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Plasma copeptin levels predict disease progression and tolvaptan efficacy in autosomal dominant polycystic kidney disease

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Abstract

In the TEMPO 3:4 Trial, treatment with tolvaptan, a vasopressin V2 receptor antagonist, slowed the increase in total kidney volume and decline in estimated glomerular filtration rate (eGFR) in autosomal dominant polycystic kidney disease (ADPKD). We investigated whether plasma copeptin levels, a marker of plasma vasopressin, are associated with disease progression, and whether pre-treatment copeptin and treatment-induced change in copeptin are associated with tolvaptan treatment efficacy. This post hoc analysis included 1,280 TEMPO 3:4 participants (aged 18–50 years, estimated creatinine clearance ≥ 60 ml/min and total kidney volume ≥ 750 mL) who had plasma samples available at baseline for measurement of copeptin using an automated immunofluorescence assay. In placebo-treated subjects, baseline copeptin predicted kidney growth and eGFR decline over 3 years. These associations were independent of sex, age, and baseline eGFR, but were no longer statistically significant after additional adjustment for baseline total kidney volume. In tolvaptan-treated subjects, copeptin increased from baseline to week 3 (6.3 pmol/L versus 21.9 pmol/L, respectively). In tolvaptan-treated subjects with higher baseline copeptin levels, a larger treatment effect was noted with respect to kidney growth rate and eGFR decline. Tolvaptan-treated subjects with a larger percentage increase in copeptin from baseline to

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DISCLOSURE

RTG, ABC, OD, RDP, and VET were members of the Steering Committee of the TEMPO 3:4 trial and are consultants for Otsuka Pharmaceutical Development & Commercialization, Inc. FSC, JDB, JL, and JO are employed by Otsuka Pharmaceutical Development & Commercialization, Inc. KS is employed by BRAHMS GmbH, a part of Thermo Fisher Scientific.

week 3 had a better disease outcome, with less kidney growth and eGFR decline after three years. Copeptin holds promise as a biomarker to predict outcome and tolvaptan treatment efficacy in ADPKD.

Keywords

ADPKD; AVP; copeptin; polycystic kidney disease; tolvaptan; vasopressin

Autosomal dominant polycystic kidney disease (ADPKD) is a progressive disorder, that leads to end-stage renal disease (ESRD) in the majority of affected patients, with a highly variable disease course between patients.¹ With the vasopressin V2 receptor antagonist tolvaptan, the first disease-modifying drug has recently become available.² It is important to select ADPKD patients for tolvaptan treatment that have a high likelihood of rapid disease progression, because these patients are expected to have the highest net benefit of disease-modifying drugs.³ Assessment of estimated glomerular filtration rate (eGFR) and total kidney volume (TKV) may be of help to select high-risk patients,^{3,4} but these biomarkers predict age at reaching ESRD with moderate specificity. Additional biomarkers are therefore needed to identify high-risk patients. Ideally, such biomarkers would also help to assess in individual patients shortly after the start of treatment whether this treatment will be effective for them in the long term.

Vasopressin signaling is increased in ADPKD and plays a pivotal role in the pathophysiology of the disease. Experimental studies have shown that vasopressin binding to the V2 receptor (V2R) causes an increase in intracellular cAMP concentration in renal collecting duct cells, leading to cyst formation and growth, and to renal function decline.^{5,6} Conversely, V2R antagonists lead to less cyst growth and better renal function outcome in polycystic kidney disease, in experimental studies^{7–11} as well as in a clinical trial.² Plasma copeptin levels, as validated surrogate for plasma vasopressin levels, are associated with ADPKD disease severity¹² and progression^{13–15} in single-center studies that were often of small scale. Shortly after the start of tolvaptan treatment plasma copeptin increases,¹⁶ reflecting via mechanisms the level of inhibition of vasopressin activity by this drug. Taken together, these observations suggest that plasma copeptin may be a valuable candidate biomarker to predict disease progression, and response to V2R antagonism in ADPKD.¹⁷

In the present study we aimed, therefore, to investigate in a large-scale multicenter study whether baseline copeptin is associated with ADPKD disease progression and tolvaptan treatment efficacy, and whether change in copeptin shortly after initiating tolvaptan treatment is associated with long-term outcome during this treatment.

RESULTS

Study participants

Baseline characteristics of the 1280 ADPKD patients included in this study are shown in Table 1, stratified according to sex-adjusted quartiles of baseline plasma copeptin level. Distribution of copeptin is shown in Supplementary Figure S1, with a median value of 6.4 (3.8 to 11.0) pmol/l, and higher values in males than females. Subjects with higher copeptin

had higher systolic and diastolic blood pressure, a higher body mass index as well as higher total kidney volume (TKV) and lower eGFR. In addition, a higher proportion of whites was noted per increasing quartile of copeptin. In a multivariate analysis, baseline copeptin was associated with age, sex, race, body mass index, plasma osmolality, eGFR, and TKV (all $P < 0.001$ except for body mass index, $P = 0.007$; Supplementary Table S1). Baseline characteristics specifically for subjects randomized to either placebo or tolvaptan are given in Supplementary Tables S2 and S3. In the placebo- and tolvaptan-treated subjects, median baseline copeptin was 6.3 (3.8 to 11.5) and 6.6 (3.8 to 10.5) pmol/l, respectively ($P = \text{NS}$). The interaction between copeptin and the treatment group for TKV growth was significant ($P = 0.008$), and for eGFR decline it was borderline significant ($P = 0.07$).

Copeptin versus ADPKD outcome in placebo-treated subjects

Subjects who received placebo treatment had an annual TKV growth rate of 5.5% (5.4%–5.7%) and an annual rate of eGFR decline of -3.7 (-3.8 to -3.6) ml/min per 1.73 m^2 . In placebo-treated subjects baseline copeptin was significantly associated with annual TKV growth ($P = 0.0004$; Table 2 and Figure 1, left panel). This association was independent of sex and age (Table 3, model 2). When additionally adjusted for baseline GFR or baseline TKV, the association of copeptin with annual TKV growth remained significant (Table 3, models 3 and 4). When sex, age, and both eGFR and baseline TKV were entered into the multivariate model, the association of baseline copeptin with annual TKV growth lost formal statistical significance ($P = 0.09$; Table 3, model 5).

A significant association was also found between baseline copeptin and annual eGFR decline in placebo-treated subjects when analyzed univariate ($P < 0.0001$; Table 2 and Figure 1, right panel). In multivariate analyses this association was independent of sex, age, and baseline eGFR (Table 4, models 2 and 3), but lost statistical significance after adding baseline TKV (Table 4, model 4).

When plasma osmolality was included instead of copeptin, the R-square values of the multivariate models explaining annual change in TKV and eGFR were lower and the associations of plasma osmolality with these outcomes was less strong (Supplementary Table S4). Copeptin also had stronger associations with increase in TKV than urine osmolality, whereas for decline in eGFR urine osmolality had slightly stronger associations (Supplementary Table S5).

In placebo-treated subjects with higher baseline copeptin, statistically fewer aquaretic adverse events were observed, especially less thirst, pollakiuria, and polydipsia. On the other hand, these subjects reported more ADPKD-related adverse events of hematuria and renal pain (Supplementary Table S6).

Copeptin versus ADPKD outcome in tolvaptan-treated subjects

Subjects who used tolvaptan had an annual decline in eGFR of -2.8 (-2.8 to -2.7) ml/min per 1.73 m^2 and an annual increase in TKV of 2.6% (2.3%–3.0%). In tolvaptan-treated subjects an association of baseline copeptin with annual decline in eGFR was observed ($P = 0.004$), but not with annual TKV growth ($P = 0.5$; Table 2).

In the tolvaptan-treated group 24.2% of subjects withdrew from the study, compared with 14.2% in the placebo-treated group ($P < 0.001$). Subjects that withdrew from tolvaptan treatment had higher baseline copeptin, but similar TKV and eGFR compared with subjects who completed the study (median: 7.9 [IQR: 4.9–13.4] versus 6.0 [IQR: 3.6–10.8], $P = 0.0001$; 1441 [IQR: 1077–1968] versus 1480 [IQR: 1077–2035], $P = 0.7$; and 80.8 ± 20.7 versus 83.2 ± 22.1 ml/min per 1.73 m^2 , $P = 0.1$, respectively). In tolvaptan-treated subjects, higher baseline copeptin was significantly associated with a higher discontinuation rate due to adverse events, but also with less thirst and pollakiuria (Supplementary Table S6). In tolvaptan-treated subjects no association was found between baseline copeptin and liver function test abnormalities (Supplementary Table S6).

Baseline copeptin and tolvaptan treatment efficacy

When compared to placebo, tolvaptan treatment led to a significant decrease in rate of TKV growth in all 4 copeptin quartiles, and this effect was stronger in subjects with higher sex-adjusted baseline levels of copeptin (P for trend = 0.001; Table 2 and Figure 2, left panel). As shown in Table 2, there were no differences in TKV growth rate between the 4 quartiles of copeptin in the tolvaptan-treated subjects. However, in placebo-treated subjects copeptin concentration showed a positive, log-linear association with TKV growth rate. In order to study the tolvaptan treatment effect, the TKV growth rates of tolvaptan-treated subjects were adjusted for the natural course of the disease, being the TKV growth rates in the placebo-treated subjects in the same quartile of copeptin. Tolvaptan treatment effect on TKV growth was stronger in subjects with higher baseline copeptin levels with a log-linear association (Figure 2, left panel). The interaction between baseline copeptin level and tolvaptan treatment effect was independent of baseline age, sex, eGFR, and TKV ($P = 0.8$, $P = 0.7$, $P = 0.8$, $P = 0.9$, respectively). eGFR decline was associated with baseline copeptin concentrations for both placebo-treated subjects, as well as tolvaptan-treated subjects ($P < 0.0001$ and $P = 0.004$, respectively). Tolvaptan treatment effect on annual decline in eGFR tended to be stronger in subjects with higher baseline levels of copeptin (P for trend 0.02; Table 2 and Figure 2, right panel). In subjects with higher baseline copeptin, tolvaptan compared with placebo treatment led to more discontinuations and more polydipsia, but less renal pain (Supplementary Table S6, last column). Of note, tolvaptan dose was not different between the 4 quartiles of baseline copeptin (at week 3 mean dosage was 111, 110, 106, and 106 mg/d, and at month 36 it was 97, 97, 95, and 97 mg/d).

Copeptin during tolvaptan treatment

Three weeks after starting treatment with tolvaptan, plasma copeptin levels were significantly higher compared with baseline (21.9 vs. 6.3 pmol/l, respectively, $P < 0.0001$; Figure 3). Copeptin in placebo-treated subjects decreased slightly but significantly during the first 3 weeks of treatment compared with baseline (6.6 vs. 6.0 pmol/l, respectively, $P = 0.02$). Copeptin levels were significantly different between tolvaptan- and placebo-treated subjects during treatment (all time points $P < 0.0001$). After withdrawal of both treatments, copeptin in previously tolvaptan-treated subjects decreased to 6.3 pmol/l, whereas in placebo-treated subjects it remained stable (7.0 pmol/l, difference with previously tolvaptan-treated subjects $P = 0.07$). After withdrawal of treatment, copeptin in tolvaptan-treated subjects was significantly lower compared with baseline ($P = 0.0007$), whereas in previously

placebo-treated subjects copeptin was significantly higher compared with baseline ($P < 0.0001$). A sensitivity analysis that included only subjects with available copeptin values at month 36 showed similar findings (data not shown). The correlation between baseline copeptin and copeptin after 3 weeks in placebo-treated subjects, an indication for intra-subject reproducibility, was high, with a Pearson correlation coefficient of 0.72 ($P < 0.0001$). The observed variability was smaller for fasting compared with nonfasting subjects (mean difference between baseline and week 3 data -1.15 [95% confidence interval -2.35 to 0.05] versus -3.29 [95% confidence interval -8.37 to 1.79] pmol/l, respectively).

Short-term change in copeptin versus long-term outcome on tolvaptan

In the subjects who were treated with tolvaptan, the percentage change in copeptin observed in the first 3 weeks of tolvaptan treatment significantly correlated with the annual change in TKV thereafter; that is, a larger percentage increase in copeptin was associated with a lower TKV growth rate ($P = 0.006$; Table 5 and Figure 4, left panel) as well as a trend for less eGFR decline ($P = 0.06$; Table 5 and Figure 4, right panel). In multivariable analysis, change in copeptin was no longer associated with TKV growth after adjustment for age and sex (Supplementary Table S7), whereas change in copeptin became significantly associated with eGFR decline after such adjustment and remained significantly associated with eGFR decline even after additional adjustment for other covariates (Supplementary Table S8).

Sensitivity analyses

The various sensitivity analyses showed similar results for the associations of baseline copeptin with annual TKV growth rate and eGFR decline in untreated subjects, as well as for the associations of baseline copeptin with tolvaptan treatment effect and of short-term change in copeptin with ADPKD outcome in tolvaptan-treated subjects. For the subgroup analyses, not every analysis reached formal statistical significance, because of the lower number of subjects per subgroup.

This held true when studying only subjects who stated they were fasting when they had blood drawn for copeptin assessment ($n = 912$), or when males ($n = 664$) and females ($n = 616$) were studied separately (data not shown). In contrast, for the per-protocol analyses (i.e., when studying only those subjects who continued tolvaptan treatment throughout the study; $n = 1157$), associations were generally stronger. For instance, in this case baseline copeptin was significantly associated with tolvaptan treatment effect on rate of eGFR decline ($P = 0.01$ vs. $P = 0.07$ in the main analysis; Figure 2).

DISCUSSION

In the present study we investigated whether baseline copeptin is associated with rate of ADPKD progression and tolvaptan treatment efficacy, and whether a change in copeptin shortly after the start of tolvaptan treatment is associated with long-term outcomes during this treatment.

Copeptin is part of the vasopressin precursor hormone pre-pro-vasopressin. When the precursor is split, copeptin and vasopressin are released from the pituitary gland in equimolar amounts into the circulation.¹⁸ Copeptin levels correlate with vasopressin levels

during physiological changes in plasma osmolality, from water excess to dehydration.^{18,19} Measurement of copeptin is increasingly preferred over measurement of vasopressin in clinical and epidemiological studies as well as in clinical routine, because of the technical constraints of the conventional assays for vasopressin.²⁰ Copeptin has a small molecular size (5 kDa) and is therefore subject to glomerular filtration. It has therefore been suggested that copeptin levels are influenced by kidney function *per se*.^{21,22} However, it has also been shown that plasma copeptin concentration was similar in healthy kidney donors prior to and 7 weeks after nephrectomy, despite a reduction in measured GFR from 105 to 66 ml/min per 1.73 m².²³ These data suggest that in these subjects renal clearance is not an important route of elimination for copeptin. Another study has shown that the copeptin-vasopressin ratio was stable across the range of kidney function in subjects with an eGFR above 30 ml/min per 1.73 m².²⁴ Taken together, these data indicate that in the present study, copeptin can be used as a surrogate marker for vasopressin, because all included subjects had an estimated creatinine clearance of more than 60 ml/min.

Subjects with ADPKD have an impaired urine concentrating capacity, already at an early stage of their disease,^{25–29} possibly resulting from an impaired osmolar gradient caused by disruption of the medullary architecture secondary to cyst formation and renal insufficiency.^{5,6} As a result, plasma osmolality will rise, and through sensing of osmoreceptors vasopressin (as reflected by copeptin) will increase to maintain water balance. Indeed, median copeptin levels in our ADPKD patients were higher than those reported for healthy subjects (6.4 vs. 3.8 pmol/l, respectively),²³ and we found a positive association between TKV and copeptin.

In a cross-sectional analysis of baseline data, we found that copeptin level was positively associated with TKV and negatively with eGFR. In subjects randomized to placebo treatment, in whom the natural course of the disease can be studied in a longitudinal setting, baseline copeptin was associated with rates of TKV growth and eGFR decline during follow-up. These data confirm in a larger-scale setting the findings from previous small-scale studies that copeptin is associated with disease severity as well as disease progression in ADPKD,^{12,30} and support the notion that vasopressin has a causal role in disease progression.

In subjects randomized to tolvaptan treatment, baseline copeptin was associated with stronger treatment efficacy with respect to rate of TKV growth. This association was independent of baseline characteristics. A similar but slightly less strong association of baseline copeptin with tolvaptan treatment efficacy was found for rate of eGFR decline. An explanation for the latter weaker association might be that GFR estimated with creatinine inherently has more variability (dependent among others on level of exercise, food intake, and hydration status³¹) than TKV. Another explanation might be that vasopressin causes cyst growth,³² whereas eGFR is more indirectly affected via cyst growth-related nephron loss.³³

During tolvaptan treatment, copeptin increased significantly compared with baseline, an effect that was already present 3 weeks after initiation of treatment. This is in line with previous findings, where also a nearly 3-fold increase in copeptin was found after 3 weeks of treatment with tolvaptan.¹⁶ This can be explained by the aquaretic effect of the V2 receptor

antagonist tolvaptan. As a result, plasma osmolality will increase, leading to an increase in vasopressin, thirst, and polydipsia. As a consequence of water reabsorption in the collecting duct and increased water intake, plasma osmolality is maintained within a relatively narrow range, at the expense of increasing vasopressin levels that contribute to more rapid disease progression.^{12,13,15} The increase in copeptin during tolvaptan treatment is assumed to reflect the degree of V2R antagonism via feedback mechanisms. These same mechanisms might also explain why baseline plasma osmolality has weaker associations with rate of disease progression than baseline copeptin (Tables 3 and 4 vs. Supplementary Table S4).

In order to investigate the hypothesis that the increase in copeptin after start of tolvaptan treatment reflects the degree of V2R antagonism, we evaluated whether change in copeptin observed in the first 3 weeks of tolvaptan treatment was associated with disease progression during treatment. The initial change in copeptin indeed predicted TKV growth ($P = 0.006$), with a higher increase in copeptin being associated with a stronger tolvaptan treatment effect on TKV growth. This association lost significance in multivariable analyses. In contrast, initial change in copeptin after the start of tolvaptan was associated with eGFR decline thereafter when adjusted for age, sex, and baseline eGFR and TKV ($P = 0.04$). It should be noted that the aforementioned associations did not show a clear “dose-response” association, as is shown in Figure 4. This may be explained at least in part by the fact that blood was not drawn for copeptin measurement in a rigorously standardized setting (i.e., that not all subjects were fasting), which will inevitably have led to additional variation in copeptin values. Notwithstanding, the associations that were observed between change in copeptin after 3 weeks and long-term disease outcome during treatment suggest that measurement of short-term change in copeptin may be of help to guide drug treatment (i.e., in case of a small change in copeptin tolvaptan treatment could either be stopped or increased in dose). Vice versa, in case of a large short-term change in copeptin, patients could be reassured that the drug they are using may be effective or that in such patients treatment could be tapered.

Another parameter that reflects urine concentrating capacity and V2R signaling is urine osmolality. In a recent *post hoc* analysis from the TEMPO 3:4 trial, urine osmolality at baseline correlated negatively with TKV and positively with eGFR, and a larger reduction in urine osmolality after start of tolvaptan treatment was associated with fewer clinical progression events.³⁴ Although under physiological conditions vasopressin and urine osmolality are positively related, their interrelation is complex. In ADPKD, in case of a severe urine concentrating defect, patients may have a high 24-hour urine volume in spite of a high vasopressin value. Indeed it has been described that in individual ADPKD patients urine osmolality can be low, whereas copeptin can be high.³⁵ Because vasopressin is causally related to disease progression in ADPKD, it may be expected that associations with disease severity and progression are stronger for copeptin than for urine osmolality. This was indeed found for TKV growth in the present study, but not for eGFR decline. Thus, whether urine osmolality and/or copeptin can best be used to predict disease progression and tolvaptan treatment efficacy needs additional study, which is beyond the scope of the present analyses.

A limitation of this study is that it is performed as a *post hoc* analysis of a randomized clinical trial with specific inclusion and exclusion criteria, limiting the external validity of

our findings for the general ADPKD population. Furthermore, not all subjects were fasting when blood was drawn. This will have affected the representativeness of the measured copeptin values, as copeptin (like vasopressin) is known to change rapidly after ingestion of salt, proteins, and fluid.^{36,37} On the other hand, measurement of TKV was conducted very precisely by planimetry of magnetic resonance images. This increases the predictive value of TKV in comparison with that of copeptin in multivariable analyses, and limits the external validity of our findings, because in clinical practice TKV is estimated using the ellipsoid formula or by ultrasound, both methods that are known to be less precise.^{4,38} This also suggests that our findings with respect to the predictive value of copeptin may be an underestimation of the potential of this biomarker in clinical practice.

Strengths of this study include the large cohort of well phenotyped ADPKD subjects, and that this is the first report on the associations of (change in) copeptin with tolvaptan treatment efficacy.

In conclusion, we found that the baseline copeptin level in placebo-treated ADPKD subjects was a predictor of disease progression. In addition, we found promising but not conclusive results indicating that a higher baseline copeptin level predicted a larger treatment effect of tolvaptan with less TKV growth and also less eGFR decline. The change in copeptin after 3 weeks of tolvaptan treatment predicted future disease progression assessed for eGFR decline ($P = 0.04$ after adjustment for covariates). Given these data, copeptin holds promise to help predict prognosis and possibly also tolvaptan treatment efficacy in subjects with ADPKD. Additional studies are required to corroborate our findings before copeptin, and especially treatment-induced change in copeptin, can be used to guide treatment. Such studies should standardize the conditions for copeptin assessment.

METHODS

Patients and study design

The present study is performed as a *post hoc* exploratory analysis of the TEMPO 3:4 trial, a prospective, double-blinded, randomized controlled trial in patients diagnosed with ADPKD ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00428948) identifier: NCT00428948). A detailed description of the study can be found in Supplementary Methods. The institutional review board or ethics committee at each site approved the protocol. The trial is conducted according to the International Conference of Harmonisation Good Clinical Practice Guidelines and all other applicable regulatory requirements and adheres to the ethical principles of the Declaration of Helsinki. Written informed consent was obtained for all participants.

Data collection, measurement, and definitions

GFR was estimated with the creatinine-based CKD-EPI equation.³⁹ TKV was assessed using standardized kidney magnetic resonance imaging at baseline and at months 12, 24, and 36 or at early withdrawal by manual boundary tracing, as described in the original protocol.⁴⁰ Copeptin was measured in plasma from blood samples obtained at baseline, during treatment at week 3 and months 12, 24, and 36, and after treatment withdrawal at follow-up by an automated immunofluorescence assay (Copeptin-proAVP KRYPTOR; BRAHMS

GmbH, Hennigsdorf, Germany).^{18,36} Urine osmolality was measured by freezing point depression osmometry and plasma osmolality (Posm) was calculated as $2 \times \text{sodium} + (\text{glucose}/18) + (\text{BUN}/2.8)$. Detailed methods can be found in the Supplementary Methods.

Statistical analyses

A detailed description of the statistical analyses can be found in the Supplementary Methods. Baseline characteristics of the study population are stratified according to sex-adjusted quartiles of baseline plasma copeptin level, because copeptin and vasopressin are known to be higher in men than in women.^{36,41} Differences between the quartiles were tested with a Cochran-Armitage trend test for binary characteristics and an analysis of variance trend test for continuous characteristics.

The prognostic value of copeptin was tested in placebo-treated and tolvaptan-treated subjects separately, first by assessing the associations of sex-adjusted quartiles of baseline copeptin with annual change in eGFR, as well as annual change in TKV during follow-up using linear mixed models (crude analysis). Second, multivariate regression analysis was used to determine if the associations of copeptin with these outcomes were independent of subject characteristics that are used in clinical practice to assess prognosis (age, gender, eGFR, and TKV). In separate analyses, copeptin was replaced in these multivariate models by plasma or urine osmolality to test the additional effect of using copeptin instead of these variables for explaining annual change in TKV and eGFR. Tolvaptan treatment-induced effects on annual change in TKV as well as eGFR were calculated using a linear mixed model with dependent variable log

TKV (or eGFR) and independent fixed effects of treatment, time, treatment time interaction, and covariate baseline log TKV (or eGFR). The intercept and slope of each subject were treated as a random effect in the linear mixed model. Tolvaptan treatment effect was estimated by the treatment time interaction term in the model. Formal interaction between baseline copeptin and tolvaptan treatment effect was tested by mixed models with annual changes in TKV and eGFR expressed on a continuous scale. Testing was performed to determine whether these interactions were dependent of the aforementioned baseline characteristics.

The effect of tolvaptan treatment on copeptin levels was assessed by comparing placebo- and tolvaptan-treated subjects at all time points where copeptin was measured, using observed case analysis on log-transformed data (mixed model of repeated measurement analysis). Change in TKV as well as eGFR has been calculated with mixed models (i.e., incorporating information on TKV or eGFR, respectively, that was collected on all time points). This was done similarly for placebo- and tolvaptan-treated subjects.

We studied whether initial change in copeptin (in quartiles) was associated with annual TKV growth and eGFR decline, and whether these associations were independent of baseline characteristics.

Various sensitivity analyses were performed. First, the association between baseline copeptin level versus tolvaptan treatment effects was investigated including only those subjects who

continued tolvaptan and placebo treatment throughout the study (per protocol analysis). Second, all analyses were repeated including only subjects that had blood drawn for copeptin measurements at baseline in a fasted state. Third, male and female subjects were studied separately.

All analyses were performed with the statistical software package SAS 9.3. A P value < 0.05 was considered to be statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1 |.

Annual change (mean and 95% confidence interval) in total kidney volume (TKV, left panel) and estimated glomerular filtration rate (eGFR, right panel) in placebo-treated subjects according to sex-adjusted quartiles of baseline copeptin.

Figure 2 |.

Tolvaptan treatment effect on annual change in total kidney volume (TKV, left panel) and estimated glomerular filtration rate (eGFR, right panel) according to sex-adjusted quartile of baseline copeptin (means and interquartile ranges).

Figure 3 |. Median copeptin levels in tol-vaptan-(dark gray) and placebo- (light gray) treated subjects at baseline, during 36 months of treatment, and after withdrawal of treatment (follow-up).

* $P < 0.05$, ** $P < 0.001$ versus baseline.

Figure 4 | Short-term change in copeptin (week 3 vs. baseline) in tolvaptan-treated subjects versus annual TKV growth rate (left panel) and annual eGFR decline (right panel).

Data are shown per quartile of change in copeptin (Q C1: <119%; Q C2: 119%–230%; Q C3: 230%–390%; Q C4: ≥ 390%).

Table 1 | Baseline characteristics of TEMPO 3:4 trial participants according to sex-adjusted quartiles of baseline plasma copeptin

Variables	Baseline copeptin (pmol/l)					P value for trend ^a
	M <4.9 F <3.2	M 4.9–8.0 F 3.2–5.1	M 8.0–12.3 F 5.1–8.5	M 12.3 F 8.5		
N	320	320	320	320	NA	
Copeptin (pmol/l)	2.8 (2.2–3.5)	5.1 (4.0–6.4)	8.3 (6.4–10.2)	16.1 (12.7–20.5)		<0.0001
Male (%)	52	52	52	52		1.0
Race (n, %)						0.009
White	252 (78.8)	269 (84.1)	270 (84.4)	278 (86.9)		
Black	2 (0.6)	3 (0.9)	8 (2.5)	4 (1.3)		
Asian	64 (20.0)	42 (13.1)	36 (11.3)	27 (8.4)		
Other	2 (0.6)	6 (1.9)	6 (1.9)	11 (3.4)		
Age (yr)	39.3 ± 7.1	38.8 ± 7.2	38.6 ± 7.4	39.1 ± 7.2		0.6
BMI (kg/m ²)	25.4 ± 4.8	25.7 ± 4.5	26.5 ± 5.2	27.3 ± 5.7		<0.0001
SBP (mm Hg)	126.4 ± 12.3	128.9 ± 13.7	129.7 ± 13.7	129.8 ± 13.8		0.001
DBP (mm Hg)	81.1 ± 8.7	81.9 ± 9.9	83.4 ± 10.0	83.8 ± 10.0		0.0001
Use of BLD (%)	73	72	66	82		0.05
Use of RAASi (%)	73	71	65	82		0.06
Hypertension (%)	82	85	77	88		0.3
Cholesterol	5.0 ± 0.9	4.9 ± 0.8	5.1 ± 0.9	5.1 ± 1.0		0.1
Use of LLD (%)	13	13	11	14		0.9
Hypercholesterolemia (%)	25	23	24	28		0.3
Plasma osmolality (mOsm/kg H ₂ O)	291.3 ± 4.2	292.0 ± 4.5	293.2 ± 4.6	294.8 ± 4.9		<0.0001
Urine osmolality (mOsm/kg H ₂ O)	448 ± 186	506 ± 189	546 ± 169	505 ± 153		<0.0001
eGFR (ml/min per 1.73 m ²)	85.6 ± 19.0	85.4 ± 20.5	82.7 ± 21.5	71.0 ± 22.3		<0.0001
TKV (ml)	1333 (981–1742)	1434 (1050–1894)	1567 (1121–1993)	1683 (1276–2338)		<0.0001

BLD, blood pressure-lowering drugs; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; LLD, lipid-lowering drugs; NA, not applicable; RAASi, reninangiotensin aldosterone system inhibitors; SBP, systolic blood pressure; TKV, total kidney volume.

Data are expressed as mean ± SD or as median (interquartile range).

^a P-value tested for baseline copeptin on a continuous scale.

Table 2 | Annual change in TKV and eGFR (means and IQR) according to sex-adjusted quartile of baseline copeptin and randomization group

Variables	Baseline copeptin (pmol/l)				P value for trend ^a
	M <4.9 F <3.2	M 4.9–8.0 F 3.2–5.1	M 8.0–12.3 F 5.1–8.5	M 12.3 F 8.5	
TKV					
Annual change					
Placebo (% per year)	4.5 (4.2–4.8)	5.1 (4.8–5.4)	5.5 (5.3–5.8)	7.0 (6.6–7.4)	0.0004
Tolvaptan (% per year)	3.2 (2.5–3.9)	2.4 (1.6–3.1)	1.9 (1.2–2.6)	3.1 (2.2–3.9)	0.5
Tolvaptan treatment effect					
Percentage (%)	–29.5 (–45.1 to –13.9) P = 0.01	–53.3 (–68.5 to –38.2) P < 0.0001	–65.5 (–78.4 to –52.5) P < 0.0001	–55.8 (–68.5 to –43.0) P < 0.0001	0.001
Absolute (% per year)	–1.33 (–2.06 to –0.60) P = 0.01	–2.72 (–3.53 to –1.91) P < 0.0001	–3.63 (–4.39 to –2.87) P < 0.0001	–3.88 (–4.84 to –2.92) P < 0.0001	0.001
eGFR					
Annual change					
Placebo (ml/min per 1.73 m ² per year)	–2.8 (–3.0 to –2.6)	–3.5 (–3.7 to –3.3)	–3.0 (–3.2 to –2.8)	–5.3 (–5.6 to –5.1)	<0.0001
Tolvaptan (ml/min per 1.73 m ² per year)	–2.5 (–2.6 to –2.4)	–2.7 (–2.8 to –2.6)	–2.4 (–2.6 to –2.2)	–3.6 (–3.7 to v3.4)	0.004
Tolvaptan treatment effect					
Percentage (%)	–11.0 (–18.4 to –3.6) P = 0.3	–23.9 (–29.9 to –17.8) P = 0.03	–20.6 (–28.3 to –12.9) P = 0.1	–33.3 (–37.9 to –28.7) P < 0.0001	0.02
Absolute (ml/min per 1.73 m ² per year)	0.31 (0.09–0.53) P = 0.3	0.84 (0.59–1.10) P = 0.03	0.62 (0.36–0.89) P = 0.1	1.78 (1.47–2.08) P < 0.0001	0.02

eGFR, estimated glomerular filtration rate; IQR, interquartile range; TKV, total kidney volume.

^a *P* value tested for baseline copeptin on a continuous scale.

Table 3 |

Models explaining annual change in TKV in placebo-treated subjects investigating the prognostic value of baseline copeptin

Variables	Model 1			Model 2			Model 3			Model 4			Model 5		
	St. β	P value	R ²	St. β	P value	R ²	St. β	P value	R ²	St. β	P value	R ²	St. β	P value	R ²
			0.121			0.185 ^a			0.265 ^b			0.340 ^c			0.359 ^d
Age	-0.109	0.07		-0.080	0.17		-0.230	0.0003		-0.139	0.009		-0.210	0.0004	
Male sex	0.320	<0.0001		0.243	<0.0001		0.224	0.0001		0.164	0.003		0.165	0.003	
Log copeptin				0.268	<0.0001		0.175	0.004		0.129	0.03		0.100	0.09	
eGFR							-0.325	<0.0001					-0.175	0.008	
Log TKV										0.432	<0.0001		0.367	<0.0001	

eGFR, estimated GFR; St. β , standardized beta; TKV, total kidney volume.

Standardized betas and *P* values were calculated using multivariate linear regression. The dependent variable is annual change in TKV, and independent variables are age, male sex, baseline log copeptin, baseline eGFR, and/or baseline log TKV.

^aSignificance compared with model 1 ($P < 0.0001$).

^bSignificance compared with model 2 ($P < 0.0001$); strength added by copeptin to model 3: $P = 0.004$.

^cSignificance compared with model 2 ($P < 0.0001$); strength added by copeptin to model 4: $P = 0.03$.

^dSignificance compared with model 3 ($P < 0.0001$) and model 4 ($p = 0.008$); strength added by copeptin to model 5: $P = 0.09$.

Table 4 |

Models explaining annual change in eGFR in placebo-treated subjects investigating the prognostic value of baseline copeptin

Variables	Model 1			Model 2			Model 3			Model 4			Model 5		
	St. β	P value	R ²	St. β	P value	R ²	St. β	P value	R ²	St. β	P value	R ²	St. β	P value	R ²
			0.006			0.037 ^a			0.070 ^b			0.088 ^c			0.097 ^d
Age	-0.069	0.27		-0.089	0.15		0.009	0.89		-0.055	0.36		-0.004	0.95	
Male sex	-0.044	0.48		0.017	0.79		0.032	0.61		0.061	0.34		0.061	0.34	
Log copeptin				-0.190	0.004		-0.137	0.04		-0.121	0.07		-0.103	0.12	
eGFR							0.209	0.003					0.123	0.10	
Log TKV										-0.243	0.0002		-0.196	0.006	

eGFR, estimated GFR; St. β , standardized beta; TKV, total kidney volume.

Standardized betas and P values were calculated using multivariate linear regression. The dependent variable is annual change in eGFR, and independent variables are age, male sex, baseline log copeptin, baseline eGFR, and/or baseline log TKV. ¹ Significance compared to model

^a $P = 0.004$.

^b Significance compared with model 2 ($P = 0.003$); strength added by copeptin to model 3; $P = 0.04$.

^c Significance compared with model 2 ($P = 0.0002$); strength added by copeptin to model 4; $P = 0.07$.

^d Significance compared with model 3 ($P = 0.006$) and model 4 ($P = 0.10$); strength added by copeptin to model 5; $P = 0.12$.

Table 5 |

Quartiles of short-term change in copeptin during tolvaptan treatment (week 3 of treatment vs. baseline) versus rate of TKV growth and eGFR decline during follow-up (means and IQR)

Variables	Short-term change in copeptin (%)				P value for trend ^a
	<119 Q C1	119–229 Q C2	230–390 Q C3	>390 Q C4	
TKV					
N	133	146	154	158	
Annual change (% per year)	3.5 (3.1–3.9)	2.1 (1.7–2.5)	3.2 (2.8–3.6)	1.4 (1.1–1.7)	0.006
eGFR					
N	142	147	156	157	
Annual change (ml/min per 1.73 m ² per year)	–4.1 (–4.7 to –3.4)	–2.4 (–3.0 to –1.8)	–2.5 (–3.1 to –1.9)	–2.2 (–2.6 to –1.8)	0.06

eGFR, estimated glomerular filtration rate; TKV, total kidney volume.