Urinary Excretion of Epidermal Growth Factor and Rapid Loss of Kidney Function

Jon Viljar Norvik, M.D., Ph.D.[*§,||], Laura R Harskamp, M.D.[*‡], Viji Nair, M.Sc.[¶], Kerby Shedden, Ph.D.[**], Marit D Solbu, M.D., Ph.D.[§,||], Bjørn O Eriksen, M.D., Ph.D.[§,||], Matthias Kretzler, M.D.[¶,††], Ron T Gansevoort, M.D., Ph.D.[‡], Wenjun Ju, Ph.D.[†¶,††] and Toralf Melsom M.D., Ph.D.[†§,||]

These authors contributed equally to the work presented as
[*] first and [†] last authors

[‡]Dept. of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands,

[§]Metabolic and Renal Research Group, UiT The Arctic University of Norway,
[||]Section of Nephrology, University Hospital of North Norway,
Tromsø, Norway,

[¶]Dept. of Internal Medicine/Nephrology and
[**]Department of Statistics,
[††]Dept. of Computational Medicine and Bioinformatics (DCMB), University of Michigan,
Ann Arbor, MI, USA.

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Corresponding author:
Jon Viljar Norvik,
Section of Nephrology,
University Hospital of North Norway,
Tromsø, Norway.
Email: jonviljar@gmail.com

ABSTRACT

Background

Lower urinary excretion of the kidney tubule-specific biomarker epidermal growth factor (uEGF) is associated with increased risk of renal function (GFR) loss in diabetes and in patients with established CKD. We investigated whether uEGF is associated with rapid GFR decline or incident CKD in the general population.

Methods

Subjects without CKD or diabetes were recruited from the general population in Tromso, Norway (RENIS; N=1,249) and Groningen, the Netherlands (PREVEND; N=4,534), with a median follow-up of 5.6 and 7.4 years. GFR was measured by iohexol clearance in RENIS and estimated using the CKD-EPI creatinine-cystatin C equation in PREVEND. Rapid GFR decline was defined as an annual GFR loss > 3.0 ml/min/1.73 m^2, and in sensitivity analyses as subjects with the 10% steepest GFR slope within each cohort.

Results

Lower baseline uEGF excretion was associated with rapid GFR loss in both cohorts (RENIS, odds ratio (OR) per 1 µg/mmol lower uEGF 1.42 (1.06 – 1.91), p=0.02; PREVEND, OR 1.29 (1.10 – 1.53), p<0.01), adjusted for baseline GFR, albumin-to-creatinine ratio (ACR) and conventional CKD risk factors. Similar results were obtained using the outcome of 10% steepest GFR slope in each cohort. Lower uEGF levels were associated with incident CKD in combined analysis of both cohorts.

Conclusions

Lower uEGF level is associated with increased risk of rapid GFR loss and incident CKD in the general population. This finding, together with previous findings in CKD and high-risk populations, supports that uEGF may serve as a broadly applicable biomarker representing the tubular component of the current glomerulus-centric clinical risk assessment system.

Key words: Renal function decline; epidermal growth factor; uEGF, chronic kidney disease; clinical epidemiology.

INTRODUCTION

Chronic kidney disease (CKD) is a major global public health problem with a high and increasing prevalence over the last decades (1). It is an independent risk factor for cardiovascular disease, end-stage kidney disease (ESKD) and all-cause mortality (2–4), highlighting the importance of identifying individuals at high CKD risk for early clinical intervention (5,6). However, the current clinical in-use methods for CKD diagnosis and risk stratification are limited to the measurement of urinary albumin-to-creatinine ratio (ACR) and the estimation of glomerular filtration rate (eGFR). These variables cover glomerular damage and function of the kidney but are insensitive to early tubular dysfunction (7).

Recently, we used a kidney biopsy transcriptome-driven approach to develop nephron segment-specific, noninvasive prognostic biomarkers for CKD progression (8). mRNA levels of epidermal growth factor (EGF), a transcript selectively expressed by distal tubular epithelial cells and considered a marker of functional tubular mass and regeneration potential, correlated with GFR (8). In patients with established CKD from various etiologies, lower urinary excretion of EGF (uEGF) was associated with increased tubular atrophy and interstitial fibrosis. Urinary EGF levels improved prediction of CKD progression independent of eGFR and ACR (8). In patients with type 2 diabetes, lower uEGF was associated with an increased risk of new-onset of GFR < 60 ml/min/1.73m^2 and rapid kidney function decline, indicating that uEGF is applicable as a prognostic biomarker in subjects at high risk for CKD (9).

Tubular atrophy, interstitial fibrosis and loss of nephrons are hallmarks of impending renal dysfunction in apparently healthy subjects (10). These early morphological changes correlate with the GFR decline in aging individuals. Age-related GFR decline is a major

contributor to the high prevalence of CKD in the elderly population, reaching approximately 30-40% of people aged 65 years and older (11). However, the rate of renal function decline rate differs significantly between people (12). We hypothesized that uEGF is a biomarker for rapid kidney function loss where early kidney damage is silent due to lack of sensitive and kidney-specific biomarkers. Therefore, we investigated whether decreased uEGF excretion at baseline is associated with rapid loss of GFR and incident CKD in two general population-based prospective cohorts with serial follow-up.

MATERIALS AND METHODS

Study populations

Figure 1 shows the flow charts of the RENIS (the Renal Iohexol Clearance Survey) and PREVEND (Prevention of REnal and Vascular END-stage disease) population-based cohort studies. Details have been described previously (13–15).

The RENIS study (Tromsø, Norway) was designed to study determinants of change in measured GFR in middle-aged subjects from the general population, and is an ancillary part of the sixth wave of the Tromsø Study, conducted in 2007-08. Subjects who did not report previous myocardial infarction, angina pectoris, stroke, diabetes mellitus (DM) or renal disease were invited to RENIS (N=2,825). A total of 2,107 gave a positive response, and 1,627 subjects were included according to a predetermined target of 1,600 based on power calculations. Because uEGF has been shown to predict GFR decline in subjects with type 2 DM and preexisting CKD (8,9), for the current study we excluded subjects with DM (fasting glucose ≥ 7.0 mmol/L or hemoglobin A1c ≥ 6.5%, N=33) or CKD (ACR ≥ 3.0 mg/mmol or mGFR < 60 ml/min per 1.73 m^2, N=61), as well as subjects with missing data for uEGF (N=2)

or GFR at follow-up, mostly due to withdrawal (N=282), leaving a cohort of 1,249 subjects (Figure 1).

The PREVEND study (Groningen, the Netherlands) was designed to study the impact of albuminuria in subjects from the general population. A cohort of 8,592 subjects was recruited from the inhabitants of the city of Groningen, aged between 28 to 75 years, enriched for subjects with higher levels of albuminuria. For the current study, subjects that completed the second screening were used (2001-2003, N=6,188) because urine samples from these subjects were available for uEGF measurement. We excluded subjects with DM (N=144), CKD (ACR ≥ 3.0 mg/mmol or eGFR < 60 ml/min per 1.73 m^2, N=756), and missing data for uEGF (N=86) or eGFR at baseline (N=25), or eGFR at follow-up screening, mostly due to withdrawal (N=643), leaving a cohort of 4,534 subjects (Figure 1).

Both studies were approved by the local ethics committees and performed in accordance with the guidelines of the Declaration of Helsinki. All subjects provided written informed consent.

Data collection

The details of sample procurement in both PREVEND and RENIS have been previously described (13–15). In both studies, the subjects completed a questionnaire regarding demographics, cardiovascular and renal disease history, smoking habits and medication use. Blood samples were drawn after an overnight fast for measurement of fasting serum glucose, triglycerides, cholesterol and hemoglobin A1c, and stored at −80°C. For both studies, urinary albumin and creatinine concentration were measured in fresh urine samples.

Assessment of uEGF

In both studies, uEGF was assessed in duplicate. uEGF was normalized for urine creatinine concentration (uEGF/Cr) to adjust for differences in urine concentration. Hereafter, uEGF/Cr will be referred to as uEGF excretion. For RENIS, uEGF was measured in the second void morning spot urine sample (stored at –80 °C) using the Human EGF Immunoassay Quantikine ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) on a 96-ELISA format, with a mean intra- and inter-assay coefficients of variation (COV) of 3.5% and 4.6%, respectively. For PREVEND, uEGF was measured in a 24-hour urine collection (stored at –20 °C) using the Immunoassay Human EGF DuoSet ELISA (R&D Systems, Inc., Minneapolis, MN, USA) on a 384-ELISA format with a mean intra- and inter-assay COV of 4.7% and 7.9%, respectively. uEGF has been shown to be stable after prolonged frozen storage, and after up to 3 freeze-thaw cycles (16). Since uEGF was measured using different assay platforms in the two studies, 200 randomly selected urine samples were reanalyzed using both platforms, showing a correlation of 0.93 and a high recovery within 70-130% and 80-120% boundaries of 94.0% and 86.1%, respectively.

Assessment of GFR

In RENIS, GFR was measured at baseline and follow-up with a single-sample plasma clearance of iohexol (mGFR). This method has been validated against gold standard methods and previously described in detail (13,17). Repeated follow-up measurements of GFR in a random sample of 87 subjects within 8 weeks showed that the mean COV for intraindividual GFR was 4.2% (3.4–4.9%) (18). The subjects in RENIS had one (baseline, N=1,531), two (follow-up, N=1,249) or three (repeated follow-up, N=86) GFR measurements. In PREVEND, GFR was estimated with the CKD-EPI equation for creatinine and cystatin C (eGFR) (19), and

has previously been described (20,21). Both measurements were calibrated against international standards. The subjects in PREVEND had one (baseline, N=5,202), two (follow-up, N=4,534), three (repeated follow-up, N=3,951) or four (repeated follow-up, N=2,849) eGFR assessments.

Statistical methods

Continuous data are presented as the means with standard deviations, or as medians and interquartile ranges in case of skewed distribution. Categorical data are presented as percentages. We used Student's t-test for normally distributed continuous variables, Wilcoxon rank-sum test for skewed continuous variables, and Pearson's chi-squared test for categorical variables to evaluate differences between the subjects below and above the combined uEGF median of both cohorts.

Rapid GFR decline was defined as an annual GFR loss > 3.0 ml/min/1.73 m^2, calculated by the difference in GFR from baseline to follow-up, dividing with the observation time; a cut-off and method used in previous studies (22–24). For comparison, similar follow-up times for both cohorts were chosen (5.6 years in RENIS and for PREVEND 5.0 years, limiting data from the baseline to the second follow-up screening for the latter cohort).

To utilize all available GFR measurements, we also defined rapid GFR decline as subjects with the 10% steepest GFR slope within each cohort (25,26). The GFR slope for each subject was calculated using all available GFRs in a linear mixed model with the baseline covariables age, sex, ACR, body mass index (BMI), systolic blood pressure, fasting glucose, total cholesterol, triglycerides, current smoking, and use of lipid and blood pressure lowering drugs using random intercepts and slopes.

The association between uEGF excretion and rapid GFR loss was analyzed using logistic regression models progressively adjusted for (1) sex, baseline age and GFR; (2) ACR; and (3) the other aforementioned risk factors for CKD. Effect modification by age and sex was tested by including a two-way cross-product with uEGF excretion. Non-linear associations between uEGF excretion and rapid GFR decline were investigated by including a quadratic term of uEGF excretion in the logistic regression models. Logistic regression analyses with restricted cubic splines with three knots were used to visualize the association of uEGF excretion with risk for rapid GFR decline.

In sensitivity analyses, we calculated the individual slope using a model adjusted for sex, baseline age and BMI only, because 8 and 252 subjects in RENIS and PREVEND, respectively, had missing value for the slope created using a model with all the baseline covariables listed above. Using this model, there were no missing values for the slope in either cohort.

To evaluate the discriminatory power of uEGF excretion for predicting rapid decline in GFR during follow-up, we compared the area under the receiver operating characteristic (ROC) curve (AUC) for nested logistic regression models using the likelihood ratio test, assessed the relative integrated discrimination index (rIDI) and the net reclassification index (NRI) (27). The continuous NRI was used because it overcomes the problem of using categories that do not naturally exist and because we tested the same biomarker in two separate populations with different characteristics and age ranges (27–29).

The association between uEGF and mean change in eGFR/GFR was analyzed in linear mixed models with random intercept and random slope.

Finally, we combined the two cohorts to obtain power for interaction analyses with CKD risk factors and for the endpoint of incident CKD using multiple logistic regression. Incident CKD was defined as GFR < 60 ml/min/1.73 m^2 at the last follow-up, or as GFR < 60 ml/min/1.73 m^2 accompanied by a GFR loss > 10 ml/min/1.73 m^2 or ≥ 25% relative to baseline GFR in accordance with recent proposals (30–33).

The analyses were performed using Stata version 14 (StataCorp. LP. College Station, TX, USA) and R version 3.5.1 (R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). A p-value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

The population characteristics of both studies are shown in Table 1. Baseline measured GFR was 94.5 ± 13.0 ml/min per 1.73 m^2 in RENIS, and estimated GFR was 95.2 ± 14.2 ml/min per 1.73 m^2 in PREVEND. Because there was oversampling of subjects with higher albuminuria at baseline in the PREVEND cohort, the median ACR level was higher in PREVEND (median ACR 0.7 mg/mmol, IQR 0.5 to 1.0) compared to RENIS (0.2 mg/mmol, IQR 0.1 to 0.5).

Associations between uEGF excretion and baseline characteristics

Table 2 shows the associations of uEGF excretion stratified by the median value (1.44 µg/mmol) with baseline demographics and established CKD risk factors. In both cohorts,

subjects with a lower uEGF excretion were older, had higher blood pressure and fasting blood glucose.

A lower uEGF excretion level at baseline correlated with lower baseline GFR (r=0.14, p<0.001 and r=0.22, p<0.001, in RENIS and PREVEND, respectively). No significant correlation between uEGF excretion and ACR was observed in either cohort.

Association of uEGF excretion with rapid GFR decline

The median annual change in GFR was -0.92 (IQR -0.36 to -1.46) ml/min/1.73 m^2 per year in RENIS (N=1,249, follow-up 5.6 years) and -0.85 (IQR -0.61 to -1.12) ml/min/1.73 m^2 per year in PREVEND (N=4,534, follow-up 7.4 years). Lower uEGF excretion was associated with increased risk of rapid GFR decline in both cohorts (Table 3). All the models were well calibrated, as judged by the Hosmer-Lemeshow statistic.

There was a borderline significant, nonlinear quadratic association between uEGF excretion and rapid GFR decline in RENIS (p=0.07 to 0.1 in Models 1 – 4, Table 3), but not in PREVEND (p>0.1). We repeated the analyses using an outcome of rapid GFR decline defined as subjects with the 10% steepest GFR slope within each cohort, calculated using linear mixed regression. The effect estimates were strengthened in RENIS and attenuated in PREVEND, although statistically significant in both cohorts (OR per 1 µg/mmol lower uEGF was 1.67 (1.23 – 2.27) and 1.20 (1.04 – 1.38) in RENIS and PREVEND, respectively, in model 3 (Figure 2 and Table S1).

We then investigated the association between baseline uEGF levels and the mean GFR change rates using linear mixed models. In PREVEND, there was a significant association between lower uEGF levels with steeper GFR slope in the crude model only, and in RENIS

there were borderline significant associations with steeper slopes in crude and adjusted models (Table S2). The associations between tertiles of uEGF and GFR using linear mixed models with age as the time-variable (age at baseline + observation time) are shown in Figure S1.

We tested the incremental value of adding uEGF to a statistical model with traditional CKD risk factors in predicting rapid GFR decline, defined as > 3.0 ml/min/1.73 m^2/year. The C statistic increased only slightly from 0.71 to 0.72 in RENIS and 0.65 to 0.66 in PREVEND (Table S3). The improvement in the net reclassification index (NRI) was 0.30 (0.12 – 0.48) in RENIS and 0.14 (0.02 – 0.26) in PREVEND (Table S3). The event and nonevent NRIs, as well as the relative integrated discrimination index (rIDI), are shown in Table S3. The predicted versus observed risk of rapid GFR decline is shown in separate calibration plots for RENIS and PREVEND in Figure S2.

Subgroup analyses and incident CKD (GFR < 60 ml/min/1.73 m^2)

The cohorts were combined to improve power for analyses of the subgroups sex, current smoking, hypertension, hypercholesterolemia, overweight, impaired fasting glucose, and age, e/mGFR and ACR above or below the median level, and for analysis with incident CKD as an outcome.

No interaction was observed between uEGF excretion and CKD risk factors (Figure 3). The results seemed robust, as all subgroups showed increased risk when uEGF was lower, although due to impaired power significance was not obtained in all subgroups (Figure 3). Data on subgroup analysis for RENIS and PREVEND separately are provided in Figure S3.

There were 189 cases of Incident CKD defined as GFR < 60 ml/min/1.73 m^2 at the last follow-up, and 96 and 41 cases of CKD defined as GFR < 60 ml/min/1.73 m^2 accompanied by a GFR loss > 10 ml/min/1.73 m^2 or ≥ 25% relative to baseline, respectively. The associations between uEGF and three separate definitions of incident CKD are shown in Table 4.

Sensitivity analyses

To test the robustness of the results, we repeated the analyses using an outcome of rapid GFR decline defined as subjects with the top 20% steepest slopes in each cohort. The results were similar (Table S4). Next, we recalculated the individual slopes using a less complex linear mixed model (adjusted for sex, baseline age and BMI only) and the results were similar (Table S5).

When subjects with DM or CKD at baseline were not excluded from the analyses similar associations were found (Table S6). When subjects with ACR 3.0-30 mg/mmol were included, the association between uEGF and incident CKD became stronger and significant in all models (Table S7).

Finally, in the RENIS cohort, we repeated the logistic regression analyses with eGFR calculated by the CKD-EPI creatinine-cystatin C equation in place of the measured GFR. In these analyses, uEGF was similarly associated with rapid eGFR decline (Table S8).

DISCUSSION

In two prospective general population derived cohorts that excluded subjects with DM and CKD, lower urinary excretion of the kidney tubule-specific biomarker uEGF was associated with rapid kidney function decline and incident CKD.

GFR and albuminuria primarily cover the hemodynamic and glomerular state of the kidney but are insensitive to early tubular dysfunction. Tubular damage is increasingly recognized as an important factor in the development and progression of kidney disease (34). Several urinary biomarkers of acute tubular injury have been identified, but only a few biomarkers reflect chronic tubular damage. EGF is expressed by tubular epithelial cells, represents functional tubular mass and is inversely associated with interstitial fibrosis and tubular atrophy (8,35). It also plays an important role in the regeneration of tubular cells through cross-talk with the JAK-STAT, ERK and PI3K pathways (36,37). Furthermore, intrarenal EGF is predicted to be the dominant upstream regulator of intrarenal transcripts significantly correlated with GFR decline in patients with CKD, providing a potential molecular mechanism that may underlie rapid loss of kidney function (8).

Our present clinical results build upon uEGF excretion as a predictor for kidney disease progression in patients with established CKD and DM (8,38). In CKD, reduced uEGF excretion is associated with progression to the composite endpoint of incident ESKD or 40% reduction in kidney function (8,38,39). In normoalbuminuric subjects with DM, lower uEGF showed an increased risk for future renal function decline (9).

EGF is only minimally detectable in plasma (40,41), and urinary EGF excretion is a readout of kidney tubular-specific expression of EGF (8,42). Hence, compared to serum creatinine, albuminuria, and other biomarker candidates, uEGF level is less likely to be confounded by extrarenal factors, which potentially contributes to its biomarker

performance and sensitivity for early kidney injury. Moreover, uEGF is stable over extended period of times and can be robustly detected using just a few microliters of urine sample, making it a clinically applicable biomarker in population level settings.

Unlike the well-defined endpoints for CKD progression, i.e., the incidence of ESKD, a doubling of serum creatinine, or a decrease in GFR of 30%-40% compared to baseline (43), there is no consensus on the definition of rapid kidney function decline in population-based studies. The aforementioned endpoints are unlikely to occur in healthy subjects from the general population within a limited duration of a typical cohort study. Endpoints defined by an absolute or percentage change from baseline to follow-up GFR have therefore been used in some studies (44). However, as this usually relies on two GFR values, this method is more prone to misclassification and confounding, compared to using a slope of GFR change calculated from all available GFR values throughout the follow-up duration. Therefore, we also defined rapid GFR decline as the top 10% of subjects with the steepest GFR slopes as calculated by linear mixed models (43). Similar results using this method further support the association between uEGF and rapid GFR decline.

Although uEGF levels were associated with rapid GFR decline in both cohorts, in adjusted models they were only borderline associated with the mean GFR decline rate in RENIS. Notably, it is not necessarily a contradiction between the neutral effect of uEGF on the mean level of GFR and its association with rapid decline or incident CKD in a minority. uEGF may be an important contributing risk factor for CKD in combination with other genetic or environmental factors, thus affecting subgroups. For the youngest age group in the PREVEND, the mean annual eGFR change rate decline was very small. Lower precision of eGFR in the normal range, confounding from non-GFR related factors (such as muscle mass)

and an abnormal increase in GFR in subgroups due to a phase of hyperfiltration may have influenced the results.

We defined incident CKD as incident GFR < 60ml/min/1.73m^2 accompanied by a GFR loss > 10 ml/min/1.73 m^2 or 25 % loss of GFR from baseline (30–33). By doing so we assure that incident CKD cases are likely to indeed have progressive CKD and preclude "contamination" of incident CKD due to variability in measurements and biological day-to-day variability (45). The association of uEGF with incident CKD is significant, independent of age, gender, cohort, baseline GFR, and ACR. After adjusting for additional risk factors, the association either remains significant or on the borderline of significance (p=0.08). An analysis by including subjects with ACR 3.0-30 mg/mmol at baseline into the analysis, which increased the sample size of the events, exhibited stronger and significant associations (Table S7).

Addition of uEGF excretion to the conventional risk factors improved prediction of rapid kidney function decline assessed by NRI and IDI in both cohorts, but the improvement of AUC was limited. Continuous NRI has been proposed as a better metric to assess the discriminatory potential of a new biomarker, particularly in comparison against models with existing baseline functionality (28,29). Although the use of continuous NRI has been criticized, in the current setting it is expected to be more sensitive than C-statistics and able to capture the enhanced detection of individuals at risk of a specific outcome where natural categories do not exist (28). Our results build upon uEGF excretion as a predictor for kidney disease progression in patients with established CKD and DM (8,38). However, the exact place of measurement of uEGF to predict rapid kidney function decline has yet to be defined, and is beyond the scope of the present study. However, it could be imagined that including uEGF into a risk assessment system may of help to better predict which patients at

high risk for CKD progression will actually progress. In addition, determination of this biomarker may also be of help to evaluate “intrarenal health”. This may be important for instance for kidney function assessment to select subjects to act as kidney transplant donor, and before prescribing medications known to cause toxicity to kidney tubular cells, including certain antibiotics, mesalazine and HIV medications (46,47).

Our study has several limitations. Differences in the methods of urine sample collection and storage were present between both cohorts. However, it is unlikely that this affected the outcomes, as earlier studies showed that uEGF excretion was similar in spot and 24-hr urine samples (40), and that uEGF was stable at different freezing conditions and during frozen storage (16). A limitation regarding the uEGF measurements is the single measurement of uEGF. Intraindividual variation (day-to-day variation) in uEGF excretion may have allowed for misclassification bias when dividing the cohorts according to uEGF levels. Because both cohorts included predominantly white European subjects, validation of the utility of uEGF in general population cohorts of multiethnic origin will be required.

Strengths of the current study include comparable results in two independent, well-described cohorts from the general population (8,9). GFR was measured using an accurate method in RENIS and estimated using the CKD-EPI creatinine-cystatin equation during long-term follow-up in PREVEND.

In conclusion, lower urinary excretion of the tubular biomarker uEGF was associated with rapid GFR loss in subjects without DM or CKD in two European population studies. Our study, together with previous reports in patients with CKD and in patients with diabetes and at high risk of CKD, indicates that uEGF may be a useful non-invasive biomarker that

captures the tubular component of the function of the kidney in a manner beyond serum creatinine and proteinuria.

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CONFLICT OF INTEREST STATEMENT

MK, VN and WJ have a patent pending on biomarkers for CKD progression (encompassing uEGF as biomarkers of CKD progression). There are no other potential conflicts of interest relevant to this article.

AUTHORS' CONTRIBUTION

Conception and design of study: JVN, LRH, BOE, MK, RTG, WJ, TM. Analysis of data: JVN, LRH, VN, KS, RTG, BOE, WJ, TM. Acquisition and interpretation of data: JVN, LRH, VN, KS, MDS, BOE, MK, RTG, WJ, TM. Drafting the manuscript: JVN, LRH, RTG, WJ, TM. Critically revising the manuscript for important intellectual content: MDS, BOE, MK, RTG. All authors read and approved the final manuscript. All authors agreed to be accountable for all aspects of the manuscript in ensuring that questions related to the accuracy or integrity of any part of it are appropriately investigated and resolved.

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Table 1. Population characteristics.

	RENIS (N=1,249)	**PREVEND (N=4,534)**
Sex, women (%)	624 (50)	2313 (51)
Age, years	57.9 ± 3.9	51.5 ± 10.9
Race		
Caucasians (%)	1249 (100)	4364 (96)
Asians	0	86 (2)
Black	0	35 (1)
Other	0	49 (1)
Body Mass index, kg/m^2	27.1 ± 3.8	26.3 ± 4.1
Systolic blood pressure, mmHg	129.0 ± 17.4	123.4 ± 16.8
Diastolic blood pressure, mmHg	83.2 ± 9.7	72.6 ± 8.7
Current smoker, n (%)	232 (19)	1203 (27)
Antihypertensive medication, n (%)	209 (17)	698 (15)
Lipid-lowering medication, n (%)	74 (6)	317 (7)
Fasting blood glucose, mmol/L	5.3 ± 0.5	4.8 ± 0.6
Total cholesterol, mmol/L	5.6 ± 0.9	5.4 ± 1.0
Triglycerides, mmol/L	1.0 (0.7 – 1.4)	1.1 (0.8 – 1.6)
$eGFR_{cyscrea}$, $ml/min/1.73m^2$	N/A	95.2 ± 14.2
$mGFR_{iohexol}$, $ml/min/1.73m^2$	94.5 ± 13.0	N/A
Urinary albumin-to-creatinine ratio, mg/mmol	0.21 (0.10 – 0.51)	0.65 (0.48 – 1.01)
uEGF, µg/l	11.6 (5.9 – 22.0)	10.5 (6.3 – 16.8)
uEGF/Cr, µg/mmol	1.8 (1.4 – 2.3)	1.3 (0.9 – 1.9)

Plus–minus values are means ± SD. Skewed data is shown as median (interquartile range). Abbreviation are: eGFR, estimated glomerular filtration rate using CKD-EPI creatinine-cystatin C equation; mGFR, measured glomerular filtration rate using iohexol clearance; uEGF, urinary epidermal growth factor; uEGF/Cr, urinary EGF normalized to creatinine.

Table 2. Associations of uEGF excretion stratified by the common uEGF/Cr median of both cohorts with baseline demographics and various established CKD risk factors.

	RENIS			PREVEND		
	uEGF/Cr < median* (N=372)	uEGF/Cr > median* (N=877)	p-value	uEGF/Cr < median* (N=2,520)	uEGF/Cr > median* (N=2,014)	p-value
Sex, women (%)	128 (34)	496 (57)	<0.001	1054 (42)	1259 (50)	<0.001
Age, years	58.4 ± 3.8	57.7 ± 3.9	0.002	53.8 ± 10.9	48.6 ± 10.2	<0.001
Body mass index, kg/m^2	27.4 ± 3.6	27.0 ± 3.9	0.08	27.0 ± 4.1	25.5 ± 4.0	<0.001
Systolic blood pressue, mmHg	130.7 ± 17.1	128.2 ± 17.5	0.02	125.9 ± 17.1	120.3 ± 15.9	<0.001
Diastolic blood pressure, mmHg	84.1 ± 9.4	82.8 ± 9.9	0.03	73.9 ± 8.9	70.8 ± 8.8	<0.001
Current smoker, n (%)	62 (17)	170 (19)	0.26	630 (25)	573 (28)	0.001
Antihypertensive medication, n (%)	75 (20)	134 (15)	0.01	491 (19)	207 (10)	<0.001
Lipid-lowering medication, n (%)	21 (6)	53 (6)	0.79	228 (9)	89 (4)	<0.001
Blood glucose, mmol/l	5.4 ± 0.5	5.3 ± 0.5	0.007	4.9 ± 0.6	4.7 ± 0.6	<0.001
Total cholesterol, mmol/l	5.6 ± 1.0	5.7 ± 0.9	0.13	5.5 ± 1.0	5.3 ± 1.0	<0.001
Triglycerides, mmol/l	1.1 (0.8 – 1.5)	1.0 (0.7 – 1.4)	0.1	1.2 (0.8 – 1.7)	1.0 (0.7–1.3)	<0.001
Urinary albumin-to-creatinine ratio, mg/mmol	0.20 (0.10 – 0.43)	0.22 (0.10 – 0.54)	0.14	0.65 (0.47 – 1.03)	0.68 (0.49 – 0.99)	0.07
$eGFR_{cyscrea}$, ml/min/1.73 m²	100.6 ± 11.4	104.7 ± 10.1	<0.001	92.5 ± 14.4	98.6 ± 13.3	<0.001
$mGFR_{iohexol}$, ml/min/1.73 m²	92.4 ± 13.0	95.4 ± 12.8	<0.001	N/A	N/A	N/A
uEGF, µg/l	6.3 (3.1 – 12.8)	14.5 (7.6 – 26.0)	<0.001	7.3 (4.6 – 10.6)	16.2 (11.4 – 22.7)	<0.001
uEGF/Cr, µg/mmol	1.17 (0.99 – 1.31)	2.06 (1.72 – 2.47)	<0.001	0.95 (0.69 – 1.19)	2.01 (1.69 – 2.59)	<0.001

*Urinary EGF/creatinine median was 1.44 µg/mmol

Values are given as means ± standard deviation or median (interquartile range) for skewed data. Abbreviation are: eGFR, estimated glomerular filtration rate using CKD-EPI creatinine-cystatin C equation; mGFR, measured glomerular filtration rate using iohexol clearance; uEGF, urinary epidermal growth factor; uEGF/Cr, urinary EGF normalized to creatinine.

Table 3. Logistic regression models with odds ratios (OR) for rapid GFR decline defined as a GFR decline rate > 3.0 ml/min/1.73 m^2/year.*

	RENIS			PREVEND		
	OR	95% CI	p-value	OR	95% CI	p-value
Crude	**N=1,249**			**N=3,951**		
uEGF/Cr, per 1 µg/mmol decrease	1.17	(0.89 – 1.53)	0.30	1.32	(1.13 – 1.54)	0.001
uEGF/Cr, 4th quartile†	Ref			Ref		
uEGF/Cr, 3rd quartile†	1.15	(0.72 – 1.82)	0.57	1.47	(0.99 – 2.17)	0.06
uEGF/Cr, 2nd quartile†	1.71	(1.06 – 2.75)	0.03	1.51	(1.03 – 2.22)	0.03
uEGF/Cr, 1st quartile†	1.29	(0.62 – 2.68)	0.50	1.87	(1.30 – 2.68)	0.001
Model 1	**N=1,249**			**N=3,951**		
uEGF/Cr, per 1 µg/mmol decrease	1.42	(1.06 – 1.90)	0.02	1.31	(1.11 – 1.54)	0.001
uEGF/Cr, 4th quartile†	Ref			Ref		
uEGF/Cr, 3rd quartile†	1.46	(0.89 – 2.39)	0.13	1.45	(0.98 – 2.16)	0.07
uEGF/Cr, 2nd quartile†	2.38	(1.41 – 4.01)	0.001	1.49	(1.00 – 2.21)	0.048
uEGF/Cr, 1st quartile†	1.95	(0.90 – 4.22)	0.09	1.78	(1.22 – 2.62)	0.003
Model 2	**N=1,245**			**N=3,945**		
uEGF/Cr, per 1 µg/mmol decrease	1.42	(1.06 – 1.90)	0.02	1.31	(1.12 – 1.55)	0.001
uEGF/Cr, 4th quartile†	Ref			Ref		
uEGF/Cr, 3rd quartile†	1.45	(0.89 – 2.37)	0.14	1.47	(0.98 – 2.18)	0.06
uEGF/Cr, 2nd quartile†	2.37	(1.40 – 4.01)	0.001	1.52	(1.02 – 2.25)	0.04
uEGF/Cr, 1st quartile†	1.98	(0.91 – 4.28)	0.08	1.81	(1.23 – 2.66)	0.003
Model 3	**N=1,241**			**N=3,758**		
uEGF/Cr, per 1 µg/mmol decrease	1.42	(1.06 – 1.91)	0.02	1.29	(1.10 – 1.53)	0.002
uEGF/Cr, 4th quartile†	Ref			Ref		
uEGF/Cr, 3rd quartile†	1.43	(0.87 – 2.35)	0.16	1.53	(1.02 – 2.31)	0.04
uEGF/Cr, 2nd quartile†	2.39	(1.41 – 4.07)	0.001	1.50	(0.99 – 2.26)	0.05
uEGF/Cr, 1st quartile†	1.93	(0.88 – 4.24)	0.10	1.74	(1.16 – 2.61)	0.01

*Calculated by subtracting eGFR at baseline from eGFR at follow-up and dividing by the observation time (median 5.6 years in RENIS and 5.0 years in PREVEND).
n=127 for rapid decline in RENIS n=293 for rapid decline in PREVEND
†Common uEGF quartiles were used for both cohorts, 0.11 – 0.99 µg/mmol, 0.99 – 1,44 µg/mmol, 1.44 – 2.02 µg/mmol, and 2.02 – 28.27 µg/mmol.
Model 1: Adjusted for sex, age and baseline GFR.
Model 2: Adjusted for sex, age, baseline gfr and albumin-to-creatinine ratio (ACR)
Model 3: As in Model 2 and adjusted for body mass index, systolic blood pressure, fasting glucose, total cholesterol, triglycerides, current smoking, use of lipid-lowering drugs, and use of antihypertensive medication.
Abbreviation are: GFR, glomerular filtration rate; 95% CI, confidence interval; OR, odds ratio; uEGF/Cr, urinary EGF normalized to creatinine.

Table 4. Logistic regression analyses with odds ratios (OR) for incident CKD stage 3a or lower in RENIS and PREVEND combined.*

	Incident GFR < 60 ml/min/1.73 m²				Incident GFR < 60 ml/min/1.73 m² and baseline GFR > 70 ml/min/1.73 m²				Incident GFR < 60 ml/min/1.73 m² and > 25% GFR decline from baseline			
	OR	95% CI		p-value	OR	95% CI		p-value	OR	95% CI		p-value
Model 1												
uEGF/Cr, per 1 µg/mmol decrease	1.63	(1.26	– 2.10)	<0.001	1.70	(1.20	– 2.42)	0.003	1.97	(1.14	– 3.40)	0.02
uEGF/Cr > median†	Ref				Ref				Ref			
uEGF/Cr < median†	2.02	(1.43	– 2.86)	<0.001	2.26	(1.41	– 3.64)	0.001	2.22	(1.08	– 4.54)	0.03
Model 2												
uEGF/Cr, per 1 µg/mmol decrease	1.23	(0.97	– 1.56)	0.09	1.44	(1.02	– 2.05)	0.04	1.83	(1.06	– 3.15)	0.03
uEGF/Cr > median†	Ref				Ref				Ref			
uEGF/Cr < median†	1.41	(0.98	– 2.04)	0.07	1.91	(1.18	– 3.11)	0.01	2.06	(1.00	– 4.24)	0.049
Model 3												
uEGF/Cr, per 1 µg/mmol decrease	1.27	(0.99	– 1.62)	0.06	1.39	(0.98	– 1.97)	0.07	1.72	(1.01	– 2.95)	0.048
uEGF/Cr > median†	Ref				Ref				Ref			
uEGF/Cr < median†	1.44	(0.98	– 2.13)	0.06	1.77	(1.07	– 2.93)	0.03	1.95	(0.92	– 4.12)	0.08

*There were 189, 96 and 41 incident cases of CKD according to the 3 different definitions, respectively.

†The common urinary EGF/creatinine median was 1.44 µg/mmol.

Model 1: Adjusted for sex, age and cohort.

Model 2: Adjusted for sex, age, cohort, baseline GFR and albumin-to-creatinine ratio (ACR).

Model 3: As in Model 2 and adjusted for body mass index, systolic blood pressure, fasting glucose, total cholesterol, triglycerides, current smoking, use of lipid-lowering drugs, and use of antihypertensive medication.

Abbreviation are: GFR, glomerular filtration rate; 95% CI, confidence interval; OR, odds ratio; uEGF/Cr, urinary EGF normalized to creatinine.

Figure 1. Flowchart of the RENIS and PREVEND cohort. Abbreviations are: FU, follow-up; CKD, chronic kidney disease; (e)GFR, (estimated) glomerular filtration rate; uEGF/Cr, urinary epidermal growth factor to creatinine ratio.

Figure 2. Urinary EGF excretion and risk for rapid GFR decline defined as belonging among the 10% of subjects with the steepest GFR slope during follow-up per cohort (RENIS mean annual rate < -2.00 ml/min/1.73 m^2, PREVEND mean annual rate < -1.43 ml/min/1.73 m^2). Upper panels show the association in subjects from the RENIS cohort (N=1,241) crude (panel A) and adjusted for age, sex, mGFR and albuminuria (panel B). Lower panels show the association in subjects from the PREVEND cohort (N=4,282) again crude (panel C) and adjusted for age, sex, eGFR and albuminuria (panel D). Gray shaded area denotes 95% confidence interval.

Figure 3. Logistic regression analyses with odds ratios (OR) for rapid GFR decline and interactions with various subgroups for both cohorts combined. Subgroups are defined by common medians or clinical class (hypertension defined as systolic BP ≥ 140 mmHg, diastolic BP ≥ 90 mmHg or use of BP lowering drugs, hypercholesterolemia defined as total cholesterol ≥ 6.5 mmol/l or use of lipid lowering drugs, overweight as BMI ≥ 25 kg/m^2, and impaired fasting glucose as fasting glucose ≥ 5.6 mmol/l). The odds ratios are adjusted for baseline sex, age, m/e GFR and ACR. Common medians were: age, 53.3 years; m/e GFR, 96.0 ml/min/1.73 m^2; ACR, 0.58 mg/mmol. Abbreviations are: BMI, body mass index; m/eGFR, measured or estimated glomerular filtration rate; ACR, albumin-over-creatinine ratio; BP, blood pressure; N, number of subjects; n, number of events; CI, confidence interval). Rapid GFR decline is defined as a GFR decline rate > 3.0 ml/min/1.73 m^2/year.