Low levels of urinary epidermal growth factor predict chronic kidney disease progression in children

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Urinary epidermal growth factor (uEGF) has recently been identified as a promising biomarker of chronic kidney disease (CKD) progression in adults with glomerular disease. Low levels of uEGF predict CKD progression and appear to reflect the extent of tubulointerstitial damage. We investigated the relevance of uEGF in pediatric CKD. We performed a post hoc analysis of the Cardiovascular Comorbidity in Children with CKD (4C) study, which prospectively follows children aged 6–17 years with baseline estimated glomerular filtration rate (eGFR) of 10–60 ml/min/1.73 m^2. uEGF levels were measured in archived urine collected within 6 months of enrollment. Congenital abnormalities of the kidney and urinary tract were the most common cause of CKD, with glomerular diseases accounting for <10% of cases. Median eGFR at baseline was 28 ml/min/1.73 m^2, and 288 of 623 participants (46.3%) reached the composite endpoint of CKD progression (50% eGFR loss, eGFR < 10 ml/min/1.73 m^2, or initiation of renal replacement therapy). In a Cox proportional hazards model, higher uEGF/Cr was associated with a decreased risk of CKD progression (HR 0.76; 95% CI 0.69–0.84) independent of age, sex, baseline eGFR, primary kidney disease, proteinuria, and systolic blood pressure. The addition of uEGF/Cr to a model containing these variables resulted in a significant improvement in C-statistics, indicating better prediction of the 1-, 2- and 3-year risk of CKD progression. External validation in a prospective cohort of 222 children with CKD demonstrated comparable results. Thus, uEGF may be a useful biomarker to predict CKD progression in children with CKD.

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Pediatric patients are particularly vulnerable to complications associated with progression of CKD. These include, but are not limited to, impairment of quality of life, growth and neurocognitive function, as well as early cardiovascular morbidity.[1–5] Identifying patients with the highest risk of CKD progression would allow for timely and effective interventions and would also facilitate the design of clinical trials of nephroprotective therapies in the pediatric population where the incidence of CKD is low and sample sizes are a major concern. Lastly, the ability to predict renal function decline may guide patient counseling and decision-making for the physician, especially when preparing for the initiation of renal replacement therapy.

The efforts to find new tools that could improve prediction of CKD progression arise from the notion of high variability in the rates and nonlinear patterns of GFR decline among pediatric and adult patients with CKD.[6–8] Numerous factors that involved the presumed common pathway of CKD progression,[9] including compounds present in body fluids, have been studied as potential biomarkers of CKD progression.[10,11] For example, we recently identified the serum levels of the soluble urokinase receptor (suPAR) as a potential progression marker in children with mild to moderate CKD.[12] Few of these factors, such as blood pressure, proteinuria, and eGFR itself, are currently routinely used to assess the risk of progression and some have evolved into therapeutic targets.[11,13] However, these are insufficient to fully explain the heterogeneity in GFR decline and can be confounded by such factors as primary renal disease, comorbidities, or therapeutic interventions.[14] Studies of novel biomarkers in the pediatric CKD population are particularly scarce and often yield equivocal results. Candidate biomarkers frequently fail to demonstrate an incremental value in predicting CKD progression as compared with the established ones. Furthermore, the small number of large prospective pediatric cohorts further limits the possibilities of identifying and validating novel biomarkers.[11]

Recently, low uEGF excretion has been shown to predict CKD progression in adult patients with glomerular CKD, together with comprehensive mechanistic evidence that deficient uEGF excretion reflects tubulointerstitial fibrosis and tubular atrophy. These findings were validated in 3 independent adult cohorts covering a variety of glomerular diseases.[15] uEGF may be a particularly useful biomarker as it appears to provide quantitative information about the extent of tubulointerstitial pathology, complementary to the traditional biomarkers of glomerular function.

The preponderance of congenital kidney malformations makes children a unique population for CKD research and in particular to determine the added value of CKD progression markers in nonglomerular kidney disease.[16] Here we aimed to analyze the association and the predictive value of uEGF excretion for CKD progression in a large prospective pediatric CKD cohort and validated the findings in another independent cohort.

RESULTS

Cohort description

A total of 623 of 704 patients from the 4C study meeting the eligibility criteria were included in the main analysis. The baseline demographic, clinical, and laboratory characteristics of the cohort are summarized in Table 1. Mean age at the time of enrollment was 12.2 ± 3.35 years. The leading primary renal diagnosis was congenital anomalies of kidney and urinary tract (N = 435; 69.8%), followed by tubulointerstitial diseases, glomerulopathies, CKD after acute kidney injury, and miscellaneous conditions. Median eGFR at baseline was 28.1 (21.6–35.4) ml/min per 1.73 m^2. Fifty-five percent (N = 344) of the patients were prescribed at least 1 antihypertensive medication, and approximately 43% (N = 269) received renin-angiotensin system antagonists.

Correlates of uEGF/Cr

Baseline characteristics stratified by uEGF/urinary creatinine (Cr) quartiles are shown in Table 1. At baseline, uEGF/Cr showed positive correlation with eGFR ($r = 0.52$, $P < 0.001$). In the univariable analysis, log-uEGF/Cr was associated with higher eGFR, higher serum bicarbonate, and younger age (Table 2). Only the associations with higher eGFR and younger age remained in the multivariable model (Table 2). The same multivariable model was repeated with log-uEGF/urine osmolality (osm) instead of log-uEGF/Cr and showed similar associations with age and baseline eGFR (Table S1).

uEGF/Cr and CKD progression

During a median follow-up of 2.8 (1.1–4.9) years, 288 patients (46.3%) reached the composite endpoint of CKD progression, corresponding to an incidence rate of 15.2 events per 100 patient-years. Median time to the primary endpoint was 2.2 (1–3.8) years. When stratified by uEGF/Cr quartiles, patients in the higher uEGF/Cr quartiles showed lower probability of CKD progression ($P < 0.001$ for between-group differences, Figure 1). Of the remaining 335 patients who did not reach the composite endpoint, 104 (31%) were censored because of the end of observation period and 231 (69%) were dropouts.

To investigate whether the association of uEGF/Cr with CKD progression is independent of established progression factors, 2 Cox proportional hazards regression models containing these variables without and with uEGF/Cr (Model 0 and Model 1, respectively) were constructed. In the basic model (Model 0), baseline eGFR, age, primary renal disease, baseline systolic blood pressure SD scores, and baseline log-transformed urine protein-to-creatinine ratio (uPCR) but not sex showed significant associations with the risk of CKD progression (Table 3, Model 0). When added to Model 0, a higher log-transformed uEGF/Cr remained significantly associated with a lower risk of CKD progression (hazard ratio,

Table 1 | Baseline characteristics of the whole sample and stratified by uEGF/Cr quartiles

Baseline characteristic	All (n = 623)	uEGF/Cr (ng/mg) quartile ≤1.92 (n = 157)	1.92–3.46 (n = 155)	3.46–6.47 (n = 155)	>6.47 (n = 156)	*P* value
Sex, no. of boys (%)	409 (65.7)	116 (73.9)	95 (61.3)	100 (64.5)	98 (62.8)	0.08
Age, mean (SD), yrs	12.2 (3.35)	13.4 (2.98)	13.1 (3.11)	11.4 (3.18)[a,b]	10.8 (3.38)[a,b]	<0.001
Primary renal diagnosis, no. (%)						0.07
Obstructive/reflux nephropathies	317 (50.9)	65 (41.4)	77 (49.7)	85 (54.8)	90 (57.7)	
Other CAKUT	118 (18.9)	34 (21.7)	31 (20)	31 (20)	22 (14.1)	
Tubulointerstitial disorders	79 (12.7)	19 (12.1)	20 (12.9)	20 (12.9)	20 (12.8)	
Glomerulopathies	51 (8.2)	21 (13.4)	14 (9)	4 (2.6)	12 (7.7)	
Post-AKI	33 (5.3)	10 (6.4)	5 (3.2)	11 (7.1)	7 (4.5)	
Other	25 (4)	8 (5.1)	8 (5.2)	4 (2.6)	5 (3.2)	
Systolic blood pressure						
mm Hg, mean (SD)	112 (15)	114 (15)	113 (15.8)	110 (13.9)	111 (14.9)	0.07
SDS, median (IQR)	0.65 (−0.13 to 1.49)	0.64 (−0.23 to 1.49)	0.59 (−0.24 to 1.37)	0.53 (−0.09 to 1.46)	0.82 (−0.07 to 1.74)	0.32
Diastolic blood pressure						
mm Hg, mean (SD)	68.6 (12.3)	68.3 (12.1)	69.6 (12.9)	67.9 (12.3)	68.5 (11.8)	0.65
SDS, median (IQR)	0.55 (−0.05 to 1.19)	0.51 (−0.29 to 1.174)	0.57 (−0.1 to 1.29)	0.51 (0.02–1.09)	0.64 (0.15–1.22)	0.33
Body mass index						
kg/m^2, mean (SD)	18.3 (4.25)	18.7 (4.11)	18.7 (4.16)	18.3 (4.47)	17.7 (4.18)	0.10
SDS, median (IQR)	0.19 (−0.59 to 0.89)	0.07 (−0.76 to 0.7)	0.26 (−0.66–0.82)	0.23 (−0.34 to 0.94)	0.16 (−0.6 to 1.06)	0.32
Height						
cm, mean (SD)	141 (20.3)	147 (19.2)	147 (18.1)	137 (19.7)[a,b]	134 (20.7)[a,b]	<0.001
SDS, median (IQR)	−1.23 (−2.08 to −0.4)	−1.20 (−2 to −0.3)	−1.01 (−1.99 to −0.24)	−1.28 (−2.13 to −0.5)	−1.36 (−2.13 to −0.58)	0.23
eGFR, median (IQR), ml/min per 1.73 m^2	28.1 (21.6–35.4)	21.4 (18.6–26.9)	26.4 (21.8–32.6)[a]	29.6 (24.2–36.8)[a,b]	35.3 (29.2–43.8)[a,b,c]	<0.001
uPCR, median (IQR), mg/mg	1.22 (0.52–2.83)	1.22 (0.56–2.64)	1.21 (0.51–2.53)	1.02 (0.52–2.44)	1.48 (0.48–3.51)	0.57
uEGF/Cr, median (IQR), ng/mg	3.46 (1.92–6.47)	1.11 (0.51–1.45)	2.65 (2.32–2.93)	4.67 (4.03–5.54)	10.17 (7.86–14.9)	<0.001
Total cholesterol, median (IQR), mg/dl	4.47 (3.75–5.22)	4.45 (3.57–5.12)	4.27 (3.59–5.28)	4.65 (4.09–5.28)	4.53 (3.85–5.25)	0.12
Bicarbonate, mean (SD), mmol/l	20.6 (5.45)	19.8 (5.29)	20.3 (4.91)	21 (5.12)	21.3 (6.3)	0.07
Serum albumin, mean (SD), g/l	39.1 (5.99)	39 (7.37)	39 (6.23)	39 (5.12)	39.3 (4.94)	0.95
Hemoglobin, mean (SD), g/dl	11.4 (2.53)	10.9 (2.19)	11.5 (2.17)	11.5 (2.63)	11.8 (2.97)[a]	0.03
No. on antihypertensive medication (%)	344 (55.2)	107 (68.2)	89 (57.4)[a]	80 (51.6)[a]	68 (43.6)[a,b]	<0.001
No. on RAS inhibitors (%)	269 (42.6)	80 (51)	67 (43.2)	69 (44.5)	53 (34)[a]	0.03

AKI, acute kidney injury; CAKUT, congenital anomalies of kidney and urinary tract; eGFR, estimated glomerular filtration rate; IQR, interquartile range; RAS, renin-angiotensin system; SDS, SD score; uEGF/Cr, urinary epidermal growth factor/urinary creatinine; uPCR, urinary protein-to-creatinine ratio.
[a]$P < 0.05$ compared with quartile 1 (≤1.92).
[b]$P < 0.05$ compared with quartile 2 (1.92–3.46).
[c]$P < 0.05$ compared with quartile 3 (3.46–6.47).
SI conversion factors: to convert total cholesterol to mmol/l, multiply values by 0.0259.

0.76; 95% confidence interval, 0.69–0.84, $P < 0.001$; Table 3, Model 1). The addition of uEGF/Cr to Model 0 also improved the fit of the model based on the Akaike information criterion (Table 3). In addition, the Cox proportional hazard model analysis was performed substituting log-uEGF/Cr by log-uEGF/osm and showed comparable results (Table S2, Model 2).

The inclusion of uEGF/Cr to Model 0 also increased the C-statistics for predicting 1-, 2-, and 3-year risk of CKD progression (C-statistics 0.826–0.840, 0.792–0.806, and 0.784–0.795, respectively, Table 4) indicating improvement in risk discrimination.

Validation of the prediction analysis

The results of C-statistics were validated internally and externally. Validation within the same cohort by leave-one-out cross-validation and bootstrapping revealed similar results (Table 4). The external validation sample consisted of 222 (of 385 enrolled) eligible participants from the ESCAPE Trial with a median follow-up of 5 (2.9–5.7) years. Eighty-three participants (37.4%) reached the primary endpoint at a median of 2.9 (1.6–4.4) years. In the ESCAPE Trial cohort, the inclusion of uEGF/Cr to Model 0 developed in the 4C cohort resulted in an increase of C-statistics at 1 and 3 years (Table 4). Compared with the 4C cohort, participants of the ESCAPE Trial were on average younger (mean age, 11.3 vs. 12.2 years), had higher eGFR (median eGFR, 48.5 vs. 28.1 ml/min per 1.73 m^2), lower proteinuria (median uPCR, 0.6 vs. 1.22 mg/mg), and all received renin-angiotensin system inhibitors (detailed comparison presented in Table S3).

DISCUSSION

In the present analysis, we sought to investigate the predictive value of uEGF as a biomarker of CKD progression in a large prospective pediatric CKD cohort. Our results demonstrate a clear association between lower uEGF/Cr and a higher risk of

Table 2 | Explaining variables of log-uEGF/Cr (univariable and multivariable regression)

	Univariable regression				Multivariable regression			
	Exp(β)	95% CI		*P* value	Exp(β)	95% CI		*P* value
eGFR, per ml/min per 1.73 m^2	1.056	1.048	1.064	<0.001	1.056	1.048	1.064	<0.001
Age, per year	0.909	0.884	0.934	<0.001	0.905	0.884	0.927	<0.001
Diagnosis group (ref: nonglomerular diseases)								
Glomerulopathies	0.811	0.573	1.148	0.24	–	–	–	–
Log uPCR, per mg/mg	1.079	0.996	1.168	0.06	–	–	–	–
Serum bicarbonate, per mmol/l	1.021	1.004	1.039	0.02	1.008	0.993	1.023	0.30

CI, confidence interval; eGFR, estimated glomerular filtration rate; uEGF/Cr, urinary epidermal growth factor/urinary creatinine; uPCR, urinary protein-to-creatinine ratio. Multivariable regression includes only the variables with $P < 0.05$ in the univariable regression.

CKD progression in children. More importantly, multivariable modeling revealed that uEGF/Cr is associated with CKD progression independent of well-established progression factors (i.e., proteinuria, systolic blood pressure, renal diagnosis, and eGFR). Finally, the addition of uEGF/Cr to the prediction model with known CKD progression factors improved risk discrimination illustrating the incremental value of uEGF/Cr over the conventional markers. In addition, we validated these results in a large independent pediatric CKD cohort. Our findings expand recent results from adult studies to the pediatric CKD population, where nonglomerular renal diseases are predominant. Collectively, these findings suggest that uEGF/Cr is a biomarker of CKD progression providing complementary information to the established risk markers that reflects a common and yet unaddressed pathway of CKD evolution involving tubulointerstitial injury.

Biological plausibility is an essential feature of a biomarker. Understanding the biology underlying the association of uEGF with CKD progression is crucial for comprehending the applicability of this biomarker. EGF is one of the most

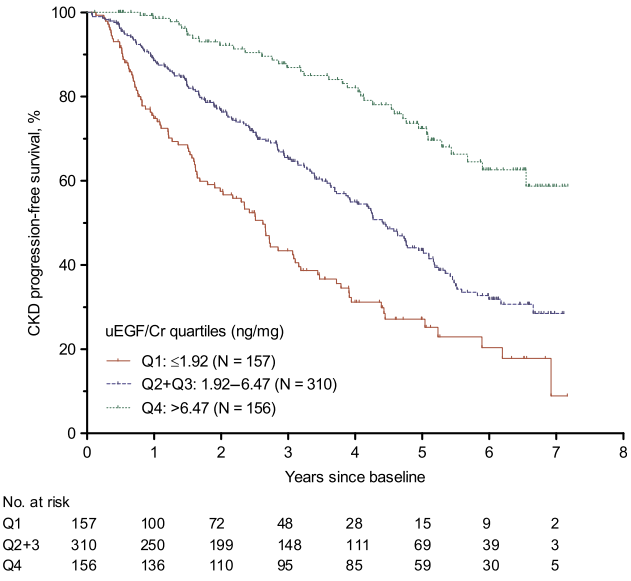

Figure 1 | Chronic kidney disease (CKD) progression-free survival by urinary epidermal growth factor/urinary creatinine (uEGF/Cr) quartiles. Quartiles 2 and 3 were superimposed and therefore were combined.

well-investigated ligands of the EGF receptor. EGF receptor and its signaling pathway play an essential role in cell physiology, including cell growth, migration, proliferation, and differentiation.[17] Based on bioinformatic analyses and experimental evidence, the role of EGF in kidney disease progression might be a combination of marking the functional tubular cell mass and its active participation in the repair process. First, EGF is reversely and tightly associated with interstitial fibrosis and tubular atrophy in patients with primary glomerular disease: the more tubulointerstitial damage, the less of functional tubular cells and less EGF.[15] Secondly, EGF represents tubular epithelial cell regenerative potential: both *in vivo* and *in vitro* studies have shown that higher EGF enhances renal tubule cell regeneration and repair, and accelerates the recovery of renal function after injury through its pro-proliferative and antiapoptotic action.[18–20] Lastly, integration of data-driven approach and prior literature analysis predicted EGF as the top upstream regulator of genes whose expression is significantly correlated with faster decline of kidney function.[15] This suggests that EGF may lead to kidney disease progression through regulation of these genes. Moreover, EGF shows highly specific renal expression and is typically detected in very low amounts in the circulation.[15,21] Therefore, urinary EGF levels accurately reflect its expression in the tubules. Indeed, significant differences in urinary but not serum EGF levels were found between children with CKD and healthy controls.[22]

This is the first large longitudinal study to investigate and show the association of urinary EGF excretion and CKD progression in children with CKD. The importance of our findings is rooted in substantial differences between the pediatric and adult CKD populations. First, children with CKD share a different spectrum of primary renal conditions compared with adults, with congenital anomalies of kidney and urinary tract and hereditary renal diseases being the leading cause of CKD development.[16] The preponderance of nonglomerular disorders combined with the relative lack of comorbid conditions (e.g., primary hypertension, diabetes mellitus, atherosclerosis, or smoking) makes children with CKD a unique population for studies of CKD. Hence, our findings not only expand the prognostic value of uEGF/Cr to the pediatric age range but also prove the applicability of uEGF/Cr in nonglomerular CKD populations.

Table 3 | Cox proportional hazard models for CKD progression

	Model 0 (AIC 3080)				Model 1 (AIC 3057)			
	Hazard ratio	95% CI		*P* value	Hazard ratio	95% CI		*P* value
Sex (female)	1.00	0.79	1.28	0.98	1.07	0.84	1.37	0.59
Age (yr)	1.08	1.05	1.12	<0.001	1.06	1.02	1.10	0.004
Log uPCR	1.62	1.46	1.80	<0.001	1.83	1.63	2.06	<0.001
Systolic blood pressure SDS	1.13	1.04	1.24	0.006	1.13	1.04	1.24	0.007
Diagnosis group (ref: nonglomerular diseases)								
Glomerulopathies	1.91	1.29	2.81	0.001	1.78	1.20	2.64	0.004
Baseline eGFR (ml/min per 1.73 m^2)	0.93	0.92	0.95	<0.001	0.95	0.93	0.97	<0.001
Log-uEGF/Cr	–	–	–	–	0.76	0.69	0.84	<0.001

AIC, Akaike information criterion; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; SDS, SD score; uEGF/Cr, urinary epidermal growth factor/urinary creatinine; uPCR, urinary protein-to-creatinine ratio.

To further investigate whether the association of uEGF/Cr with CKD progression is independent of conventional markers of progression risk, we compared Cox proportional hazard models before and after inclusion of uEGF/Cr. The basic model yielded results in accordance with previous studies: proteinuria, baseline eGFR, systolic blood pressure, age, and primary renal diagnosis were associated with the risk of CKD progression.[23] Most importantly, the association of uEGF/Cr and CKD progression remained significant even after the adjustment for these progression risk factors. Given that both urine creatinine excretion and the rate of CKD progression are age dependent and increase in puberty, the association observed with uEGF normalized to urine creatinine could be a spurious effect. Therefore, to rule out collinearity-driven associations, we repeated the same analysis using normalization to urine osmolality, which is age independent across childhood. The very similar results obtained with both models validate the findings with uEGF/Cr.

Most importantly, we sought to investigate whether uEGF/Cr is associated with incremental improvement in the prediction of CKD progression in children. Because of increasing censoring with longer follow-up times, the prediction analysis was limited to the prediction of 1-, 2-, and 3-year risk of CKD progression. The increase of C-statistics for CKD progression at all time points after the addition of uEGF/Cr to the basic model indicates its added value for the risk discrimination. To demonstrate the reproducibility of our findings and to adjust for overoptimism, we performed internal and external validation of the prediction analysis that confirmed the results of our main analysis. It is important to note that urinary EGF/Cr improved risk discrimination also in the external validation cohort that differed from the 4C sample by age, underlying disease distribution and eGFR range, and was subject to pharmacological interventions during the observation period. Hence, uEGF/Cr appears to be useful in risk prediction across diverse CKD populations.

Even after adjustment for other covariates, uEGF/Cr showed significant negative association with age. Considering age-related changes in urine creatinine excretion, we repeated the analysis with uEGF/osm. This adjustment, however, did not abolish the association of uEGF with patient age. A recent study reported that uEGF excretion decreases with age in healthy children,[24] which suggests that higher uEGF levels in younger children may represent a physiological phenomenon.

Our analysis is subject to several limitations. These include the use of one-time uEGF/Cr measurements, the lacking representation of young infants, children with early CKD stages 1 and 2, and children with African American and Asian background. On the other hand, the large multi-center study cohort of well-phenotyped pediatric CKD patients encompassing a wide range of primary renal disease and ethnic backgrounds is a major strength of our study. Moreover, the positive results of internal and external validation measures add additional strength by documenting the reproducibility of our findings.

None of the current routine markers of progression specifically address tubulointerstitial damage, an important common segment in the CKD-to-ESKD pathway. Our results expand the recent evidence from adult studies to the congenital anomalies of kidney and urinary tract predominant populations of childhood

Table 4 | C-statistics with internal and external validation for Models 0 and 1 at different time points

	C-statistic [95% CI]			Leave-one-out cross-validation		Bootstrapping		External validation (ESCAPE cohort)	
Time point	Model 0	Model 1	ΔC-statistic	Model 0	Model 1	Model 0	Model 1	Model 0	Model 1
1-yr	0.826 [0.776, 0.876]	0.840 [0.790, 0.890]	0.014 [0.002, 0.027]	0.822	0.836	0.822	0.835	0.917	0.942
2-yr	0.792 [0.755, 0.830]	0.806 [0.771, 0.841]	0.013 [0.004, 0.023]	0.788	0.801	0.788	0.800	0.843	0.843
3-yr	0.784 [0.754, 0.815]	0.795 [0.765, 0.826]	0.011 [0.003, 0.019]	0.780	0.791	0.781	0.791	0.814	0.816

Model 0 included sex, age, log-urine protein-to-creatinine ratio, systolic blood pressure SD score, primary renal diagnosis group (glomerular vs. nonglomerular), and baseline estimated glomerular filtration rate.
Model 1 included sex, age, log-urine protein-to-creatinine ratio, systolic blood pressure SD score, primary renal diagnosis group (glomerular vs. nonglomerular), baseline estimated glomerular filtration rate, and log-urine epidermal growth factor/urine creatinine ratio.
CI, confidence interval.

CKD. Collectively, these results strongly support uEGF/Cr, a specific marker of tubulointerstitial damage, as an independent biomarker of CKD progression in diverse populations of adult and childhood CKD. Future large multiethnic validation studies including patients with early-stage CKD will be needed to assess the usefulness of uEGF in predicting the risk of CKD progression before adding this novel biomarker to the routine care of children and adults with CKD.

METHODS

Study design and setting

Study cohort. The association of uEGF with CKD progression was analyzed in the 4C study cohort. The 4C study is a prospective, observational, multicenter cohort study aiming to uncover the causes and natural course of cardiovascular disease in children with CKD. The design and setting of the 4C study have been described in detail elsewhere.[25] Briefly, children aged 6–17 years with the baseline eGFR of 10–60 ml/min per 1.73 m^2 and without prior renal replacement therapy were enrolled at 55 centers across 12 European countries. Study participants were followed with 6-monthly clinical assessments along with collection of blood and urine samples from 2010 to 2016.

Validation cohort. The cohort followed in the ESCAPE Trial, an investigator-initiated randomized clinical trial investigating the effect of intensified blood pressure control on CKD progression, was used for external validation of the principal findings of this analysis. This cohort comprised children aged 3–17 years with a baseline eGFR of 15–80 ml/min per 1.73 m^2. The trial design and procedural details are available in Appendix S1 and have been published previously.[26]

Participants of the 4C study and the ESCAPE Trial were considered eligible for this analysis based on the (i) availability of frozen urine samples collected within 6 months of enrollment and (ii) the availability of data on CKD progression.

The protocols of both studies were approved by the central ethics committee of the Heidelberg University Medical Faculty and by each local institutional review board.

Laboratory assessments

All blood and urine samples were analyzed centrally following standardized procedures. Enzymatically measured serum creatinine and cystatin C determined by turbidimetric assay were used to calculate eGFR using a previously described equation.[27] uPCR (mg/mg) was used to define proteinuria. Urine EGF levels were determined by the enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) in spot urine samples collected within 6 months of enrollment. A clean catch spot urine sample was collected and the urine samples were immediately aliquoted and frozen and stored at −80 °C until tested. Two urine samples with an intraplate coefficient of variation of uEGF concentration more than 20% were excluded from further analysis. Urine EGF levels were then normalized by calculating uEGF/Cr and uEGF/osm ratios.

Clinical assessments

Anthropometric and clinical data were collected at each 6-monthly study visit according to the study protocol. [4] Age- and sex-dependent clinical variables (weight, height, and blood pressure) were standardized to calculating SD scores.[28]

Statistical analysis

Baseline characteristics for the whole sample and stratified by uEGF/Cr quartile groups are described by mean with SD or median with interquartile range or frequencies, as appropriate. Analysis of variance, Kruskal-Wallis, or chi-squared tests with respective *post hoc* tests were applied for group comparisons, and Spearman's rank correlation coefficient was used to quantify correlations. Primary renal diseases were classified as obstructive/reflux nephropathies, other congenital anomalies of kidney and urinary tract, tubulointerstitial diseases, glomerulopathies, CKD after acute kidney injury, and miscellaneous (Table S4). The association of baseline eGFR, age, primary renal disease, log-transformed uPCR, and serum bicarbonate with log-transformed uEGF/Cr levels was analyzed by univariable regression and the multivariable linear model that included the variables with P value < 0.05 in univariable analysis.

The primary endpoint of CKD progression was defined as time to a composite event of 50% reduction in eGFR, eGFR < 10 ml/min per 1.73 m^2 or start of renal replacement therapy, whichever occurred first. If a 50% reduction in eGFR occurred between 2 study visits, interpolation was used to determine the time point of the event. Two Cox proportional hazard models (with and without log-transformed uEGF/Cr) were applied to assess factors (baseline eGFR, age, sex, primary renal disease, baseline systolic blood pressure SD scores, and baseline log-transformed uPCR) associated with CKD progression. The Akaike information criterion was used to assess and compare the quality of the 2 models with lower values indicating a better fit. Kaplan Meier survival curves stratified by quartiles of uEGF/Cr were constructed to illustrate and the log-rank test was used to compare CKD progression-free survival between different uEGF/Cr quartiles.

Prediction model performance was evaluated by C-statistics, a measure of discrimination. Truncated version of C (C_τ) was calculated with the R package *survC1* for $\tau = 1, 2, 3$ years.[29] To compare the predictive performance of the 2 models, the difference between the C-statistics with 95% confidence interval was calculated with $0 \notin$ 95% confidence interval, indicating significant improvement. To adjust for optimism, leave-one-out cross-validation and bootstrap (1000 repetitions) corrected estimates were calculated.[30] In addition, models were evaluated in an external validation data set. The baseline hazard function and regression coefficients from the developed model were fixed and applied to the validation data set.

The two-sided P value of <0.05 was considered significant. Statistical analysis was performed using SAS Software Version 9.4 (SAS Inc., Cary, NC) or R version 3.2.2.

APPENDIX

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DISCLOSURE

VN, WJ, and MK hold a patent PCT/EP2014/073413 "Biomarkers and methods for progression prediction for chronic kidney disease." All the other authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Appendix S1. Design and methods of the ESCAPE Trial.
Table S1. Determinants of log-uEGF/osm (multivariable linear regression model).
Table S2. Cox proportional hazard models for CKD progression using log-uEGF/osm.
Table S3. Comparison of baseline characteristics between the 4C Study and the ESCAPE Trial cohorts.
Table S4. The categories of primary renal diseases in the 4C study.
Supplementary material is linked to the online version of the paper at www.kidney-international.org.

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