

Copeptin Is Associated with Kidney Length, Renal Function, and Prevalence of Simple Cysts in a Population-Based Study

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ABSTRACT

Arginine vasopressin (AVP) has a key role in osmoregulation by facilitating water transport in the collecting duct. Recent evidence suggests that AVP may have additional effects on renal function and favor cyst growth in polycystic kidney disease. Whether AVP also affects kidney structure in the general population is unknown. We analyzed the association of copeptin, an established surrogate for AVP, with parameters of renal function and morphology in a multicentric population-based cohort. Participants from families of European ancestry were randomly selected in three Swiss cities. We used linear multilevel regression analysis to explore the association of copeptin with renal function parameters as well as kidney length and the presence of simple renal cysts assessed by ultrasound examination. Copeptin levels were log-transformed. The 529 women and 481 men had median copeptin levels of 3.0 and 5.2 pmol/L, respectively ($P < 0.001$). In multivariable analyses, the copeptin level was associated inversely with eGFR ($\beta = -2.1$; 95% confidence interval [95% CI], -3.3 to -0.8 ; $P = 0.002$) and kidney length ($\beta = -1.2$; 95% CI, -1.9 to -0.4 ; $P = 0.003$) but positively with 24-hour urinary albumin excretion ($\beta = 0.11$; 95% CI, 0.01 to 0.20 ; $P = 0.03$) and urine osmolality ($\beta = 0.08$; 95% CI, 0.05 to 0.10 ; $P < 0.001$). A positive association was found between the copeptin level and the presence of renal cysts (odds ratio, 1.6 ; 95% CI, 1.1 to 2.4 ; $P = 0.02$). These results suggest that AVP has a pleiotropic role in renal function and may favor the development of simple renal cysts.

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The antidiuretic hormone arginine vasopressin (AVP) plays an essential role in osmoregulation by facilitating water transport in the collecting duct.¹ This effect is mediated by the stimulation of the G protein-coupled vasopressin V2 receptor (V2R), increasing cAMP levels and leading to a transient increase in osmotic water transport across the principal cells.² Furthermore, it was recently demonstrated that V2Rs are also expressed in the thick ascending limb of the human kidney.^{3,4} In addition to the V2R, AVP acts on the V1a receptor (Ca²⁺ as second messenger), which is distributed in the endothelial cells lining systemic and renal blood vessels and capillaries (including

vasa recta), as well as the glomerulus, the macula densa, the medullary interstitial cells, and the cortical collecting duct.^{5–7} Of note, V1a receptors seem to

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antagonize V2 effects on urinary concentration by activating prostaglandin synthesis, but higher levels of AVP are required to activate those receptors.⁷ The functional relevance of the complex expression pattern of V1a receptors and V2Rs remains debated. Experimental studies using the Brattleboro rat, which is genetically deficient in AVP, as well as pharmacologic inhibition based on V2R-specific or V1a/V2R antagonists showed a decrease in kidney disease progression, kidney hypertrophy, and proteinuria.^{8–10} Abnormal AVP signaling and increased cAMP levels are also involved in renal cyst formation in various rodent models of autosomal dominant polycystic kidney disease (ADPKD), as evidenced by the protective effect of high water intake, selective V2R antagonists, and genetic deletion of AVP in such models.^{9,11,12} The role of AVP/V2R signaling on cystogenesis was recently confirmed by a large randomized, double-blind placebo-controlled trial testing the effect of a V2R antagonist in patients with ADPKD.¹³ Taken together, these studies suggest that AVP exerts pleiotropic effects on the kidney, beyond regulation of water balance. Whether such effects can be detected in the general population remains unknown.

Thus far, population-based studies addressing the role of AVP were limited by technical issues related to AVP measurement,^{14–16} such as high preanalytical instability,^{16,17} low sensitivity linked to competitive assay,¹⁸ and a long turn-around time (4–10 days). AVP is a small nonapeptide that must be isolated from plasma by chromatography and further concentrated before the assay, which notoriously complicates the analytical procedure.^{14,19} Even with RIAs, AVP remains difficult to measure: Its plasma $t_{1/2}$ is short, it is not stable *ex vivo*, and it interferes with plasma components or factors such as heparin.^{15,20,21} In addition, storage at -20°C and freeze-thaw cycles result in a decline of plasma AVP levels over time. These issues have limited the use of AVP measurement in clinical practice, explaining the small number of cohort studies. Copeptin (also named CT-proAVP) is a peptide derived from the cleavage of the precursor of AVP (provasopressin), produced in an equimolar ratio.¹⁷ Copeptin is strongly correlated to AVP over a large range of plasma levels and has the same kinetics in response to changes in plasma osmolality or during hemorrhagic shock.^{18,22–26} Furthermore, copeptin has a longer $t_{1/2}$ than AVP, is stable at room temperature, and does not need extraction nor special sample handling procedures. For these reasons, copeptin is now considered as a robust surrogate for AVP, overcoming the technical problems related to AVP dosage.¹⁷

In this study, we investigated the potential link of copeptin with renal function parameters, including eGFR, urine albumin excretion and urine osmolality, the morphology of the kidney, and the presence of simple renal cysts, in a large, multicentric population-based cohort.

RESULTS

From December 2009 to March 2013, 1128 participants were included in the Swiss Kidney Project on Genes in Hypertension (SKIPOGH) study. Renal ultrasonography measurements were

obtained in 1125 participants (99.7%). We excluded 6 (0.5%) participants because of missing copeptin measures and 111 (9.8%) with incomplete data for the outcomes of interest or covariables, mainly urinary ones. Another participant was excluded because of a fortuitous diagnosis of typical ADPKD on ultrasonography. Finally, we included 1010 participants in these analyses.

The characteristics of the 529 women and 481 men are presented in Table 1. Men had higher plasma copeptin levels than women. They also had higher body mass index (BMI), higher BP, eGFR, triglycerides, urinary osmolality values, and 24-hour osmolar excretion, as well as a higher prevalence of other cardiovascular risk factors such as smoking, treated hypertension, and diabetes.

Ultrasonography examination revealed that men had significantly larger kidneys and more cysts than women. Only one participant had missing information on kidney length because of a horseshoe kidney. The prevalence of renal simple cysts was 12% ($n=121$) in the studied population, and increased with age: 2.4%, 12.3%, and 24.6% for those aged <40 , 40–59, and ≥ 60 years, respectively. A significantly higher prevalence of cysts was observed in men than in women in each age category: 3.1% versus 1.7% before 40 years ($P=0.39$), 16.7% versus 9.1% at 40–59 ($P=0.03$), and 31.5% versus 18.3% after 60 years ($P=0.01$). The median of the largest diameter measured in the biggest cyst was 17.0 mm (interquartile range [IQR], 10.5–24.8).

In addition to cysts, the presence of kidney stones was suspected by ultrasonography in 65 participants (6.4%), without significant differences between men and women (7.5% versus 5.5%; $P=0.20$).

Association Analyses

In univariate analyses, participants aged >60 years had higher median copeptin levels than younger participants (4.4 pmol/L [IQR, 3.0–6.8] versus 3.8 pmol/L [2.6–5.7]; $P<0.001$), and copeptin was higher in participants with higher BMI (BMI ≥ 30 $\text{kg}/\text{m}^2=5.1$ pmol/L [3.4–7.9] versus BMI 25–29.9 $\text{kg}/\text{m}^2=4.4$ pmol/L [3.2–6.5] versus BMI <25 $\text{kg}/\text{m}^2=3.4$ pmol/L [2.4–5.2]; P for trend, $P<0.001$). Copeptin levels were also significantly higher in smokers than in nonsmokers (4.4 pmol/L [IQR, 2.9–6.6] versus 3.8 pmol/L [2.6–5.7]; $P=0.003$) and in patients with diabetes than in patients without diabetes (6.1 pmol/L [4.0–9.7] versus 3.9 pmol/L [2.7–5.7]; $P<0.001$) or CKD (5.1 pmol/L [3.8–10.4] versus 3.9 pmol/L [2.7–5.9]; $P<0.001$). Participants with a pathologic 24-hour albumin-to-creatinine ratio (ACR) also presented higher copeptin levels (5.0 pmol/L [IQR, 3.2–8.7] versus 3.9 pmol/L [2.7–5.8]; $P<0.001$). Copeptin levels were higher in individuals taking diuretics than in those not taking them (4.5 pmol/L [IQR, 3.3–7.9] versus 3.9 pmol/L [2.7–5.9]; $P=0.03$) but not when all hypertension treatment were considered (4.4 [2.8–6.6] versus 3.9 [2.7–5.8]; $P=0.10$).

Copeptin levels were positively associated with both the presence of simple cysts (4.8 pmol/L [IQR, 3.6–7.9] versus

Table 1. Characteristics of the participants according to sex (N=1010)

Characteristic	Women (n=529)	Men (n=481)	P Value
Age (yr)	47.9±17.3	46.5±17.8	0.20
BMI (kg body wt/m ²)	24.2±4.7	25.9±4.2	<0.001
Height (cm)	164.8±6.5	177.6±6.9	<0.001
Systolic BP (mmHg)	115.3±17.5	120.9±15.5	<0.001
Diastolic (mmHg)	73.6±9.6	77.9±9.1	<0.001
Copeptin (pmol/L)	3.0 (2.2–4.2)	5.2 (3.7–7.8)	<0.001
eGFR (ml/min per 1.73 m ²)	94.7±17.2	98.5±18.4	0.001
Plasma sodium (mmol/L)	140.3±2.6	140.3±2.5	0.88
Estimated plasma osmolality (mOsm/L)	288.7±5.2	288.8±5.0	0.68
Plasma triglycerides (mmol/L)	0.8 (0.6–1.1)	1.0 (0.7–1.4)	<0.001
24-h urine flow rate (ml/min)	1.2 (0.8–1.5)	1.1 (0.8–1.5)	0.17
24-h urine sodium excretion (mmol)	117.5(88.3–150.5)	158.2(121.6–198.4)	<0.001
24-h urine osmolality (mOsm/kg of H ₂ O)	417 (314–563)	570 (430–773)	<0.001
24-h urine creatinine excretion (mg/kg of body wt)	18.1 (14.7–20.8)	22.1 (19.0–25.0)	<0.001
24-h urine albumin excretion (mg)	5.8 (3.8–10.1)	6.0 (3.7–10.6)	0.52
24-h osmolar excretion (mOsm)	669.4 (560.1–800.7)	888.9 (735.7–1047.6)	<0.001
Hypertension treatment (yes)	66 (12.5)	90 (18.7)	0.01
Diabetes (yes)	15 (2.8)	34 (7.1)	0.002
Smoking (yes)	103 (19.5)	137 (28.5)	0.001
CKD (yes)	16 (3.0)	16 (3.3)	0.78
Albuminuria (yes) ^a	22 (4.2)	38 (7.9)	0.01
Kidney length (mm)	107.0±7.9	113.7±8.2	<0.001
Cysts	48 (9.1)	73 (15.2)	0.003
Number of cysts			
None	481 (90.6)	408 (85.0)	0.003
1	38 (7.2)	47 (9.8)	
≥2	10(1.9)	26 (5.6)	

Continuous variables are expressed as the mean±SD or median and IQR (25th–75th percentiles) as appropriate. Categorical variables are expressed as n (%). CKD was defined as an eGFR<60 ml/min per 1.73 m². eGFR was calculated with the CKD-EPI equation. Plasma osmolality was calculated as 2×(Na+K). The 24-hour osmolar excretion was calculated as urine osmolality×24-hour urine volume.

^aPresence of albuminuria was defined as urinary ACR≥25 μg/mg in women and ≥17 μg/mg in men.

3.8 pmol/L [2.6–5.7]; $P<0.001$) and the number of cysts in the kidneys (Figure 1A). Kidney size increased with the number of cysts (Figure 1B). Participants with numerous kidney cysts were significantly older and presented higher systolic BP and 24-hour urinary albumin excretion and lower eGFR compared with those with only one cyst or without cysts (Table 2). The association of cysts with copeptin was stronger in younger participants (<61 years; odds ratio [OR], 2.27; 95% confidence interval [95% CI], 1.29 to 3.00; $P=0.01$) than in older participants (≥61 years; OR, 1.26; 95% CI, 0.70 to 2.15; $P=0.40$). These associations remained similar upon adjustment for eGFR. A higher urinary osmolality was observed in participants with numerous kidney cysts ($P=0.03$).

Compared with participants without evidence of kidney stones, those with suspected stones had higher median 24-hour urine osmolality (569.0 mOsm/kg H₂O [IQR, 385.7–795.2] versus 489.3 mOsm/kg H₂O [355.5–646.5]; $P=0.02$), a lower urinary flow rate (0.9 ml/min [0.7–1.3] versus 1.1 ml/min [0.8–1.5]; $P=0.02$), and higher copeptin levels (4.9 pmol/L [3.0–8.4] versus 3.9 pmol/L [2.7–5.8]; $P=0.01$).

We found a linear association between copeptin and most of the continuous variables in Table 1. Because sex was a major confounder for many of the associations, we presented

univariate correlations separately for men and women (Table 3). Only BMI, height, eGFR, 24-hour urinary flow rate, and urinary osmolality had a significant association with copeptin in men and women.

The association of copeptin with eGFR, 24-hour urine albumin excretion, 24-hour urine osmolality, and kidney length adjusted for age, sex, and center is presented graphically in Figure 2. Table 4 describes the β coefficients, ORs, and 95% CIs for the associations between copeptin levels and these variables of interest (dependent variables) in different multivariate models progressively integrating other covariates. Because all of the models are adjusted for sex, results are presented altogether for men and women. In all models, urinary albumin excretion and urinary osmolality were positively associated with adjusted copeptin levels. On the contrary, eGFR and kidney length presented an inverse association with adjusted copeptin levels. Adding mean BP as a covariate to the last model did not change these associations. The inverse association with kidney length was maintained even when the model was adjusted for body height instead of BMI, although the β coefficients were smaller. Of note, we observed that kidney length remained associated with eGFR ($\beta=0.16$; 95% CI, 0.12 to 0.20; $P<0.001$), urine osmolality ($\beta=0.004$; 95% CI, 0.001 to 0.01; $P=0.03$), and

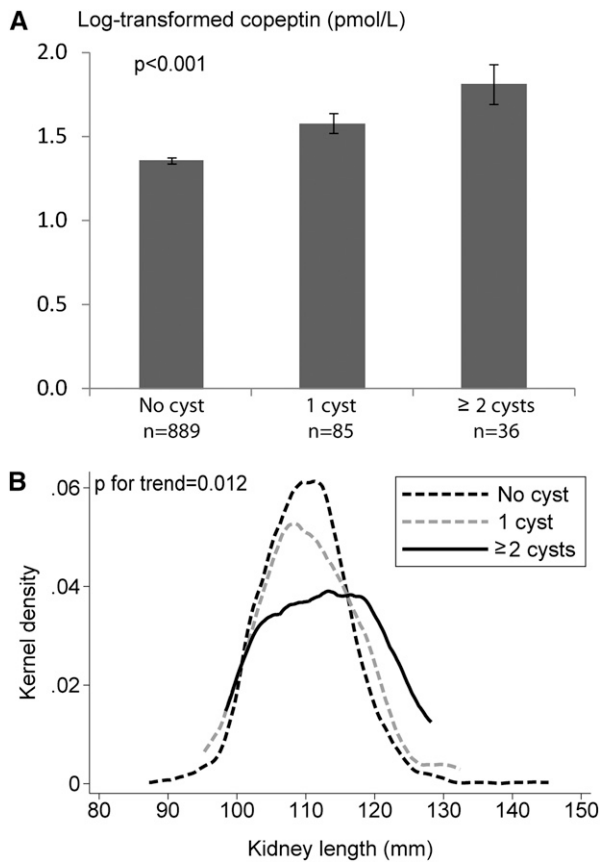


Figure 1. Association of the number of renal cyst with copeptin levels and kidney length. (A) Unadjusted analysis. (B) Analysis adjusted for age, sex, center, and body height. Bars represent the SEM.

24-hour osmolar excretion ($\beta=0.01$; 95% CI, 0.003 to 0.01; $P<0.001$) beyond the fully adjusted model 3. These associations were linear.

We also looked at the association between copeptin and the presence of CKD ($n=32$) or albuminuria ($n=60$) using dichotomized eGFR and sex-specific cutoffs for ACR. In univariate analyses, the odds of having CKD or pathologic ACR with higher levels of copeptin were 2.86 (95% CI, 1.65 to 4.98; $P<0.001$) and 2.08 (95% CI, 1.39 to 3.11; $P<0.001$), respectively. In the models adjusting for sex, age, and center, copeptin remained associated with CKD (OR, 2.82; 95% CI, 1.45 to 5.50; $P=0.002$) and ACR (OR, 1.70; 95% CI, 1.08 to 2.68; $P=0.02$).

Copeptin was positively associated with the presence of cysts (Table 4). Even after adjusting for age, sex, and center, participants with higher copeptin levels had a higher probability of having renal cysts (Figure 3). The association remained significant when adjusting for kidney size and urine concentrating ability. As described in the univariate analyses, the association was stronger in younger participants than in older ones, even when adjusting for age, sex, center, and eGFR. Of note, the largest diameter of the biggest cyst measured, used as a surrogate of cyst size, was not associated with copeptin levels neither in univariate nor in any of the multivariable models (data not shown).

The association of kidney stones with copeptin observed in univariate analyses was less significant when adjusting for age, sex, and center (OR, 1.62; 95% CI, 1.02 to 2.58; $P=0.04$) and was no longer statistically significant upon further adjustments.

Sensitivity Analyses

Analyses repeated after winsorizing copeptin values led to similar results. We also obtained similar results after excluding participants with suspected inaccurate urine collection ($n=6$), as defined in the Concise Methods. We obtained similar results for the association of renal size with copeptin after excluding five participants with a single kidney. Sensitivity analyses including only participants having a kidney length within the normal range of 90–120 mm ($n=868$) led to similar significant associations, although the β coefficients were smaller, as expected. Excluding participants with renal cysts did not change the association of eGFR, urinary osmolality, and kidney length with copeptin, but the association with albuminuria was no longer significant in any of the models.

Heritability estimates (\pm SEM) for copeptin levels were 34.7% (6.1%; $P<0.001$) without a sibship component and 32.7% (5.5%; $P<0.001$) with a sibship component.

DISCUSSION

Several lines of evidence have demonstrated a link between AVP, renal cyst development, and total kidney volume in rodent models and patients with ADPKD.²⁷ However, to the best of our knowledge, the potential association of AVP with the presence of simple renal cysts, or with kidney size, has not been investigated in normal individuals without ADPKD. For the

Table 2. Characteristics of participants according to the number of cysts

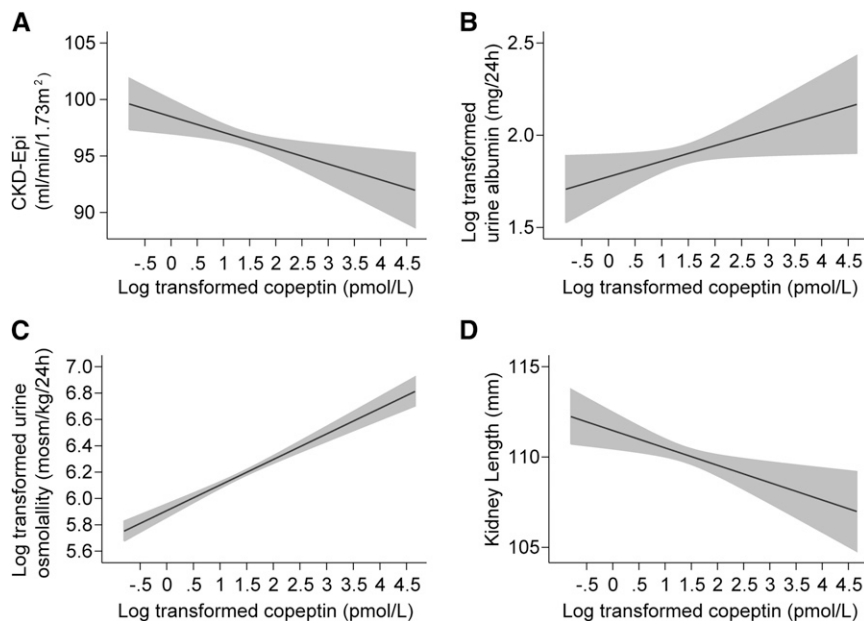
Variable	No Cysts (n=889)	1 Cyst (n=85)	≥2 Cysts (n=36)	P for Trend
Age (yr)	45.4±17.1	58.0±14.6	67.5±11.0	<0.001
Systolic BP (mmHg)	116.8±16.4	125.5±17.3	130.8±16.8	<0.001
eGFR (ml/min per 1.73 m ²)	98.0±17.3	88.8±18.8	79.8±18.7	<0.001
24-h urine osmolality (mOsm/kg of H ₂ O)	486 (357–647)	527 (338–740)	602 (419–723)	0.03
Urine albumin excretion (mg/24 h)	5.7 (3.6–9.7)	8.4 (3.9–14.5)	10.0 (5.5–14.9)	<0.001
Kidney length (mm)	109.9±8.5	110.8±9.0	114.5±11.5	0.01

Data are expressed as the mean±SD or the median and IQR (25th–75th percentiles).

Table 3. Associations between copeptin and continuous variables according to sex

Variable	Women (n=529)		Men (n=481)	
	Correlation	P Value	Correlation	P Value
Age (yr)	0.12	0.01	0.07	0.13
BMI (kg of body wt/m ²)	0.24	<0.001	0.10	0.02
Height (cm)	-0.13	0.002	-0.19	<0.001
Systolic BP (mmHg)	0.16	<0.001	0.07	0.14
Diastolic BP (mmHg)	0.10	0.02	-0.02	0.66
eGFR (ml/min per 1.73 m ²)	-0.12	0.01	-0.12	0.01
Plasma sodium (mmol/L)	0.01	0.79	0.08	0.08
Estimated plasma osmolality (mOsm/L)	0.02	0.73	0.08	0.09
Plasma triglycerides (mmol/L)	0.03	0.53	-0.01	0.88
24-h urine flow rate (ml/min)	-0.38	<0.001	-0.41	<0.001
24-h urine sodium excretion (mmol)	0.001	0.98	-0.02	0.62
24-h urine osmolality (mOsm/kg H ₂ O)	0.37	<0.001	0.44	<0.001
24-h urine creatinine excretion (mg/kg of body wt)	-0.16	<0.001	-0.02	0.67
24-h urine albumin excretion (mg)	0.04	0.38	0.13	0.004
24-h osmolar excretion (mOsm)	-0.03	0.54	-0.01	0.81
Kidney length (mm)	-0.06	0.20	-0.12	0.01

Data are Pearson's correlation coefficients and corresponding *P* values. Copeptin, triglycerides, urine flow rate, 24-hour urine osmolality, and albumin excretion were log-transformed. Urine sodium and creatinine excretion were square-root transformed. Plasma osmolality was calculated as $2 \times (\text{Na} + \text{K})$. The 24-hour osmolar excretion was calculated as urine osmolality \times 24-hour urine volume.

**Figure 2.** Association of copeptin with CKD-EPI, 24-hour urine albumin excretion, 24-hour measured osmolality, and kidney length. A–D are adjusted for age, sex, and center. Shaded areas represent the 95% CI.

first time, this cross-sectional study describes the association of plasma copeptin levels with renal function, kidney length, and renal simple cysts in a large, multicentric population-based cohort. We demonstrate that higher copeptin levels are associated with lower eGFR, higher urinary albumin excretion, higher

urinary osmolality, smaller kidney length, and the presence of simple renal cysts even after multiple adjustments.

Kidney length measured by ultrasonography is an easy and reproducible way of estimating kidney size, and is generally considered as a proxy of renal mass.^{28,29} In previous studies, kidney length correlated positively with eGFR in living kidney donors and the general population.^{28,30} Conversely, a shorter kidney length has been associated with higher BP and albuminuria in an Australian community.³¹ Accordingly, it is tempting to hypothesize that the negative association between copeptin levels and kidney length, persisting even after adjusting for osmolar excretion, may reflect an influence of the later parameter on urinary concentrating ability. The association of a shorter kidney length with lower urinary concentrating ability is in fact supported by our results showing a positive correlation between kidney length and eGFR and urine osmolality even after adjustment for several covariates. It must be noted that the negative correlation between copeptin and kidney length is observed in participants without kidney cysts and in participants with kidney length within the normal range (90–120 mm). Taken together, these data suggest that the urinary concentrating ability determined by kidney length is probably the main factor driving AVP/copeptin levels in a normal population and support the hypothesis that kidney length might somehow reflect kidney function.

The association between copeptin levels and the presence and number of simple cysts in a general population is a novel finding. We show that this association is independent of age, renal function, kidney length, and urinary concentrating ability. Thus far, AVP/copeptin levels have only been considered in patients with ADPKD. In ADPKD, increased levels of AVP contribute to the abnormal, cAMP-mediated signaling in collecting duct cells, promoting cyst development by several mechanisms.²⁷ The positive association of copeptin levels with total kidney volume in ADPKD also reflects the cystic disruption of the medulla, leading to the defective urinary concentrating ability that is consistently observed early in the disease.^{19,27} By contrast, our results suggest that AVP may also play a role in the formation of simple renal cysts in the general population.

Table 4. Multilevel multivariable regression analyses showing associations of GFR, 24-hour urine albumin excretion, urine osmolality, kidney length, and the presence of renal cysts with log-transformed copeptin levels

Dependent Variable	Model 1		Model 2		Model 3	
	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
eGFR (ml/min per 1.73 m ²)	-2.06 (-3.33 to -0.79)	0.001	-2.12 (-3.41 to -0.84)	0.001	-2.05 (-3.33 to -0.76)	0.002
Urine albumin excretion (mg/24 h) ^a	0.12 (0.03 to 0.22)	0.01	0.10 (0.001 to 0.19)	0.05	0.11 (0.01 to 0.20)	0.03
24-h urine osmolality (mOsm/kg) ^b	0.28 (0.24 to 0.32)	<0.001	0.28 (0.24 to 0.32)	<0.001	0.08 (0.05 to 0.10)	<0.001
Kidney length (mm)						
Adjusted for BMI	-1.42 (-2.26 to -0.57)	0.001	-1.70 (-2.52 to -0.89)	<0.001	-1.62 (-2.43 to -0.81)	<0.001
Adjusted for body height and body wt	-1.01 (-1.83 to -0.19)	0.02	-1.22 (-1.99 to -0.45)	0.002	-1.16 (-1.93 to -0.39)	0.003
Renal cysts (present, n=121) ^c	1.62 (1.11 to 2.38)	0.01	1.55 (1.05 to 2.31)	0.03	1.61 (1.08 to 2.41)	0.02

Model 1 is adjusted for age, sex, and center. Model 2 is adjusted for model 1 plus diabetes, hypertension treatment, triglycerides, smoking, BMI, and eGFR. Model 3 is adjusted for model 2 plus estimated plasma osmolality, 24-hour urine creatinine excretion, and 24-hour osmolar excretion.

^aFor 24-hour urine albumin excretion analyses, all models were adjusted for 24-hour urine creatinine excretion. Models 2 and 3 were adjusted for fasting glucose instead of diabetes. Urinary albumin was log-transformed.

^bThe 24-hour urinary osmolality was log-transformed and model 3 included urine flow rate alone instead of osmolar excretion.

^cORs are presented for renal cysts.

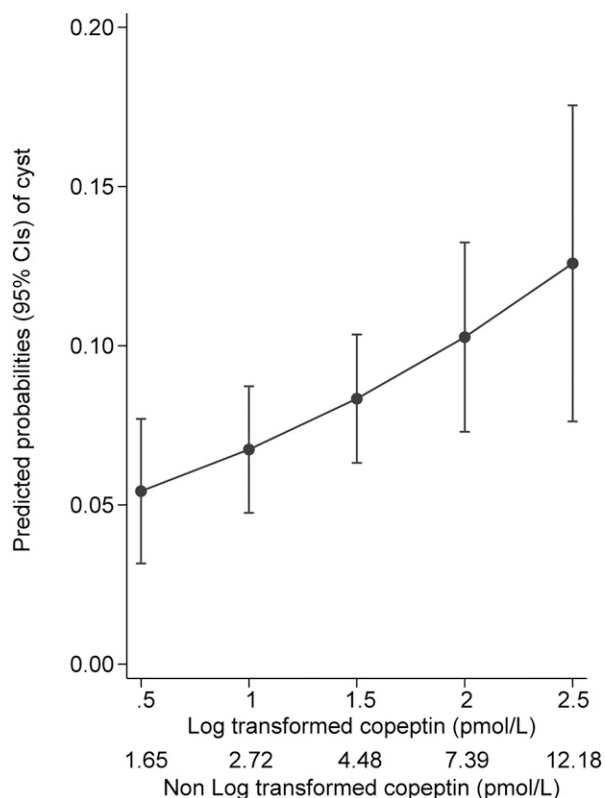


Figure 3. Higher copeptin levels are associated with higher probability of having cysts adjusted for age, sex, and center.

The prevalence of simple cysts tends to increase with age and may reflect kidney aging.^{32,33} The 12% prevalence of cysts detected by ultrasonography in our series, as well as the increased risk with age and the sex difference are in line with previous reports.^{32,34,35} The mechanisms leading to the formation of simple cysts in the kidney remain largely unknown. By definition, these cysts are acquired and fundamentally

distinct from the ADPKD cysts that appear *in utero* as the result of complex genetic mechanisms.³⁶ Simple cysts likely originate from the distal convoluted or collecting duct and are thought to arise from renal tubular diverticula, or from tubular cell hyperplasia secondary to nephron loss.^{37–39} Previous studies in healthy subjects described associations between hypertension and benign renal cysts.^{40–42} In a cross-sectional analysis of hospitalized patients without overt renal disease undergoing an abdominal computed tomography scan, the presence of cysts was associated with a reduced renal function estimated by creatinine clearance.³³ In our cohort, the association between copeptin and cysts was independent of age, hypertension, kidney function, and even urinary concentration, suggesting a distinct pathway. If elevated copeptin was simply a consequence of kidney damage rather than being causally involved in cyst formation, the association of copeptin with cysts should be stronger in older people who have accumulated kidney damage over time and have lower eGFR, and would decrease upon adjustment for eGFR. By contrast, stratified analyses revealed that the association of copeptin with renal cysts was stronger in younger participants than in older participants in this study. The mechanisms linking the potential association of AVP with simple cyst formation, as well as the clinical relevance of such association, will require further investigations.

Our results confirm the previously reported negative association between copeptin levels and eGFR. This association has been observed in prospective studies involving patients with ADPKD as well as patients with a renal transplant and patients with diabetes.^{43–48} Only two studies have used a population-based sample to test the hypothesis that AVP might be involved in the development of albuminuria. In the Prevention of Renal and Vascular Endstage Disease (PREVEND) study, which includes 7593 nondiabetic participants with an urinary albumin concentration ≥ 10 mg/L, circulating copeptin was positively associated with albuminuria and negatively with renal function in a cross-sectional analysis.⁴⁹ In 2064 participants from the

Malmö Diet and Cancer study, baseline circulating copeptin was associated with the incidence of diabetes, obesity, and albuminuria after 16 years of follow-up.⁵⁰ In healthy volunteers, eGFR was also found to be an independent predictor of copeptin levels.⁵¹ Some studies have measured GFR using iothalamate, ^{99m}Tc-DTPA, or inulin clearance.^{43–46} Other studies estimated eGFR using the Modification of Diet in Renal Disease formula or cystatin C but not the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, which is known to be the most reliable formula.^{47,49–51} In our study, we observe an inverse linear association with copeptin levels even in participants with normal eGFR and confirm an almost 3 times increased odds of CKD in patients with higher copeptin levels.

Several hypotheses could explain the association of AVP/copeptin levels with renal function. It could be that copeptin (molecular mass, 5 kD) is cleared by the kidneys and accumulates in the circulation when kidney function decreases. However, longitudinal studies in humans have shown that plasma copeptin levels increase before eGFR decreases.^{44,45,47,48} A decreased urinary concentrating capacity precedes the decrease of GFR in numerous kidney diseases. Increased copeptin levels might be a marker of this early phase of renal dysfunction. From an adaptive response, increased AVP/copeptin levels might become maladaptive and enhance CKD progression. Water ingestion, which readily decreases circulating AVP/copeptin levels, may modify CKD progression.⁵² Studies in the 5/6 nephrectomized rat model suggested that increased water intake decreases circulating AVP levels and slows down the progression of kidney disease.⁵³ Taken together, these data suggest that high levels of AVP may be deleterious for kidney function, either by a direct hemodynamic effect through V1a vascular receptors or through V2 tubular receptors.

Our results also suggest an association between copeptin levels and albuminuria (24-hour collection) in the general population. These data support earlier reports in ADPKD,⁴³ and the general population, although the significance levels were lower in our study.^{49,50} This can be due to the higher number of patients in those studies, the fact that the PREVEND study used a cutoff of 10 mg/L albuminuria for inclusion, and the Malmö study analyzed ACR in spot urine instead of 24-hour urine collection. Previous studies have shown an association between albuminuria and urinary concentrating ability in diabetic rats,⁵⁴ as well as in healthy rats and humans,⁵⁵ suggesting that increased AVP has a proalbuminuric effect. Because only V2 antagonists decrease proteinuria in animal models, one can hypothesize that albuminuria is somehow related to tubular V2Rs.^{9,54} Whether AVP antagonists can reduce albuminuria in humans is actually unknown and should be assessed in clinical studies. *Post hoc* analysis from the TEMPO 3.4 trial, presented at the 2013 American Society of Nephrology Annual Meeting by Gansevoort *et al.* shows that tolvaptan reduces albuminuria in patients with ADPKD.⁵⁶ Our results raise the question of whether targeting AVP to decrease kidney disease progression could be an option in patients with kidney diseases other than ADPKD.

Because kidney stones are associated with concentrated urines and lower urinary flow rate, we explored their potential association with copeptin in our sample. The observed positive association was no longer statistically significant upon multivariable adjustment. Although we found a prevalence of stones similar to a previous study in a very selected population of kidney donors,⁵⁷ assessment by ultrasonography is associated with underdetection of stones compared with computed tomography.^{57,58} The administration of vasopressin antagonist in order to reduce cystine stone formation was described in a case report.⁵⁹ To the best of our knowledge, there is no evidence for the association of AVP or copeptin with kidney stones. Thus, further studies are needed to determine whether AVP or copeptin are associated with kidney stones.

This study has some limitations. Its cross-sectional nature limits causal inferences: We see associations but we cannot confirm the direction of these associations or the causality. However, because the heritability integrates the genetic pattern that occurs before the disease, this suggests causality. We only included participants of European ancestry and therefore our findings might not be valid for other populations. There was no difference in terms of age and sex between the participants and the sample from the general population from which it was drawn, and it is unlikely that this would induce spurious associations as strong and consistent as the ones we detected. Although renal ultrasonography is a reliable tool for estimating kidney length, it is not as sensitive as computed tomography or magnetic resonance imaging to detect renal cysts, and very small cysts might have been missed.⁶⁰ The significance of very small cysts is unknown but we may have underestimated the association between copeptin and renal cysts. Given the cross-sectional design of our study, whether high copeptin levels are the consequence rather than the cause of simple renal cysts remains uncertain. Only long-term follow-up studies will be able to confirm whether individuals with higher baseline copeptin levels are at risk of having shorter kidneys and more cysts or the reverse. Finally, the positive association of copeptin with urinary osmolality is similar to that reported for AVP. Although the physiologic regulation of copeptin release seems to be similar than that of AVP,²⁶ we cannot exclude that other regulatory factors are involved. In addition, it is unknown whether copeptin acts on the same receptors as AVP. Copeptin is currently the most widely accepted surrogate marker for AVP despite the fact that these peptides are different entities.

In conclusion, we show a novel association of circulating copeptin with kidney length and the prevalence of simple renal cysts in a large multicentric population-based study. We also confirm that higher copeptin is associated with increasing urine osmolality, similar to AVP, and with lower renal function and higher 24-hour urine albumin excretion.

These results suggest that copeptin is a marker of renal morphology and function in the general population. By extension, they give new insights into the pleiotropic role of AVP, including the formation of simple renal cysts.

CONCISE METHODS

Study Population

SKIPOGH is a family-based cross-sectional study exploring the role of genes and kidney hemodynamics in BP regulation and kidney function in the general population. It is nested in the European Project on Genes in Hypertension study and shares the same validated protocol. A detailed description of the methods was provided elsewhere and is briefly described here.⁶¹

From December 2009 to March 2013, adult participants were recruited in two regions (Berne and Geneva) and one city (Lausanne) of Switzerland. A random sample of the inhabitants was drawn using different strategies. Inclusion criteria were as follows: (1) having a minimum age of 18 years; (2) being of European ancestry; (3) having at least one and ideally three first-degree family members willing to participate; and (4) providing a written informed consent. Pregnant or breastfeeding women were not included. The SKIPOGH study adhered to the Declaration of Helsinki and was approved by the institutional ethical committees of each participating university hospital.

Measurement and Definitions

Participants were seen in the morning after an overnight fast. In each center, the same experienced operator performed renal grayscale and Doppler ultrasonography according to a standardized procedure.²⁸ Briefly, the renal length of each kidney was measured in the supine position or left/right decubitus if needed. The mean of three optimal measures was reported. Because there was no difference between the left and right kidneys, the mean length of both kidneys was used for the analysis. Benign renal cysts were diagnosed based on known criteria: echolucent structure, round or oval in shape with a thin wall, well defined and smooth contours with a sharp demarcated posterior wall, no calcification, no septa, and no Doppler signal within the cyst.⁶² The number of cysts and the maximal diameter of the biggest cyst were reported. We also reported the presence of kidney stones suspected on the same ultrasound examination.

Fasting blood venous samples were drawn after the participants had been lying in a supine position for at least 30 minutes. A 24-hour urine sample was collected separately for day and night, and oral and written instructions were given on how to perform the urinary collection. Blood glucose, lipid profile, and renal function tests as well as serum and urinary electrolytes were analyzed by standard clinical laboratory methods in each center. Creatinine was measured using IDMS-traceable Jaffe kinetic compensated methods. Urine albumin was measured quantitatively by immunonephelometry on a BN Prospec automated analyzer (Siemens Healthcare Diagnostics, Marburg, Germany) or immunoturbidimetry (Roche/Hitachi Modular P). In addition to the continuous urinary albumin excretion in mg per 24 hours, we used urinary creatinine and albumin to calculate the ACR in milligrams per millimole. The correlation between albumin excretion and ACR was strongly positive ($r=0.93$; $P<0.001$). Urinary osmolality was measured centrally using an Advanced Osmometer (Advanced Instruments, Norwood, MA) based on the freezing-point depression. A control (Clinitrol 290) and a set of calibration standards (50, 850, and 2000 mOsm/kg) were used before running each sample

batch. The coefficient of variability was 0.19% in urine. The 24-hour osmolar excretion was calculated as the product of 24-hour urine osmolality and 24-hour urine volume. Copeptin was measured on -80° frozen EDTA-plasma samples in a batch using a previously described sandwich immunoluminometric assay (Thermo Fisher Scientific CT-proAVP KRYPTOR; BRAHMS GmbH, Hennigsdorf, Germany).¹⁷ The lower detection limit was 0.9 pmol/L and the functional assay sensitivity (20% interassay coefficient of variation) was <2 pmol/L. Estimated plasma osmolality was calculated as $2 \times (\text{plasma sodium} + \text{plasma potassium})$.

Participants filled out a questionnaire at home, which requested information on current and past medical history, medication, and habits (nutrition, smoking, and alcohol).

Diabetes was defined as reported, treated, or fasting glycemia ≥ 7 mmol/L. In this analysis, antihypertensive treatment was used as a covariate to account for hypertension. The CKD-EPI formula was used to estimate the eGFR.⁶³ CKD was defined as present for eGFR < 60 ml/min per 1.73 m^2 . The presence of albuminuria was defined using published sex-specific cut-points: whenever ACR (multiplied by 8.84) was $\geq 25 \mu\text{g}/\text{mg}$ in women and $\geq 17 \mu\text{g}/\text{mg}$ in men.⁶⁴

Statistical Analyses

All of the continuous variables with normal distribution are expressed as the mean \pm SD and as the median (IQR) in case of skewed distribution. Categorical variables are expressed as numbers and frequencies. The normality of the distribution of each continuous variable was assessed graphically. Serum triglycerides, copeptin, 24-hour urinary albumin excretion, and urine osmolality levels were log-transformed for statistical analyses. Finally, *t* tests and chi-squared tests were performed to compare baseline characteristics for continuous and categorical variables, respectively.

Association Analyses

We first conducted univariate analyses to look for associations between copeptin and the outcomes of interest (CKD-EPI, 24-hour urinary albumin excretion, 24-hour urinary osmolality, renal length, and presence of CKD, cysts, or albuminuria) and the other covariates. We calculated Pearson's correlation coefficients for continuous variables (transformed whenever needed) and used the *t* test or trend test for categorical variables.

We applied different multivariable mixed linear regression models to determine the independent association of copeptin with CKD-EPI, albuminuria, urinary osmolality, and renal length, while taking familial correlation into account. In the first model, we adjusted for sex, age, and center. In the second model, we additionally adjusted for cardiovascular risk factors (diabetes, hypertension treatment, triglycerides, smoking, BMI, and CKD-EPI). In the third model, we adjusted for estimated plasma osmolality, 24-hour osmolar excretion, and urinary creatinine excretion. For 24-hour urine osmolality, we adjusted for urinary flow rate instead of osmolar excretion. Because hypertension treatment was included as a covariate in the models, we did not adjust for BP. Although the results are not shown, we systematically checked that adding BP did not change the associations.

For the continuous albuminuria analysis, 24-hour urinary creatinine excretion (in milligrams per kilogram) was added as a covariate in all

models to account for the quality of urine collection. For kidney length, we first generated a model including BMI as for the other outcomes of interest. Because body height is a major determinant of kidney length,²⁸ we then repeated the models adjusting for body height and weight instead of BMI. Results of all linear regression analyses are presented as β coefficients and their 95% CIs.

Linearity between log-transformed copeptin and the outcomes of interest was tested in three ways: (1) graphically, (2) using sex-specific tertiles of copeptin, and (3) including the square of the log-transformed copeptin as a covariate in the models to confirm the absence of a quadratic association.

We also looked into the association of copeptin with the presence of cysts, CKD, or albuminuria (ACR) using mixed logistic regression taking familial correlations into account. Sex, age, and center were used as covariates. For CKD and dichotomized albuminuria, we did not conduct additional adjustment models because of the small number of events. For the association of copeptin with cysts, we also stratified the analyses by age, using a cutoff of 61 years to obtain two groups with the same number of cysts.

Interaction of copeptin with sex, diabetes, smoking, and center was tested for their effects on continuous outcomes of interest (CKD-EPI, 24-hour urine albumin, 24-hour urine osmolality, and renal length) but not for the categorical ones (presence of albuminuria and CKD), because of the small number of events. Interaction was found between diabetes and copeptin when looking at the association with continuous albuminuria ($P < 0.001$), but stratified multivariate analyses could not be conducted because of the small number of participants with diabetes ($n = 49$). In that case, models 2 and 3 were adjusted for fasting glucose instead of diabetes, as done in previous studies.^{49,50}

To generate the panels in Figure 2, for each dependent variable separately, we first generated adjusted residuals from mixed linear models, including age, sex, and center as covariates, and then scaled them by adding the mean of the dependent variable. In Figure 3, we generated the adjusted predicted probability of cyst at selected levels of log-transformed copeptin, including age, sex, and center as covariates in the model, using the margin function in Stata.

Statistical significance was considered for a two-sided $P < 0.05$. Statistical analyses were conducted using STATA 12.0 software (StataCorp, College Station, TX).

Sensitivity Analyses

We repeated the analyses replacing extreme values of copeptin by percentiles 99 (18.77) and 1 (1.161) in order to determine the influence of outliers (winzoration).

We repeated the analyses excluding participants that could have inaccurate urine collections defined as a 24-hour volume < 300 ml or a 24-hour creatinine excretion of < 4 mmol or > 25 mmol in women and < 6 mmol or > 30 mmol in men.⁶⁵

The same multivariate analyses were conducted in participants without cysts and with kidney length within the normal range (90–120 mm).

Heritability Analyses

Heritability is a measure of familial resemblance. It is useful in family-based studies to estimate the role of genes in a specific phenotype.

Because heritability takes into account the genetic pattern that occurs before the disease, it gives an argument for causality.

To estimate heritability, we applied a maximum likelihood approach, as implemented in the ASSOC procedure of the S.A.G.E. package, as previously described.⁶⁶ We also estimated heritability in the presence of an additional sibship component of variance, which captures both the common environment and a dominance genetic variance. Heritability estimates are expressed as h^2 values with SEM.

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DISCLOSURES

None.

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