Clinical Kidney Journal, 2023, vol. 16, no. 11, 2194–2204

https://doi.org/10.1093/ckj/sfad118
Advance Access Publication Date: 25 May 2023
Original Article

ORIGINAL ARTICLE

Copeptin in autosomal dominant polycystic kidney disease: real-world experiences from a large prospective cohort study

Sita Arjune[1,2,3,4,*], Simon Oehm[1,*], Polina Todorova[1], Ron T. Gansevoort[5], Stephan J.L. Bakker[5], Florian Erger[3,6], Thomas Benzing[1,2,3,4], Volker Burst[1,7], Franziska Grundmann[1], Philipp Antczak[1,4] and Roman-Ulrich Müller[1,2,3,4]

[1]Department II of Internal Medicine, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany, [2]Center for Molecular Medicine Cologne, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany, [3]Center for Rare Diseases Cologne, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, [4]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Cologne, Germany, [5]Department of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, [6]Institute of Human Genetics, University Hospital Cologne, Faculty of Medicine, University of Cologne, Cologne, Germany and [7]Emergency Department, University of Cologne, Faculty of Medicine and University Hospital of Cologne, Cologne, Germany

*These authors contributed equally.
Correspondence to: Roman-Ulrich Müller; E-mail: roman-ulrich.mueller@uk-koeln.de; Philipp Antczak; E-mail: pantczak@uni-koeln.de

ABSTRACT

Background. The identification of new biomarkers in autosomal-dominant polycystic kidney disease (ADPKD) is crucial to improve and simplify prognostic assessment as a basis for patient selection for targeted therapies. *Post hoc* analyses of the TEMPO 3:4 study indicated that copeptin could be one of those biomarkers.

Methods. Copeptin was tested in serum samples from patients of the AD(H)PKD study. Serum copeptin levels were measured using a time-resolved amplified cryptate emission (TRACE)-based assay. In total, we collected 711 values from 389 patients without tolvaptan treatment and a total of 243 values (of which 64 were pre-tolvaptan) from 94 patients on tolvaptan. These were associated with rapid progression and disease-causing gene variants and their predictive capacity tested and compared with the Mayo Classification.

Results. As expected, copeptin levels showed a significant negative correlation with estimated glomerular filtration rate (eGFR). Measurements on tolvaptan showed significantly higher copeptin levels (9.871 pmol/L vs 23.90 pmol/L at 90/30 mg; $P < .0001$) in all chronic kidney disease stages. Linear regression models ($n = 133$) show that copeptin is an independent predictor of eGFR slope. A clinical model (including eGFR, age, gender, copeptin) was nearly as good ($R^2 = 0.1196$) as our optimal model (including height-adjusted total kidney volume, eGFR, copeptin, $R^2 = 0.1256$). Adding copeptin to the Mayo model improved future eGFR estimation.

Received: 22.2.2023; Editorial decision: 13.4.2023

Conclusion. Copeptin levels are associated with kidney function and independently explained future eGFR slopes. As expected, treatment with tolvaptan strongly increases copeptin levels.

LAY SUMMARY

Autosomal-dominant polycystic kidney disease (ADPKD) is a genetic condition that can cause kidney damage. Researchers have been looking for new ways to predict how the disease will progress, so that patients can be offered the best treatment options. Copeptin is a substance that has been identified as a possible new marker for ADPKD. In this study, researchers measured copeptin levels in the blood of tolvaptan-naive ADPKD patients and ADPKD patients taking tolvaptan, a medication that can slow the progression of the disease. They found that copeptin levels were linked to kidney function and could help predict how the disease would progress in the future. The study also showed that tolvaptan increased copeptin levels. This research could help doctors make better treatment decisions for ADPKD patients.

Keywords: ADPKD, biomarker, copeptin, tolvaptan, vasopressin

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease and the fourth leading cause of kidney failure worldwide [1, 2]. Primarily, mutations in either of two genes, *PKD1* and *PKD2*, lead to cyst formation and a progressive decline in kidney function [3]. More than 70% of patients reach kidney failure by the age of 70 years. However, the interindividual disease course is highly variable even within families, hampering assessment of future outcome [4]. Two randomized clinical trials have shown the benefits of treatment with the V_2R-antagonist tolvaptan, leading to the approval of the first disease-modifying drug [4–6].

As only patients with evidence of rapidly progressing disease benefit from tolvaptan treatment and tolvaptan is associated with significant side effects (above all polyuria and polydipsia), patient selection for reasonable treatment recommendations has become of substantial importance [7]. However, prediction of disease progression in ADPKD remains a challenging task. Currently available predictive markers of progression including past estimated glomerular filtration rate (eGFR) loss and age-adapted total kidney volume (TKV) using the Mayo Classification are very helpful in routine clinical care but are hampered by, e.g. the necessity for MRI scans and volumetry or the lack of past creatinine measurements [8, 9]. Consequently, the identification of new and easily obtainable biomarkers remains an important goal to improve patient counselling and selection for targeted therapies. Moreover, new biomarkers would ideally allow the prediction of long-term treatment responses [10].

Vasopressin signaling plays a crucial role in the pathophysiology of ADPKD. It has been shown that vasopressin and the subsequently upregulated second messenger adenosine-3′-5′-cyclic monophosphate (cAMP) enhance cyst proliferation and intracystic fluid secretion [11–13]. Measurement of vasopressin is complex, e.g. due to its short half-life and instability in plasma. Therefore copeptin, which is part of the same precursor molecule and is released in an equimolar amount, is commonly determined as a surrogate [14]. Initial small, single-center studies pointed towards an association of plasma copeptin levels with ADPKD disease severity [15, 16] and disease progression [17–19]. More recently, in a large *post hoc* analysis of the TEMPO 3:4 (Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and Its Outcomes) trial copeptin levels were associated with kidney function loss, TKV increase and effectiveness of tolvaptan therapy [10].

The present work now aims to investigate whether copeptin is a valid biomarker in a real-life scenario and whether it may improve the predictive accuracy of the established criteria in current clinical use.

MATERIALS AND METHODS

Patient population

A total of 483 adult (≥18 years) patients with ADPKD enrolled in the AD(H)PKD registry were included in the analysis at their presentations between March 2017 and October 2020 and were followed up for up to 3 years. The AD(H)PKD registry comprises tolvaptan-naïve patients and patients starting therapy after baseline visit as well as patients already on targeted therapy. The AD(H)PKD cohort study was set up when tolvaptan was approved for use in ADPKD by European Medicines Agency in 2015 to follow up on the landscape of ADPKD patient care upon approval of the first targeted therapy. Consequently, the name reflects the mode of action, i.e. inhibition of ADH signaling. Follow-up visits are conducted for all patients and include yearly measurements of serum creatinine and other laboratory parameters (serum osmolality, urine osmolality, etc.). Written informed consent was obtained from all patients and approval was retrieved from the institutional review board of the University of Cologne. The cohort study is conducted in accordance with the Declaration of Helsinki and the good clinical practice guidelines by the International Conference on Harmonization, and is registered on www.clinicaltrials.gov (NCT02497521), where detailed information about the study can be found. Healthy control measurements were derived from a previously published cohort of healthy kidney donors using only the values obtained pre-donation [20]. More clinical information regarding the control cohort can be found in Supplementary data, Table S1.

Data collection, copeptin measurements and descriptions

Clinical and genetic data as well as laboratory parameters of all patients were available from the AD(H)PKD registry. Data collection was completed by obtaining serum creatinine and eGFR values from outpatient nephrologists or general practitioners with written informed consent available for all patients. eGFR was calculated using the 2009 Chronic Kidney Disease Epidemiology Collaboration equation [21]. Patients were asked

Table 1: Baseline characteristics of ADPKD cohort and retrospective healthy control cohort.

Characteristics	ADPKD		Control
Tolvaptan	−	+	−
N	389	94	134
Men, *n* (%)	180 (46.27)	49 (52.13)	66 (49.25)
Age (years), mean ± SD	44.14 ± 12.96	43.61 ± 9.68	51.56 ± 9.85
eGFR (mL/min/1.73 m^2), mean ± SD	69.20 ± 30.71	58.79 ± 26.76	104.70 ± 16.98
TKV (mL), mean ± SD	1864 ± 1686	2797 ± 3230	
Without baseline MRI, *n*	28	9	
MRI, *n*	363	85	
1A	10		
1B	87	5	
1C	129	26	
1D	83	39	
1E	46	15	
2	8		
CKD stage, *n*	389	94	134
1 eGFR >90 mL/min/1.73 m^2, *n* (%)	114 (29.31)	14 (14.89)	106 (79.10)
2 eGFR 60–89 mL/min/1.73 m^2, *n* (%)	113 (29.05)	20 (21.28)	28 (20.90)
3a eGFR 45–59 mL/min/1.73 m^2, *n* (%)	64 (16.45)	31 (32.98)	
3b eGFR 30–44 mL/min/1.73 m^2, *n* (%)	66 (16.97)	23 (24.47)	
4 eGFR 15–29 mL/min/1.73 m^2, *n* (%)	25 (6.43)	5 (5.32)	
5 eGFR <15 mL/min/1.73 m^2, *n* (%)	7 (1.80)	1 (1.05)	

to present to our outpatient clinic after overnight fasting for sampling of serum copeptin levels. Serum copeptin measurements were performed using the B·R·A·H·M·S Copeptin proAVP KRYPTOR assay (BRAHMS GmbH, Hennigsdorf, Germany) based on time-resolved amplified cryptate emission (TRACE) and carried out by MVZ Labor Dr Limbach & Kollegen GbR, Heidelberg, Germany. At all visits, patients were advised to present in a fasted state. TKV at baseline was assessed using standardized kidney magnetic resonance imaging (MRI) and manual segmentation, while TKV follow-up measurements were not regularly performed.

Statistics

Baseline patient characteristics are reported as mean ± standard deviation (SD) for normal distributions. Data were tested for normality using the Shapiro–Wilk test. The *P*-values for statistical difference were computed using paired Student's *t*-test for normally distributed, Mann–Whitney U test for nonparametric data and Wilcoxon signed-rank test for copeptin values. Copeptin values were analyzed for both their distribution in different patient groups (e.g. age, Mayo Class) as well as their response to tolvaptan treatment. A *P*-value <.05 was considered to be statistically significant. eGFR slopes were calculated using the robust linear modelling function implemented in the MASS library within R (Version 4.1.2). Briefly, all eGFR values were plotted against the number of days since the first copeptin treatment. Derived parameters were multiplied by 365 to represent an annual eGFR loss per patient. Quality control plots, using the ggplot library, were used to ensure that slopes were not over- or underestimated. In addition, mean eGFR slopes for each Mayo Class were calculated. For all models shown in this publication linear regression analysis was used to establish associations between eGFR slope and relevant clinical features using R. The built-in summary.lm function was used to extract the significance of parameters.

RESULTS

Baseline characteristics of the ADPKD and control cohort are shown in Table 1. The ADPKD cohort consisted of 483 patients, of whom 94 initiated treatment with tolvaptan prior to or during the study. A total of 954 individual copeptin values were used for the analysis of the ADPKD cohort, resulting in 1.98 values per patient (mean time between two copeptin measurements: 458.1 ± 164.1 days). The eGFR distribution in the treated patient group (58.8 ± 26.8 mL/min/1.73 m^2) was lower than in the tolvaptan-naïve subset (69.2 ± 30.7 mL/min/1.73 m^2), likely indicating selection by rapid progression criteria prior to initiation. The control cohort consisted of 134 healthy control individuals with a mean eGFR of 104.7 ± 18.0 mL/min/1.73 m^2. A total of 134 individual copeptin values was used for the analysis of the control cohort, resulting in one value per patient. Figure 1 provides an overview of the two study cohorts.

To obtain an insight into the distribution of copeptin values in our cohort, clinical factors known to be associated with a modulation of copeptin were examined including only tolvaptan-naïve patients. Males showed significantly higher copeptin levels than female participants (Fig. 2a). Copeptin positively correlated with the age of male (Spearman r = 0.30, $P < .0001$) and female (Spearman r = 0.35, $P < .0001$) patients with ADPKD (Fig. 2b). While copeptin levels increased with age in both male and female patients with ADPKD, the increase was less pronounced in women (Fig. 2b). The sex-specific difference in copeptin levels was still significant when only including patients with an eGFR ≥90 mL/min/1.73 m^2 (Fig. 2c). In the healthy control cohort, men also had significantly higher copeptin levels than women (Fig. 2a and c). There was no significant difference in copeptin levels between ADPKD patients in chronic kidney disease (CKD) stage 1 and healthy controls with a measured GFR ≥90 mL/min/1.73 m^2. Copeptin levels depended on kidney function with an increase starting in CKD stage 3 (Fig. 2d) and a strong negative correlation with eGFR (Fig. 2e). Serum osmolality positively correlated with copeptin in both males and females

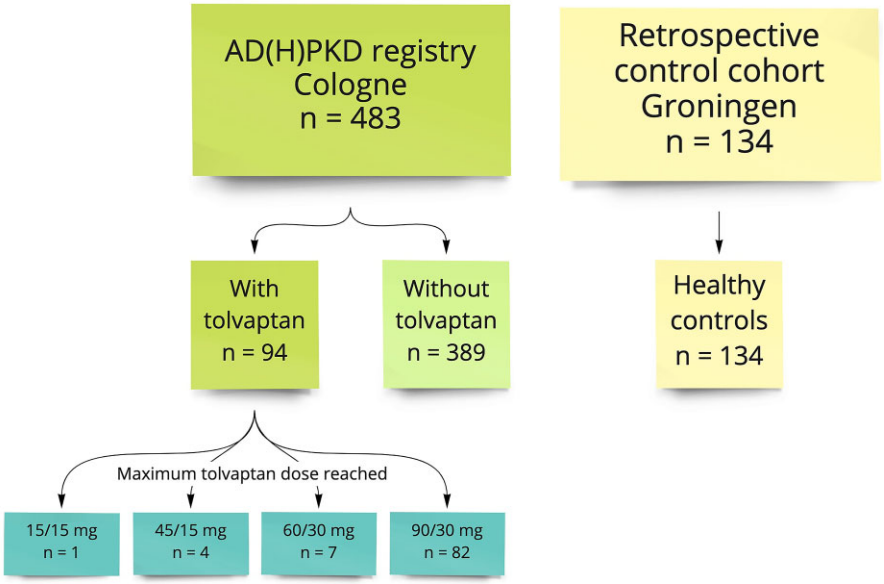

Figure 1: Study flow chart. The maximum tolvaptan dose reached shows individual unique patients reaching the respective maximum dose during the observation period. Copeptin levels were also measured at several time points or other dose steps for these patients resulting in 4 values for 15/15 mg, 9 for 45/15 mg, 27 for 60/30 mg and 139 for 90/30 mg in total.

(Fig. 2f) and increased with loss of kidney function starting in CKD stage 3 (Fig. 2g).

In longitudinal analyses in tolvaptan-naïve ADPKD patients, copeptin was shown to be stable over a follow-up time of up to 3 years (Fig. 3a). To assess the degree of fluctuations of copeptin levels over time in individual patients we analyzed the annual change of copeptin levels including patients with copeptin measurements with and without tolvaptan therapy (Fig. 3b). Here, patients without tolvaptan showed a non- significant higher relative annual change of copeptin.

Copeptin levels were significantly higher in patients taking tolvaptan than in participants not taking tolvaptan at the time of sampling (Supplementary data, Fig. S4A, median copeptin level 21 pmol/L vs 5.8 pmol/L). No difference between the three dosing steps of tolvaptan was observed (Supplementary data, Fig. S4B). However, the small number of measurements at some of the doses as well as the fact that the dose steps contain different subsets of patients need to be noted. Despite increasing copeptin levels in most patients after starting tolvaptan therapy, a drop in copeptin levels was observed in four patients (Supplementary data, Fig. S4C). Changes in copeptin associated with tolvaptan dose changes in individual patients are shown in Supplementary data, Fig. S5 and again revealed a sharp increase upon the change to any dose of tolvaptan but no dose-dependency.

Furthermore, we assessed whether copeptin was associated with factors known to be indicative of rapid progression in tolvaptan-naïve ADPKD patients. Copeptin increased with increasing Mayo Class (Fig. 4a), a classification used to assess the risk of progression based on the patient's height-adjusted TKV (htTKV) and age. Supplementary data, Fig. S1 shows the association between serum copeptin and TKV itself. No significant differences were detected between groups with early and late onset of arterial hypertension (including patients without arterial hypertension) (Fig. 4b). The same applied to patients with early or late onset of urological complications (including patients without urological complications) (Fig. 4c). This is also the case when analyzing CKD G1 and CKD G2–5 patients separately (Supplementary data, Fig. S3).

The disease-causing genetic variant was available for 221 of 389 tolvaptan-naïve patients with ADPKD. Kidney function was similar in patients with and without truncating PKD1 and PKD2 variants (Fig. 4d). Truncating *PKD1* variants showed a non-significant tendency towards higher copeptin compared with both non-truncating *PKD1* and *PKD2* variants (Fig. 4e). The PROPKD score has been shown to be a predictor of renal survival in ADPKD. Serum copeptin did not change with increasing PROPKD score (Fig. 4f and g).

In the resulting 133 patients with a total of 1467 values (minimum 3, median 8, maximum 34 creatinine values), the mean time between copeptin measurement and first historical creatinine value was 4.9 years (median 4.9, maximum 9 years). Based on these data, copeptin levels were significantly higher per increasing quartile of yearly eGFR loss and copeptin showed a significant correlation with the delta eGFR after 2 years (Fig. 5a, Supplementary data, Table S3). The effect of copeptin was not driven by differences in baseline eGFR as confirmed by a linear regression model containing baseline eGFR (Supplementary data, Table S4). The Mayo Classification is the most prevalent tool for patient stratification at present. Considering mean eGFR loss by Mayo Class, the Mayo model again proved to be an effective method for classifying patients according to disease severity (Fig. 5b). The Mayo model can also be used to estimate future eGFR. Adding copeptin to the Mayo model improved future eGFR estimation and copeptin was significant in this model (see Table 2). This is also the case regarding the estimation of future eGFR slopes (see Table 3).

We then went on to determine whether copeptin could serve as an independent variable to explain kidney function loss in addition to htTKV and different clinical variables. As a first step, we used htTKV to develop a linear regression model to explain the eGFR slope, confirming htTKV as a significant factor ($R^2 = 0.06$, Model I, Table 4). Inclusion of copeptin improved this model

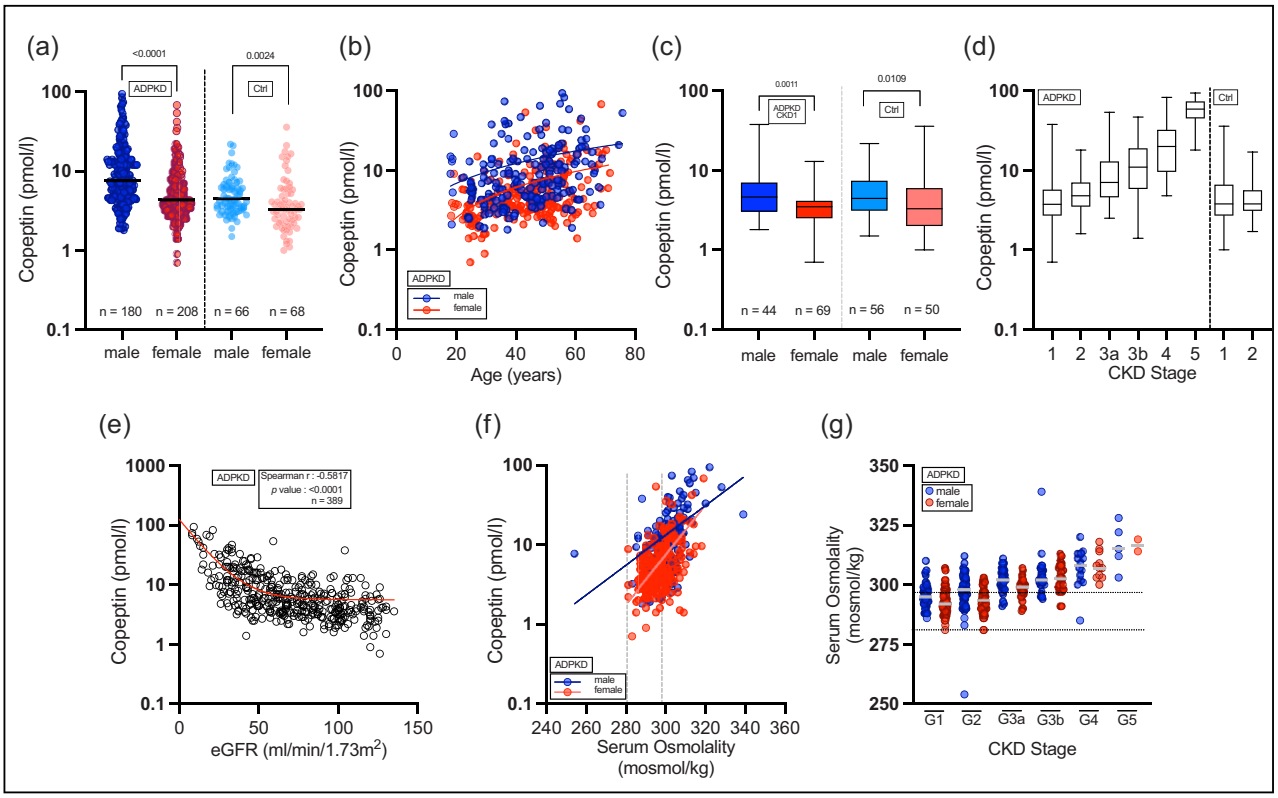

Figure 2: Association with clinical characteristics known to influence copeptin levels. All graphs include only values of measurements obtained without tolvaptan. (**a**) Sex differences of copeptin levels in ADPKD patients and healthy controls. Black line indicates median. (**b**) Correlation of copeptin and age in male (Spearman r = 0.30, $P < .0001$) and female (Spearman r = 0.35, $P < .0001$) ADPKD patients. (**c**) Copeptin levels for ADPKD patients and healthy controls with maintained kidney function (eGFR ≥90 mL/min/1.73 m²) divided by sex. Only significant differences are shown. (**d**) Copeptin according to CKD stages in tolvaptan-naïve ADPKD patients and healthy controls. Supplementary data, Table S1 shows statistics for (d). Supplementary data, Table S1 shows significant differences among CKD stages. (**e**) Correlation of copeptin with eGFR in ADPKD patients. (**f**) Relationship between copeptin and serum osmolality in male (Spearman correlation r = 0.50, $P < .0001$) and female (Spearman correlation r = 0.46, $P < .0001$) ADPKD patients. Non-linear regression (exponential growth) was performed for both sexes (continuous line). The vertical dotted lines show the reference range of serum osmolality (280–296 mOsm/L). (**g**) Relationship between CKD stages and serum osmolality. The horizontal dotted lines show the reference range of serum osmolality (280–296 mOsm/L). Supplementary data, Table S2 shows significant changes between individual CKD stages among genders.

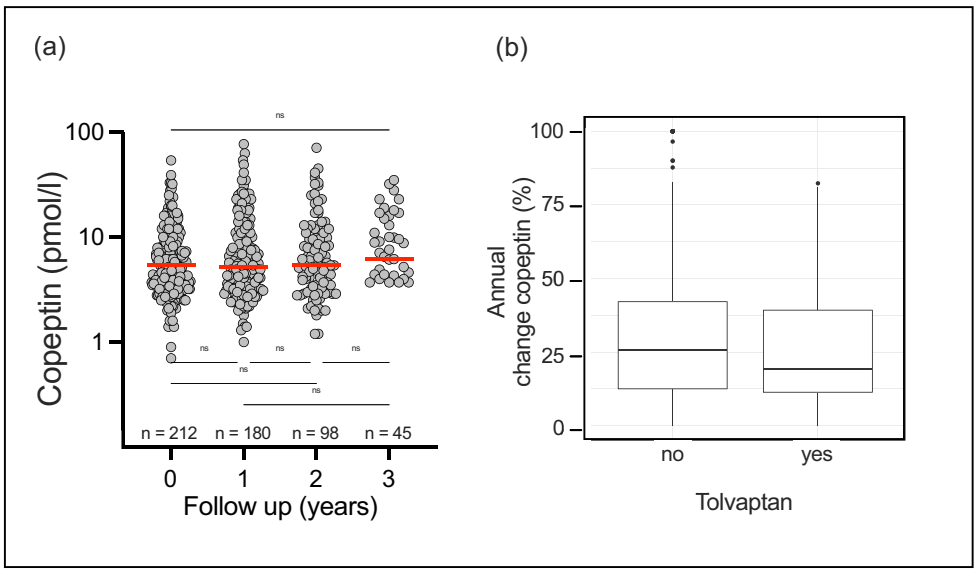

Figure 3: Treatment with tolvaptan leads to a marked increase in copeptin levels. (**a**) Stability of copeptin values over time in tolvaptan-naïve ADPKD patients, where at least one follow-up was available. Red line indicates median. (**b**) Annual change (%) of serum copeptin levels, including patients with copeptin measurements with and without tolvaptan therapy. The differences between both groups were not significant ($P = .15$) The line indicates median percentage change.

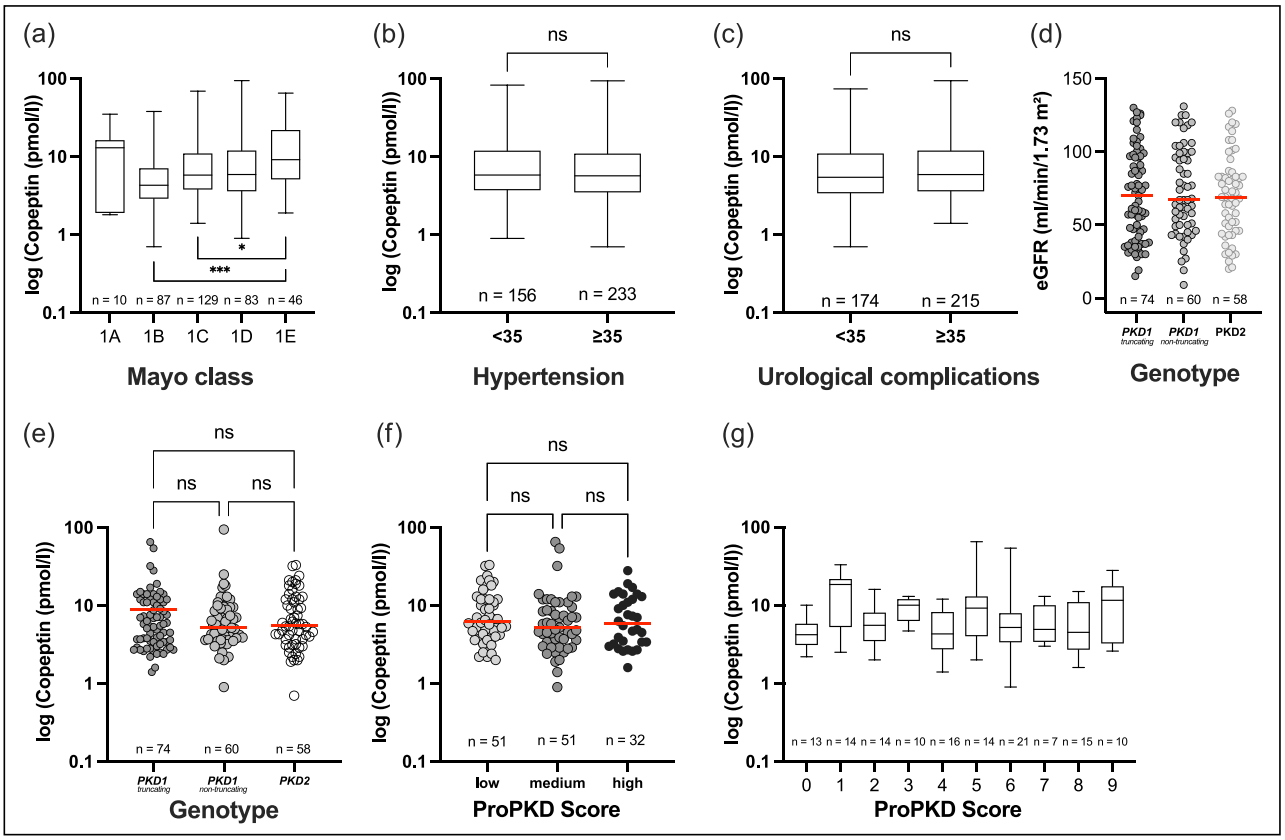

Figure 4: Copeptin and clinical criteria for rapid disease progression in ADPKD. Only values from patients not taking tolvaptan at the time of sampling are shown. (**a**) Serum copeptin levels between different Mayo Classes (1B vs 1E $P = .0001$, 1C vs 1E $P = .0334$). (**b**, **c**) Serum copeptin levels in relation to age at onset of hypertension (b) and urological complications (c). These analyses included only patients that had reached 35 years of age at the time of the analysis and the group with complication onset ≥35 years also contains all patients not suffering from arterial hypertension or urological complications. Urological complications included hematuria, cyst infection, flank pain and kidney stones. (**d**) eGFR distribution in ADPKD patients with either truncated or non-truncated PKD1 or PKD2. (**e**) Copeptin levels between respective mutation types in *PKD1* and *PKD2*. Truncating mutations, including nonsense, frameshift, splicing mutations, large rearrangements and non-truncating mutations, including missense mutations and in-frame short deletions and insertions. (**f**) Risk of progression to end-stage renal disease (ESRD) was determined using the ProPKD Score classes (low = 0–3, medium = 4–6, high = 7–9). (**g**) Copeptin levels among different ProPKD scores. Error bars indicate standard deviation. Significant differences are indicated by asterisks; not significant (ns).

Table 2: Comparison between the Mayo Classification model and a new extended model with added copeptin values for estimation of future eGFR.

Characteristics	All available eGFR values ($n = 451$ patients)				Prospective eGFR values ($n = 388$ patients)			
Model	Mayo + Copeptin		Mayo		Mayo + Copeptin		Mayo	
R^2	0.9177		0.863		0.945		0.924	
	Parameter	P-value	Parameter	P-value	Parameter	P-value	Parameter	P-value
Mayo-predicted eGFR	0.9348	2.00E-16	0.9502	2.00E-16	0.9846	2.00E-16	1.0339	2.00E-16
Log(copeptin)	−3.0322	2.00E-16			−1.8816	7.65E-07		

The table compares the Mayo Classification model and an extended model incorporating copeptin values for predicting future eGFR, using two approaches. On the left side all available eGFR values were used and the copeptin level was combined with the Mayo equation to estimate eGFR 1 year later for each individual value (left side). Since the use of historical eGFR values does not reflect the real-life situation, we added an analysis in which only eGFR values prospective to the copeptin measurements were used (right side).

($R^2 = 0.12$), but only copeptin remained significant as independent variable (Model II, Table 4). When further adding eGFR, age and gender to this model, again only copeptin remained significant (Model III, Table 4, $R^2 = 0.16$).

We then asked the question of whether a model merely depending on clinical characteristics (eGFR, age and gender) and copeptin without the need for MRIs could perform similarly well (Model IV, Table 4). With an adjusted R^2 of 0.12, this purely clinical model is nearly as good as Model III. Since ADPKD-independent mechanisms [e.g. acute kidney injury (AKI)] may lead to slopes with high fluctuations we applied the same models in a cohort fulfilling a slope threshold

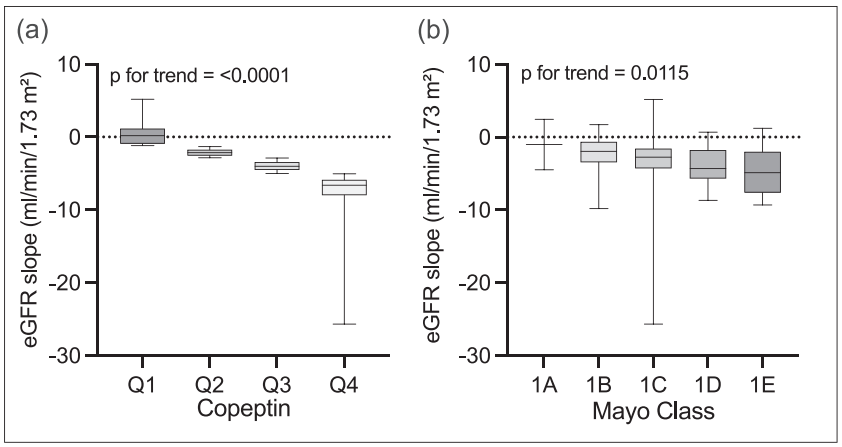

Figure 5: eGFR slope in relation to copeptin (**a**) and Mayo Classes (**b**). Only values from tolvaptan-naïve patients at the time of sampling are shown. The mean eGFR loss for the respective Mayo Classes was 1A: −1.0 mL/min/1.73 m^2, 1B: −2.2 mL/min/1.73 m^2, 1C: −3.3 mL/min/1.73 m^2, 1D: −3.8 mL/min/1.73 m^2, 1E: −4.6 mL/min/1.73 m^2.

Table 3: Comparison between the Mayo Classification model (Model V) and a new extended model (Model IV) that includes copeptin values regarding the prediction of future eGFR slopes ($n = 133$).

	Estimate	Std error	Pr(>|t|)
Model V: slope∼mayo_slope Adjusted R^2: 0.02, *P*-value: .05			
mayo_slope	0.49	0.24	0.05
Model VI: slope∼mayo_slope + copeptin Adjusted R^2: 0.11, *P*-value: .0001			
mayo_slope	0.27	0.24	0.26
log(copeptin)	−1.57	0.41	0.0002

Table 4: Linear regression models for eGFR slope prediction ($n = 133$).

	Estimate	Std error	Pr(>|t|)
Model I: slope∼htTKV Adjusted R^2: 0.06, *P*-value: .003			
log(htTKV)	−1.47	0.48	−0.003
Model II: slope∼htTKV + copeptin Adjusted R^2: 0.1165, *P*-value: .000118			
log(htTKV)	−0.64	0.54	0.24
log(copeptin)	−1.52	0.49	0.003
Model III: slope∼htTKV + copeptin + eGFR + age + gender Adjusted R^2: 0.16, *P*-value: .0003			
log(htTKV)	−0.53	0.56	0.34
log(copeptin)	−1.17	0.58	0.04
eGFR	0.03	0.02	0.09
age	0.05	0.04	0.17
gender	−0.64	0.62	0.31
Model IV: slope∼copeptin + eGFR + age + gender Adjusted R^2: 0.12, *P*-value: .0002			
log(copeptin)	−1.32	0.56	0.02
eGFR	0.04	0.02	0.05
age	0.05	0.04	0.16
gender	−0.54	−0.88	0.38

For Model III, the slope was computed using the current and subsequent year's eGFR values.

removing the strongest outliers (i.e. delta eGFR between −30 and +15 mL/min/1.73 m^2/year). In this subcohort (110 of 133 patients included), both the optimal model (Supplementary data, Table S5, Model IIIs, $R^2 = 0.29$) and the clinical model (Supplementary data, Table S5, Model IVs, $R^2 = 0.34$) showed considerably higher performance.

Since the disease-causing mutation was only available in 221 of 389 tolvaptan-naïve patients, we tested the impact of genotype on the explanation of outcome separately. In this subcohort, adding genetic information to the optimal or clinical model did not result in a significant improvement (Supplementary data, Table S6, Model IIIa, IIIb, IVa and IVb). When applying the slope threshold and including the genetic information, both, the optimal model (Model IIId, Supplementary data, Table S7, $R^2 = 0.43$) and the clinical model (Model IVd, Supplementary data, Table S7, $R^2 = 0.34$) were improved significantly.

DISCUSSION

Prediction of disease progression in ADPKD is challenging and is currently based on established biomarkers such as TKV. The identification of new biomarkers and risk factors is important to accurately predict prognostic statements and facilitate patient selection regarding tolvaptan therapy. Furthermore, new biomarkers should allow the assessment of long-term treatment responses. In this study we explored the role potential of copeptin, as part of the precursor protein pre-pro-vasopressin. Copeptin is produced in equimolar amounts [22] and thus a validated surrogate parameter to plasma vasopressin [23].

Existing information on copeptin in patients with ADPKD comes mostly from a *post hoc* analysis of the interventional TEMPO 3:4 trial [10], and all studies from a real-world setting are too small to be considered conclusive and do not provide a homogenous picture [16–20, 24]. However, real-world data are necessary because copeptin values are expected to vary depending on factors such as sampling technique. Consequently, our study of 483 ADPKD patients represents a larger sample size that provides real-world data. We were able to confirm previous results by Gansevoort *et al.* [10], showing both clear eGFR dependency of copeptin levels and higher values in male

patients with ADPKD. In ADPKD, males tend to show a faster age-dependent eGFR decline and thus male gender is viewed as a risk factor for disease progression [4]. Consequently, the finding could either be a consequence of worse kidney function in men or copeptin/vasopressin could vice-versa be a factor that explains sex-dependent differences regarding disease progression. Hence, we investigated whether copeptin is higher in male CKD1 patients with ADPKD than in healthy male kidney donors, which was not the case in our analysis. Comparing CKD1 patients with ADPKD of both genders with healthy controls with a GFR >90 mL/min/1.73 m^2 yielded no significant differences. Importantly, in contrast to our results, previous work by Zittema *et al.* [20]. had found higher copeptin levels in patients with ADPKD compared with the same healthy kidney donor cohort with males showing higher copeptin levels in both groups. However, a direct comparison between males of both groups was not carried out and patients with ADPKD with an impaired kidney function were included. These different findings may be explained by differences in kidney function between ADPKD cohorts and the retrospective use of data from the healthy cohort in the present study. Beyond this, no uniform picture emerges from the published literature: while some studies found significantly higher copeptin levels in patients with ADPKD compared with healthy controls [16], others found only non-significant tendencies to higher copeptin levels at baseline [25] or no significant differences at all [24]. Usually, gender-specific comparisons between ADPKD and healthy individuals were not conducted [16, 24, 25].

As Morgenthaler *et al*. found no major differences in copeptin values in different age groups of their healthy cohort [23], the age-dependency of copeptin in our study might be explained by the progressive deterioration of kidney function in ADPKD. However, Morgenthaler *et al*. found significantly higher copeptin values in healthy males than in females [23]. The comparison of male and female patients with a maintained kidney function in our cohort confirmed the sex-dependency of copeptin levels.

Due to disruption of the medullary architecture secondary to cyst formation and renal insufficiency, patients with ADPKD experience an impairment of their urine concentrating capacity possibly resulting from an impaired osmolar gradient and a deregulation of the vasopressin–cAMP–osmolality axis [26]. As plasma osmolality rises, vasopressin secretion is increased to maintain the overall water balance [27]. Copeptin levels can decrease rapidly [23] suggesting extrarenal clearance as predominant clearance mechanism. As expected, serum osmolality showed a positive correlation with copeptin. Besides, increased serum osmolality became apparent in patients with ADPKD from CKD stage G3a and higher and was accompanied by rises in copeptin. Higher copeptin values in later stage diseases and in people at risk for rapid disease progression can result from impaired urine concentrating capacity. Also, an increase in salt intake leads to an increase in serum osmolality and therefore to an increase of vasopressin secretion [28]. In general, increased vasopressin levels can lead to glomerular hyperfiltration which can lead to a progressive deterioration in all forms of CKD while in ADPKD, the aforementioned vasopressin-related cyst formation can cause further renal function loss [29]. In the past, several studies found evidence of an association between salt intake or sodium excretion and disease progression in patients with ADPKD [30]. Although studies that only included patients with early-stage ADPKD [CRISP (Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease) cohort, *post hoc* HALT Progression of Polycystic Kidney Disease (HALT-PKD) Study A] [31, 32] did not show a clear effect on renal function loss, both studies found an association between salt intake and TKV increase. In contrast, studies including patients with advanced disease found an association between salt intake and eGFR loss (*post hoc* HALT-PKD B, Kramers *et al.* [32, 33]).

To gain a preliminary understanding of whether high copeptin levels may indicate rapid progression, we examined the association between copeptin and established progression factors. In the earliest stages of ADPKD, copeptin levels have been shown to increase, according to previous research [25]. Despite age differences between the two cohorts, no difference in copeptin between controls and CKD1 patients was found and further no correlation between copeptin and age was found (Supplementary data, Fig. S6A).

Serum copeptin positively correlated with increasing TKV and Mayo Classes. However, no association with the PROPKD score and its individual components was found. At first sight, it was unexpected to see a lack of correlation between PROPKD score and copeptin levels, in contrast to future eGFR and TKV. This could be a consequence of limited cohort size with availability of genotype, a general problem in routine care in Germany. However, it is also intriguing to speculate that copeptin may carry information that goes beyond genotype (e.g. environmental factors) and that may partly explain the intrafamilial variability in ADPKD [34]. This question will be an interesting topic for future studies. ADPKD is genetically heterogeneous with two genes being responsible for the majority of mutations: *PKD1* encoding polycystin-1 (around 85% of cases) and *PKD2* encoding polycystin-2 (around 15% of cases) [35]. To exclude whether patients with ADPKD harbor truncated *PKD1* already progressed to an advanced disease stage and therefore showed higher copeptin levels, we compared eGFR values between both truncated and non-truncated *PKD1* patient groups and did not detect a significant difference.

If copeptin is to be used to guide patient selection for targeted therapies, further evidence whether copeptin levels improve current predictive algorithms for disease progression of ADPKD is required. An earlier study showed that baseline copeptin predicts TKV growth and eGFR decline [10]. Since only one MRI was available for the majority of the patients in the study at hand, we focused our analyses regarding the capacity of copeptin to explain the future eGFR slope.

Our linear model confirms copeptin as an independent variable to explain the future eGFR slope and this finding was independent from baseline eGFR, age and htTKV. Copeptin was indeed independently associated with the eGFR slope for the optimal model including eGFR, htTKV and copeptin ($R^2 = 0.13$), a result in a similar range compared to the previously published *post hoc* analysis of the TEMPO 3:4 trial [10]. The explanation of future eGFR slope was considerably improved (optimal model $R^2 = 0.30$, clinical model $R^2 = 0.34$) when applying a slope threshold to exclude eGFR slopes that were most likely caused by ADPKD-independent mechanisms (e.g. AKI). Adding the genetic mutation to the models did not significantly improve them, which could be a consequence of the fact that this information was only available for a subgroup of patients and suggests that additional data are necessary to develop a valid model connecting the eGFR slope and, e.g. the PROPKD score.

Since the Mayo Classification is frequently used in clinical practice to assess the risk of disease progression, we also tested a combination with this model and found a significant impact of copeptin. While the Mayo Classification was again very useful for stratifying patients into risk classes in our cohort, the explanation of future eGFR slopes using this model showed limited

accuracy which is in line with previously published literature [36]. The explanation of eGFR slopes could also be improved by adding copeptin to the model.

We further investigated the impact of tolvaptan on serum copeptin levels and detected higher copeptin levels under tolvaptan intake, which is in line with previous findings and well explained by V_2R-antagonism [10]. Gansevoort *et al.* has also shown that copeptin may be a predictor for the response to tolvaptan [10]. Patients with higher baseline copeptin showed a larger effect size, in terms of both eGFR loss per year and TKV increase. The association with therapy response was even closer when analyzing the copeptin increase after treatment association. It will be interesting to see whether this result can also be confirmed in the real-world setting. However, at this point, the data from our cohort do not provide sufficient follow-up for patients taking tolvaptan to answer this question.

In four patients an unexpected decrease of serum copeptin levels was detected. Consequently, we looked at clinical characteristics that might explain this decline, however no clinical abnormalities were found. Indeed, possible reasons for the decline in serum copeptin levels could be (i) non-compliance (meaning suspension of tolvaptan or mentioning of wrong time point or (ii) additional intake of other medication that might influence metabolization.

The findings of this study have to be seen in the light of the following limitations: creatinine measurements were conducted at various laboratory sites resulting in potential technical fluctuations. Furthermore, the number of creatinine values obtained to calculate the eGFR slope is limited in a subset of patients. Estimation of disease severity was done by estimating eGFR slopes using the baseline copeptin measurement. Importantly, the discordance between eGFR and measured GFR, which can be up to ±30% according to various cross-sectional studies presented in a meta-analysis by Porrini *et al.* [37], must be noted. As not all patients provided consent for genetic analysis, generation of a prediction model including the PROPKD score resulted in a small cohort and a low predictive value, and was therefore not included in the present analysis. Patients were asked to present to our outpatient clinic after overnight fasting for sampling of copeptin levels. However, this advice may not always have been followed in the real-life setting. Besides, data on the disease-causing genetic variant was not available in all patients.

In conclusion, our findings underline the GFR dependency of copeptin levels as well as its predictive capacity of eGFR slope in the real-world setting. The fact that a model based merely on clinical parameters including copeptin resulted in an accuracy comparable to models including imaging and genetic data is of interest but will require additional confirmation. Importantly, all models explain variation only to a rather limited degree, underlining that additional parameters such as MCP1 and a holistic approach using as much information as possible will be required to optimize risk prediction in ADPKD.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

ACKNOWLEDGEMENTS

We thank Dr Ingrid Becker for her support. Also, we thank Cornelia Böhme and Israa Karimov for excellent technical support. Database generation was supported by “clinicalsurveys.net” (Sebastian Heimann, Jörg Janne Vehreschild).

CONFLICT OF INTEREST STATEMENT

The study was supported by research funding from ThermoFisher Scientific. R.T.G. reports having received grants and consultation fees from Otsuka Pharmaceutical (the producer of tolvaptan) and ThermoFisher Scientific (the producer of the copeptin assay). All money was paid to his employing institution. The Department II for Internal Medicine (UHC) received research funding from Otsuka Pharmaceutical. R.-U.M. is member of the scientific advisory board at Santa Barbara Nutrients. The results presented in this paper have not been published previously in whole or part, except in abstract format. All other authors declare no conflict of interest.

AUTHORS’ CONTRIBUTIONS

R.-U.M. and F.G. conceptualized the study. S.A., S.O. and P.T. performed the investigation. P.A., S.O. and S.A. were involved in data curation, analysis, validation and visualization, and performed statistical analysis. R.-U.M. and F.G. acquired funding. R.T.G. and S.J.L.B. provided the data of the healthy control cohort. F.E. performed genetic analyses. S.A. and S.O. wrote the original manuscript. All authors edited and reviewed the final manuscript.

FUNDING

This project received funding through the Köln Fortune Program (Faculty of Medicine, University of Cologne) to F.G. R.-U.M. was supported by the Ministry of Science North Rhine-Westphalia (Nachwuchsgruppen.NRW 2015-2021) and the German Research Foundation (CRU 329, DFG MU 3629/6-1, DFG DI 1501/9, DFG MU 3629/3-1). We acknowledge support for the Article Processing Charge from the DFG (German Research Foundation, 491454339).

DATA AVAILABILITY STATEMENT

The data underlying this article cannot be shared publicly for the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

REFERENCES

1. Chapman AB, Devuyst O, Eckardt KU *et al.* Autosomal-dominant polycystic kidney disease (ADPKD): executive summary from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int* 2015;**88**:17–27. https://doi.org/10.1038/ki.2015.59
2. Ong AC, Devuyst O, Knebelmann B *et al.* Autosomal dominant polycystic kidney disease: the changing face of clinical management. *Lancet* 2015;**385**:1993–2002. https://doi.org/10.1016/S0140-6736(15)60907-2
3. Muller RU, Benzing T. Management of autosomal-dominant polycystic kidney disease-state-of-the-art. *Clin Kidney J* 2018;**11**:i2–i13. https://doi.org/10.1093/ckj/sfy103
4. Gabow PA, Johnson AM, Kaehny WD *et al.* Factors affecting the progression of renal disease in autosomal-dominant polycystic kidney disease. *Kidney Int* 1992;**41**:1311–9. https://doi.org/10.1038/ki.1992.195
5. Torres VE, Chapman AB, Devuyst O *et al.* Tolvaptan in patients with autosomal dominant polycystic kidney

disease. *N Engl J Med* 2012;**367**:2407–18. https://doi.org/10.1056/NEJMoa1205511
6. Torres VE, Chapman AB, Devuyst O *et al.* Tolvaptan in later-stage autosomal dominant polycystic kidney disease. *N Engl J Med* 2017;**377**:1930–42. https://doi.org/10.1056/NEJMoa1710030
7. Muller RU, Benzing T. Cystic kidney diseases from the adult nephrologist's point of view. *Front Pediatr* 2018;**6**:65. https://doi.org/10.3389/fped.2018.00065
8. Gansevoort RT, Arici M, Benzing T *et al.* Recommendations for the use of tolvaptan in autosomal dominant polycystic kidney disease: a position statement on behalf of the ERA-EDTA Working Groups on Inherited Kidney Disorders and European Renal Best Practice. *Nephrol Dial Transplant* 2016;**31**:337–48. https://doi.org/10.1093/ndt/gfv456
9. Irazabal MV, Rangel LJ, Bergstralh EJ *et al.* Imaging classification of autosomal dominant polycystic kidney disease: a simple model for selecting patients for clinical trials. *J Am Soc Nephrol* 2015;**26**:160–72. https://doi.org/10.1681/ASN.2013101138
10. Gansevoort RT, van Gastel MDA, Chapman AB *et al.* Plasma copeptin levels predict disease progression and tolvaptan efficacy in autosomal dominant polycystic kidney disease. *Kidney Int* 2019;**96**:159–69. https://doi.org/10.1016/j.kint.2018.11.044
11. Belibi FA, Reif G, Wallace DP *et al.* Cyclic AMP promotes growth and secretion in human polycystic kidney epithelial cells. *Kidney Int* 2004;**66**:964–73. https://doi.org/10.1111/j.1523-1755.2004.00843.x
12. Reif GA, Yamaguchi T, Nivens E *et al.* Tolvaptan inhibits ERK-dependent cell proliferation, Cl(-) secretion, and in vitro cyst growth of human ADPKD cells stimulated by vasopressin. *Am J Physiol Renal Physiol* 2011;**301**:F1005–13. https://doi.org/10.1152/ajprenal.00243.2011
13. Rinschen MM, Schermer B, Benzing T. Vasopressin-2 receptor signaling and autosomal dominant polycystic kidney disease: from bench to bedside and back again. *J Am Soc Nephrol* 2014;**25**:1140–7. https://doi.org/10.1681/ASN.2013101037
14. Christ-Crain M, Fenske W. Copeptin in the diagnosis of vasopressin-dependent disorders of fluid homeostasis. *Nat Rev Endocrinol* 2016;**12**:168–76. https://doi.org/10.1038/nrendo.2015.224
15. Meijer E, Bakker SJ, Halbesma N *et al.* Copeptin, a surrogate marker of vasopressin, is associated with microalbuminuria in a large population cohort. *Kidney Int* 2010;**77**:29–36. https://doi.org/10.1038/ki.2009.397
16. Raptis V, Loutradis C, Boutou AK *et al.* Serum copeptin, NLPR3, and suPAR levels among patients with autosomal-dominant polycystic kidney disease with and without impaired renal function. *Cardiorenal Med* 2020;**10**:440–51. https://doi.org/10.1159/000510834
17. Boertien WE, Meijer E, Li J *et al.* Relationship of copeptin, a surrogate marker for arginine vasopressin, with change in total kidney volume and GFR decline in autosomal dominant polycystic kidney disease: results from the CRISP cohort. *Am J Kidney Dis* 2013;**61**:420–9. https://doi.org/10.1053/j.ajkd.2012.08.038
18. Boertien WE, Meijer E, Zittema D *et al.* Copeptin, a surrogate marker for vasopressin, is associated with kidney function decline in subjects with autosomal dominant polycystic kidney disease. *Nephrol Dial Transplant* 2012;**27**:4131–7. https://doi.org/10.1093/ndt/gfs070
19. Lacquaniti A, Chirico V, Lupica R *et al.* Apelin and copeptin: two opposite biomarkers associated with kidney function decline and cyst growth in autosomal dominant polycystic kidney disease. *Peptides* 2013;**49**:1–8. https://doi.org/10.1016/j.peptides.2013.08.007
20. Zittema D, van den Berg E, Meijer E *et al.* Kidney function and plasma copeptin levels in healthy kidney donors and autosomal dominant polycystic kidney disease patients. *Clin J Am Soc Nephrol* 2014;**9**:1553–62. https://doi.org/10.2215/CJN.08690813
21. Levey AS, Stevens LA, Schmid CH *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;**150**:604–12. https://doi.org/10.7326/0003-4819-150-9-200905050-00006
22. de Bree FM, Burbach JP. Structure-function relationships of the vasopressin prohormone domains. *Cell Mol Neurobiol* 1998;**18**:173–91. https://doi.org/10.1023/A:1022564803093
23. Morgenthaler NG, Struck J, Alonso C *et al.* Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clin Chem* 2006;**52**:112–9. https://doi.org/10.1373/clinchem.2005.060038
24. Corradi V, Martino F, Gastaldon F *et al.* Copeptin levels and kidney function in ADPKD: case-control study. *Clin Nephrol* 2016;**86**:147–53. https://doi.org/10.5414/CN108894
25. Zittema D, Boertien WE, van Beek AP *et al.* Vasopressin, copeptin, and renal concentrating capacity in patients with autosomal dominant polycystic kidney disease without renal impairment. *Clin J Am Soc Nephrol* 2012;**7**:906–13. https://doi.org/10.2215/CJN.11311111
26. Ho TA, Godefroid N, Gruzon D *et al.* Autosomal dominant polycystic kidney disease is associated with central and nephrogenic defects in osmoregulation. *Kidney Int* 2012;**82**:1121–9. https://doi.org/10.1038/ki.2012.225
27. Devuyst O, Torres VE. Osmoregulation, vasopressin, and cAMP signaling in autosomal dominant polycystic kidney disease. *Curr Opin Nephrol Hypertens* 2013;**22**:459–70. https://doi.org/10.1097/MNH.0b013e3283621510
28. Zerbe RL, Robertson GL. Osmoregulation of thirst and vasopressin secretion in human subjects: effect of various solutes. *Am J Physiol* 1983;**244**:E607–14.
29. Bankir L, Bichet DG. What can copeptin tell us in patients with autosomal dominant polycystic disease? *Kidney Int* 2019;**96**:19–22. https://doi.org/10.1016/j.kint.2019.02.037
30. Torres VE. Salt, water, and vasopressin in polycystic kidney disease. *Kidney Int* 2020;**98**:831–4. https://doi.org/10.1016/j.kint.2020.06.001
31. Torres VE, Grantham JJ, Chapman AB *et al.* Potentially modifiable factors affecting the progression of autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol* 2011;**6**:640–7. https://doi.org/10.2215/CJN.03250410
32. Torres VE, Abebe KZ, Schrier RW *et al.* Dietary salt restriction is beneficial to the management of autosomal dominant polycystic kidney disease. *Kidney Int* 2017;**91**:493–500. https://doi.org/10.1016/j.kint.2016.10.018
33. Kramers BJ, Koorevaar IW, Drenth JPH *et al.* Salt, but not protein intake, is associated with accelerated disease progression in autosomal dominant polycystic kidney disease. *Kidney Int* 2020;**98**:989–98. https://doi.org/10.1016/j.kint.2020.04.053
34. Harris PC, Hopp K. The mutation, a key determinant of phenotype in ADPKD. *J Am Soc Nephrol* 2013;**24**:868–70. https://doi.org/10.1681/ASN.2013040417

35. Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. *Lancet* 2007;**369**:1287–301. https://doi.org/10.1016/S0140-6736(07)60601-1
36. Borrego Utiel FJ, Esteban de la Rosa RJ, Merino Garcia E *et al*. Predicting future renal function decline in patients with autosomal dominant polycystic kidney disease using Mayo Clinic classification. *Am J Nephrol* 2021;**52**:630–41. https://doi.org/10.1159/000518255
37. Porrini E, Ruggenenti P, Luis-Lima S *et al*. Estimated GFR: time for a critical appraisal. *Nat Rev Nephrol* 2019;**15**:177–90. https://doi.org/10.1038/s41581-018-0080-9