[26,27] In the REPRISE study, which considered the initial decline, eGFR recovered to baseline levels equivalent to the initial decline at approximately 1 month after ending the treatment.[6] In this study, we revealed an approximate 5% decline in eGFR from baseline at 1 month after treatment initiation. The activation by AVP of the facilitated urea transporter UT-A1 in the terminal inner medullary collecting duct initiates an intrarenal recycling of urea which brings a

Table 5. Multivariable analysis: factors associated with annual changes in eGFR (1M to end of study) in study B ($n = 43$)

Variables	Partial r	*P* value
Age	0.28	0.0948
Sex (male)	0.13	0.4437
Hypertension	−0.06	0.7089
TKV (BL)	−0.15	0.4569
eGFR (BL)	0.53	0.0050[a]
U-Prot/Cr (BL)	−0.21	0.1735
Initial change in U-AQP2/Cr (BL to 1M)	−0.31	0.0277[a]

1M, 1 month after tolvaptan initiation; BL, baseline; eGFR, estimated glomerular filtration rate; Partial r, standardized partial regression coefficient; TKV, total kidney volume; U-AQP2/Cr, urinary aquaporin 2 per mg urinary creatinine; U-Prot/Cr, urinary protein per mg urinary creatinine.
[a]$P < 0.05$.
Variables of interest (initial change in U-AQP2/Cr), including general risk factors for the outcome based on existing knowledge (age, sex, hypertension, TKV, eGFR, U-Prot/Cr), were included in the multivariable model.

higher urea concentration and a lower sodium chloride concentration in the loop of Henle and at the macula densa. This in turn reduces the tubuloglomerular feedback control of GFR and allows GFR to go up.[28] Tolvaptan, by inhibiting the effect of AVP on UT-A1, reduces or abolishes this vasopressin-dependent urea recycling, thus letting the tubuloglomerular feedback increase and consequently reduce the GFR.[29,30]

Tolvaptan-induced inhibition of AVP activity also causes a decrease in AQP2 production and U-AQP2 elimination.[31] Shoaf *et al*.[21] reported that U-AQP2 decreases by approximately 50% between days 1 and 5 after tolvaptan administration; however, the observed changes were not significant. Here, also, we observed an approximate 60% decrease in U-AQP2/Cr at 1 month (Figure 3), with the initial change in U-AQP2/Cr found to correlate with initial changes in eGFR at 1 month (Figure 4). Multivariable analysis further identified baseline U-AQP2/Cr, followed by baseline eGFR and initial changes in eGFR as associated with the initial changes in U-AQP2/Cr (Table 3). The high baseline U-AQP2/Cr suggests that AVP secretion is high, thereby predicting a strong inhibitory effect of V2R by tolvaptan. As noted, the initial changes in eGFR are thought to be caused by the suppression of urea recycling by tolvaptan direct effects. Surprisingly, baseline eGFR was significantly associated with the initial change in U-AQP2/Cr in multivariable analysis (Table 3). The reason that a lower baseline eGFR associated with a larger decrease in U-AQP2/Cr could be due to the fewer residual V2Rs in the collecting duct, leading to more effective suppression of V2R by relatively high tolvaptan concentration per receptor.

We then evaluated whether U-AQP2/Cr affects renal prognosis by using annual changes in eGFR and those in TKV as outcomes. First, we investigated whether an initial decrease in U-AQP2/Cr affects annual changes in eGFR in a mean observation period of 2.6 years. A larger initial decrease in U-AQP2/Cr predicted a slower long-term decline in eGFR (Tables 4, 5). In both TEMPO and REPRISE studies, tolvaptan reduced the rate of decline in eGFR.[6,7] The reduction in albuminuria in the TEMPO study and the above-mentioned TGF-associated decrease in intraglomerular pressure indicated a glomerular protective effect of tolvaptan.[32] Second, we evaluated whether U-AQP2/Cr affects renal prognosis by using annual changes in TKV in 1 year as an outcome. Larger decreases in U-AQP2/Cr tended to be associated with slower changes in TKV in 1 year, indicating a direct effect on renal cysts (Table 6). Nevertheless, U-AQP2 levels reflect the effect of tolvaptan on the collecting duct cells rather than its direct effect on cystic epithelial cells. Furthermore, nevertheless, the correlation observed between the initial decrease in U-AQP2/Cr and suppression of cyst enlargement indicates overlap between patient responsiveness to tolvaptan. Hence, initial changes in U-AQP2/Cr may serve as an effective prognostic indicator for evaluating suppression of cyst enlargement and suppression of renal function decline.

AVP action inserts AQP2 molecules in the apical membrane of the collecting duct principal cells, and some of this AQP2 is shed into the urine. Tolvaptan, by preventing the action of AVP on V2 receptors and the resulting cyclic adenosine monophosphate formation, inhibits cyst growth. It also markedly reduces insertion of AQP2 in the luminal membrane of the collecting duct cells and, thus, reduces the release of AQP2 into the urine.

U-AQP2 is considered an indicator for response to treatments for cardiac failure and liver cirrhosis.[18,33] Imamura *et al*.[33] revealed that urinary excretion of AQP2 in patients with decompensated heart failure was a predictor of the response to tolvaptan. Because both disorders cause fluid overload, therapeutic effects are determined according to increased urine volume alone, whereas changes in U-AQP2 correlate with the therapeutic effect.[18,33,34] Furthermore, daily variation in U-AQP2 levels is reportedly negligible, unlike that in AVP and urinary osmolarity levels.[10] In this study, we also confirmed that U-AQP2/Cr remained relatively constant after tolvaptan administration (see Supplementary Figure S2), suggesting U-AQP2/Cr can be measured at any time in outpatient clinics and could be used not only to determine adherence but also as an indicator for the determination of tolvaptan dose when administering drugs that affect cytochrome P450 3A4. An assay kit is already commercially available, and we look forward to the ready use of this kit in clinical practice.

One limitation was noted in this study. We investigated the clinical practices at a single institution, resulting in a relatively small sample size and lack of a fixed observation period. Nevertheless, we believe that the findings of this study could be verified in large-scale clinical studies.

CONCLUSION

In summary, our investigation of renal prognosis in patients with ADPKD undergoing tolvaptan treatment revealed a significant correlation between initial changes in U-AQP2/Cr and annual changes in eGFR. Furthermore, U-AQP2 can be evaluated irrespective of the water intake status of the patient during the outpatient visit, making it a potential indicator of renal prognosis based on the pharmacologic effect of tolvaptan in clinical practice.

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Bivariable correlation plots between baseline U-AQP2/Cr and each variable.

Figure S2. Transition in U-AQP2/Cr after initial tolvaptan administration.

Figure S3. Bivariable correlation plots between initial change in U-AQP2/Cr and each variable.

Figure S4. Changes in clinical data (baseline to 1 month) (study B; n = 73).

Figure S5. Bivariable correlation plots between annual changes in eGFR (%/yr) and each variable.

Figure S6. Bivariable correlation plots between annual changes in eGFR (%/yr) and each variable.

Figure S7. Bivariable correlation plots between annual changes in eGFR (%/yr) and each variable.

Figure S8. Bivariable correlation plots between annual changes in TKV (%/yr) and each variable.

Table S1. Univariable analysis: factors associated with baseline U-AQP2/Cr in study A (n = 84).

Table S2. Univariable analysis: factors associated with initial changes in U-AQP2/Cr (BL to 1M) in study A (n = 84).

Table S3. (A) Univariable analysis: factors associated with annual changes in eGFR (1M to end of study) in study B (n = 73). (B) The other models of multivariable analysis: factors associated with annual changes in eGFR (1M to end of study) in study B (n = 73).

Table S4. (A) Univariable analysis: factors associated with annual changes in eGFR (1M to end of study) in study B (n = 43). (B) The other models of multivariable analysis: factors associated with annual changes in eGFR (1M to end of study) in study B (n = 43).

Table S5. Univariable analysis: factors associated with annual changes in TKV in study B (n = 42).

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