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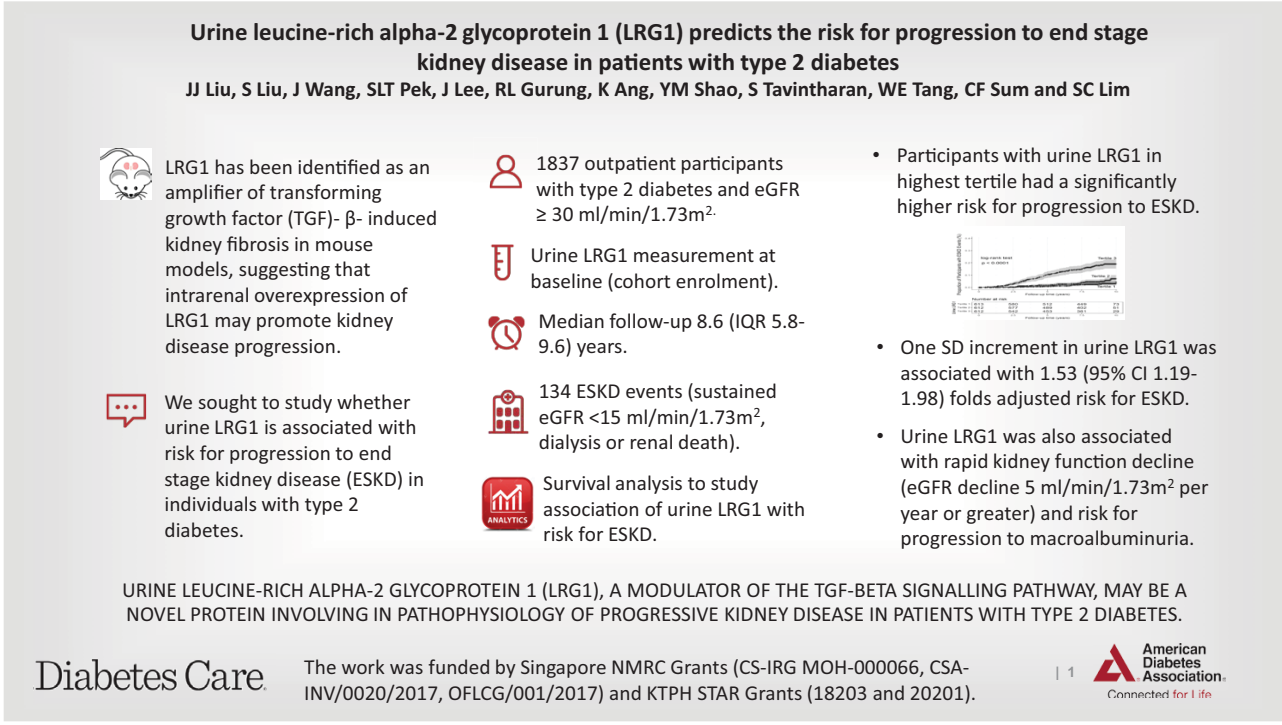

ARTICLE HIGHLIGHTS

- Leucine-rich α-2 glycoprotein 1 (LRG1) promoted kidney fibrosis by modulating transforming growth factor-β signaling in a mouse model. Clinical data on urine LRG1 and kidney disease outcomes are still lacking.
- We sought to study whether urine LRG1 predicts risk of progression to end-stage kidney disease (ESKD) in individuals with type 2 diabetes.
- Baseline urine LRG1 was associated with risk of ESKD independently of traditional clinical predictors. It was also associated with rapid kidney function decline and progression to macroalbuminuria, two common pathways leading to ESKD.
- Urine LRG1 is a novel biomarker for progression of kidney disease in patients with type 2 diabetes.

Urine Leucine-Rich α-2 Glycoprotein 1 (LRG1) Predicts the Risk of Progression to End-Stage Kidney Disease in Patients With Type 2 Diabetes

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OBJECTIVE

Leucine-rich α-2 glycoprotein 1 (LRG1) was recently identified as an amplifier of transforming growth factor-β (TGF-β)–induced kidney fibrosis in animal models. We aimed to study whether urine LRG1 is associated with risk of progression to end-stage kidney disease (ESKD) in individuals with type 2 diabetes.

RESEARCH DESIGN AND METHODS

A total of 1,837 participants with type 2 diabetes and estimated glomerular filtration rate (eGFR) >30 mL/min/1.73 m^2 were recruited from a regional hospital and a primary care facility. Association of urine LRG1 with risk of ESKD (progression to sustained eGFR <15 mL/min/1.73 m^2, dialysis, or death resulting from renal causes) was assessed by survival analyses.

RESULTS

During a median follow-up of 8.6 (interquartile range 5.8–9.6) years, 134 incident ESKD events were identified. Compared with those in the lowest tertile, participants with baseline urine LRG1 in the highest tertile had a 1.91-fold (95% CI 1.04–3.50) increased risk of progression to ESKD, after adjustment for cardiorenal risk factors, including eGFR and albuminuria. As a continuous variable, 1 SD increment in urine LRG1 was associated with a 1.53-fold (95% CI 1.19–1.98) adjusted risk of ESKD. Of note, the association of urine LRG1 with ESKD was independent of plasma LRG1. Moreover, urine LRG1 was associated with rapid kidney function decline and progression to macroalbuminuria, two common pathways leading to ESKD.

CONCLUSIONS

Urine LRG1, a TGF-β signaling modulator, predicts risk of progression to ESKD independently of clinical risk factors in patients with type 2 diabetes, suggesting that it may be a novel factor involved in the pathophysiological pathway leading to kidney disease progression.

Diabetes is the leading cause of end-stage kidney disease (ESKD) in many countries (1). The pathophysiological mechanisms underlying the pathogenesis and progression of diabetic kidney disease (DKD) are only partially understood. Metabolic

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dysregulation and hemodynamic perturbation may interact and drive the proinflammatory and profibrotic cascade, a process that is closely related to loss of kidney function (2). Intensive control of glycemia and blood pressure and administration of renal protective agents may greatly ameliorate the risk of development of DKD or delay its progression (3). However, considerable residual risk remains, even under intensive treatment in a clinical trial setting (4). In-depth understanding of the pathophysiological pathways that drive DKD progression may shed light on new interventional targets.

Leucine-rich α-2 glycoprotein 1 (LRG1) is a secretory protein characterized by the conserved leucine-rich repeat motif in structure (5). Plasma LRG1 is mainly synthesized and secreted from liver and immune cells as an acute-phase response protein (6,7). LRG1 may also be secreted locally and function in an autocrine or paracrine manner in other organs (8). Of note, LRG1 has been identified as a modulator of transforming growth factor-β (TGF-β) signaling by binding to the accessory receptor endoglin (9). Preclinical and clinical studies showed that LRG1 might be involved in the pathogenesis of diabetic vascular complications such as retinopathy and nephropathy by modulating the proangiogenic and profibrotic TGF-β signaling pathway (9,10).

In the kidney, LRG1 was found to be primarily expressed in glomerular endothelial cells. Global ablation of LRG1 markedly attenuated the development of diabetic glomerulopathy (10). Interestingly, recent studies found that LRG1 was also expressed in tubular cells in the kidney. Expression of LRG1 in the tubular epithelial cells was markedly increased upon stimulation by albumin overload or tumor necrosis factor-α treatment in animal models (11,12). Further mechanistic study showed that LRG1 secreted from tubular epithelial cells drove the profibrotic pathway and extracellular matrix deposition in the kidney by amplifying the TGF-β signaling pathway (13). Data mining showed that LRG1 RNA expression was fivefold higher in the tubulointerstitial compartment of kidney samples from patients with diabetes compared with healthy counterparts (13). On the basis of these data, it is reasonable to hypothesize that intrarenal expression of LRG1 may drive profibrotic processes by amplifying the TGF-β signaling pathway in the kidneys of patients with diabetes.

We and others reported that plasma LRG1 predicted rapid loss of kidney function in patients with type 2 diabetes (10,14). However, to our knowledge, clinical studies of the role of urine LRG1 in kidney disease are still scarce, despite the fact that measurement of LRG1 in urine may better reflect its expression inside the kidney. A small cross-sectional study (N = 13) found that urine LRG1 was associated with severity of immunoglobulin A nephropathy (15). In our early study of a surrogate renal outcome (40% estimated glomerular filtration rate [eGFR] decline), we reported that urine LRG1 was not associated with rapid loss of kidney function after adjustment for the level of albuminuria in patients with type 2 diabetes (14). With the emergence of new preclinical data pointing to the proangiogenic effect of LRG1 in the glomerulus and profibrotic action in the tubulointerstitial compartment as potential drivers of the pathogenesis of DKD (10,13), we sought to study whether urine LRG1 may predict the risk of progression to ESKD during long-term follow-up in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Participants

Information on the SMART2D (Singapore Study of Macro-angiopathy and Microvascular Reactivity in Type 2 Diabetes) cohort has been described previously (16). In brief, 2,057 participants with type 2 diabetes age between 21 and 90 years were recruited from outpatient clinics in a regional hospital and an adjacent primary care facility in northern Singapore between August 2011 and March 2014. Type 2 diabetes was diagnosed by attending physicians after excluding type 1 diabetes and diabetes attributable to specific causes. Other exclusion criteria were pregnancy, point-of-care fasting plasma glucose >15.0 or <4.5 mmol/L, HbA_{1c} >12% (108 mmol/mol), antibiotic treatment for overt infection, autoimmune disease, and active treatment for cancer.

Participants were invited for an in-person research visit in the hospital every 3 years. They were also followed by review of their electronic medical records in a centralized database, which includes integrated biochemical measurements, imaging examinations, hospitalization discharge summaries, surgery procedures, and medication dispensing records. We pooled data from the research visit and routine clinical visits into one combined data set for the current analysis. A total of 484 participants who did not respond to the research visit invitation or did not visit the hospital or its affiliated medical facilities for routine clinical care for >1 year were considered as lost of follow-up. The date of the last clinical visit was considered the date of loss to follow-up. The average rate of loss to follow-up was 3.1% per year. The actual follow-up duration for those lost to follow-up was 4.8 (interquartile range [IQR] 3.1–7.0) years.

We excluded 67 participants with eGFR between 15 and 29 mL/min/1.73 m^2 because of the distinctively high risk of progression to ESKD in this subgroup. After excluding another 45 participants with baseline eGFR <15 mL/min/1.73 m^2, one with no baseline eGFR, and another 107 with no urine sample available, a total of 1,837 participants were included in the current analysis.

The study was approved by the Singapore National Healthcare Group Domain Specific Review Committee. Each participant provided written consent.

Clinical Outcomes

The primary outcome was progression to ESKD, which was defined as *1*) progression to eGFR <15 mL/min/1.73 m^2 with at least one confirmation eGFR measurement 3 months apart, *2*) sustained dialysis for >3 months, or *3*) death attributable to renal causes, whichever occurred first. Death events were identified in electronic medical records and cross-validated with the national death registry. Death resulting from renal causes was identified from the death certificate. Follow-up was censored on 31 December 2021.

The secondary outcomes were rapid kidney function decline and progression to macroalbuminuria. Rapid kidney function decline was defined as eGFR decline by ≥5 mL/min/1.73 m^2 per year (17). Progression to macroalbuminuria was defined as the first urine albumin-to-creatinine ratio (ACR) measurement >300 μg/mg. Additionally, we defined persistent macroalbuminuria as progression to macroalbuminuria with at least one confirmation measurement 3 months apart. Only participants with ACR <300 μg/mg at

baseline were included in the analysis of albuminuria progression.

Clinical and Biochemical Variables

Smoking status, ethnicity, and sex were self-reported. History of cardiovascular diseases (CVDs), including acute myocardial infarction and stroke, was ascertained by review of electronic medical records after cohort enrollment. Blood pressure was measured three times in a sitting position by a semiautomated blood pressure monitor, and the mean of three readings was used. HbA_{1c} was measured by a point-of-care immunoassay analyzer (DCA Vantage Analyzer; Siemens, Munich, Germany). Plasma HDL and LDL cholesterol, triacylglycerol, and serum creatinine levels were measured by enzymatic methods (Roche Cobas Integra 700; Roche Diagnostics, Basel, Switzerland). eGFR was calculated by the Chronic Kidney Disease Epidemiology Collaboration creatinine equation (18). Urinary albumin was measured by an immunoturbidimetric assay (Roche Cobas c; Roche Diagnostics, Mannheim, Germany).

Urine and plasma specimens were collected after overnight fasting and stored at −80°C. Plasma and urine LRG1 concentrations were measured singly by a sandwich ELISA kit (Immuno-Biological Laboratories, Hamburg, Germany). The intra-assay coefficient of variation (CV) was 3.0–4.9%, inter-assay CV was 4.2–5.1%, and sensitivity was 0.17 ng/mL as reported by the manufacturer. In the current study, based on 12 urine samples randomly selected from the cohort, the estimated intra-assay CV was 4.0% and the inter-assay CV was 5.9%.

Statistical Analysis

Missing values were <0.5% in all variables and handled by listwise deletion. Clinical and biochemical variables are presented as mean ± SD, median (IQR), or proportion. Between-group differences were compared by the Student *t* test. Urine ACR and plasma LRG1 were natural logarithmically transformed because of skewed distributions. Urine LRG1 concentration (ng/mL) was normalized to urine creatinine concentration (mM), natural logarithmically transformed, and standardized to SD. Urine LRG1 concentration was imputed as half of the lowest detection limit if it was below the detection threshold.

The Kaplan-Meier method was used to plot the proportion of participants who experienced progression to ESKD stratified by the level of urine LRG1 in tertiles (R package survminer). Differences in risk of ESKD across tertiles were compared by the log-rank test. Cox proportional hazards (PH) regression models were fitted to study the association of urine LRG1 with the risk of progression to ESKD. Time at risk started from cohort enrollment (baseline when urine and plasma LRG1 were measured) until renal event, death resulting from nonrenal causes, loss to follow-up, or censor date, whichever occurred first. Urine LRG1 was modeled as a continuous variable (per 1 SD of log-transformed LRG1) and a categorical variable (tertiles), respectively. Covariates were a priori selected according to biological plausibility. Age, sex, ethnicity (Chinese as reference), active smoking (yes or no), CVD history (yes or no), BMI, diabetes duration, HbA_{1c}, systolic blood pressure, diastolic blood pressure, baseline eGFR, and urine ACR were adjusted as covariates in the multivariable model. A similar approach was used to study the association of urine LRG1 with risk of progression to macroalbuminuria and the association of plasma LRG1 with risk of ESKD. We used competing risk regression to study the association of urine LRG1 with risk of ESKD by modeling death resulting from nonrenal causes as a competing risk (Fine and Gray subdistribution; R package cmprsk). PH assumption was assessed by Schoenfeld residual. We plotted the hazard ratio (HR) curves allowing a nonlinear relationship between urine LRG1 and ESKD to check linearity when LRG1 was modeled as a continuous variable. Specifically, urine LRG1 was entered as a natural or penalized spline term in the Cox regression models, taking the median as reference (R package smoothHR). The product of sex × LRG1 was entered as a covariate in the multivariable Cox regression model to examine whether sex modified the association of urine LRG1 with ESKD.

The eGFR slope was estimated by a linear mixed model (random intercept, random slope, time coefficient; R package lme4). All eGFR readings from research and clinical visits were pooled into one data set after excluding those measured during hospitalization. We used multivariable logistic regression to assess the association of urine LRG1 with rapid kidney function decline (≥5 mL/min/1.73 m^2 per year). As a sensitivity analysis, we directly modeled the association of urine LRG1 with eGFR change by a linear mixed model in which urine LRG1, interaction term LRG1 (tertile) × time (years), and other covariates were modeled as fixed factors while intercept and slope were modeled as random factors. Model fit was assessed by marginal and conditional R^2.

Data analyses were performed using SPSS (version 27) and R software (version 3.4.2). Two-sided *P* values <0.05 were considered statistically significant.

Data and Resource Availability

Anonymized data are available upon reasonable request,subject to approval from the Singapore National Healthcare Group.

RESULTS

Participant Characteristics

A total of 1,837 participants were included in the current analysis (age 57 ± 11 years; diabetes duration 11 ± 9 years; 51% male; and 51% Chinese, 22% Malay, and 27% Asian Indian). Seven had a urine LRG1 value below the detection threshold. Baseline characteristics of participants included in this study were comparable to those of patients who were excluded because of missing urine samples (Supplementary Table 1). Participant baseline clinical and biochemical variables are presented in Table 1. Bivariate correlation analyses showed that urine LRG1 was only modestly correlated with age, diabetes duration, blood pressure, HbA_{1c}, and eGFR (all Spearman $\rho < 0.25$) and was moderately correlated with urine ACR (Supplementary Table 2).

During a median 8.6 (IQR 5.8–9.6) years of follow-up (13,650 patient-years), 134 incident ESKD events were identified. Specifically, 127 participants experienced progression to sustained eGFR <15 mL/min/1.73 m^2, one underwent sustained dialysis, and six died as a result of renal causes. No participant underwent kidney transplantation. The crude incidence rate of ESKD was 0.98 (95% CI 0.83–1.17) per 100 patient-years. Baseline characteristics of participants who experienced progression to ESKD and those who did not have renal events during follow-up are presented in Supplementary Table 3.

Association of Urine LRG1 With Risk of Progression to ESKD

Compared with those with no events, participants experiencing progression to ESKD had a significantly higher level of urine LRG1 at baseline (median [IQR] 72 [24–202] vs. 327 [74-1,073] ng/mL; $P < 0.001$). As shown in Fig. 1, participants with urine LRG1 in tertile 3 had a significantly higher risk of progression to ESKD compared with those in the lower tertiles (log-rank test $P < 0.0001$). The unadjusted Cox regression model showed that participants with urine LRG1 in tertile 3 had a 6.71-fold (95% CI 3.94–11.42) increased risk of ESKD compared with those in tertile 1. The association remained statistically significant (HR 1.91; 95% CI 1.04–3.50) after adjustment for demographic and cardiorenal risk factors, including eGFR and urine ACR. As a continuous variable, 1 SD increment in urine LRG1 was associated with a 1.53-fold (95% CI 1.19–1.98) increased risk of ESKD in the multivariable model (Table 2 and Supplementary Table 4). No violation of PH assumption was identified in the Cox regression models. No overt nonlinear relationship between urine LRG1 and ESKD were observed when LRG1 was modeled as a continuous variable (Supplementary Fig. 1). No interaction between urine LRG1 and sex was seen in the association with ESKD (P for interaction = 0.85).

Association of Urine LRG1 With Rapid Kidney Function Decline and Risk of Progression to Macroalbuminuria

Next, we studied whether urine LRG1 was associated with rapid kidney function decline, the common pathway leading to ESKD (17). A median of nine (IQR four to 16) eGFR measurements were recorded for each participant during follow-up. The median eGFR slope in the cohort was −1.58 (IQR −0.71 to −3.07) mL/min/1.73 m^2 per year, and 202 participants were identified as experiencing rapid kidney function decline. The multivariable logistic regression model suggested that urine LRG1 was independently associated with risk of rapid kidney function decline (adjusted odds ratio [OR] [95% CI] 3.88 [2.43–6.20]; tertile 3 vs. 1) after adjustment for clinical risk factors including baseline eGFR and urine ACR. A similar outcome was obtained when urine LRG1 was analyzed as a continuous variable (adjusted OR [95% CI] 1.36 [1.09–1.69] per SD increment in urine LRG1) (Supplementary Table 5).

Given that eGFR decline and progression of albuminuria may be driven by overlapping but distinct pathophysiological pathways (19), we studied whether urine LRG1 was also associated with risk of progression to macroalbuminuria, which was

Table 1—Participant baseline clinical and biochemical characteristics stratified by urine LRG1 tertile

		Tertile		
	Total (*N* = 1,837)	1 (*n* = 613)	2 (*n* = 612)	3 (*n* = 612)
Urine LRG1, ng/mL*	78 (26–229)	15 (7.0–26)	78 (56–111)	360 (229–698)
Index age, years	57.2 ± 10.7	56.5 ± 10.2	57.1 ± 11.4	58.1 ± 10.5
Male sex	51.2	45.0	52.0	56.7
Ethnicity				
Chinese	51.4	50.9	49.7	53.8
Malay	21.7	18.9	23.5	22.5
Asian Indian	26.9	30.2	26.8	23.7
Diabetes duration, years	11.0 ± 8.9	9.9 ± 8.5	10.7 ± 8.8	12.5 ± 9.4
Active smoker	8.7	6.2	8.2	11.6
CVD history	7.7	5.7	7.4	10.1
BMI, kg/m^2	27.7 ± 5.2	27.7 ± 5.1	27.4 ± 5.1	27.9 ± 5.4
HbA_{1c}, %	7.8 ± 1.3	7.5 ± 1.2	7.7 ± 1.3	8.1 ± 1.4
HbA_{1c}, mmol/mol	61.4 ± 14.4	58.2 ± 13.0	60.8 ± 14.0	65.3 ± 15.2
Blood pressure, mmHg				
Systolic	140 ± 18	136 ± 17	137 ± 17	147 ± 19
Diastolic	79 ± 9	78 ± 9	78 ± 9	81 ± 9
Lipid profile, mM				
HDL cholesterol	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.4
LDL cholesterol	2.7 ± 0.8	2.7 ± 0.8	2.7 ± 0.8	2.8 ± 0.9
Triacylglycerols	1.4 (1.0–1.9)	1.4 (1.0–1.9)	1.4 (1.0–1.9)	1.5 (1.1–2.0)
Baseline renal function				
eGFR, mL/min/1.73 m^2	89 ± 23	90 ± 21	89 ± 23	87 ± 24
Urine ACR, μg/mg	21 (6–82)	10 (3–29)	18 (6–53)	70 (19–424)
Plasma LRG1, ng/mL	15.6 (11.3–22.0)	14.5 (10.6–19.8)	15.5 (11.6–21.6)	17.0 (12.0–24.0)
Medication use				
Insulin	27.0	22.0	25.9	33.0
RAS blocker	59.9	53.1	56.0	70.6

Data are given as mean ± SD, median (IQR), or percentage. RAS, renin-angiotensin system. *Urine LRG1 was normalized to 1 mmol/L urine creatinine concentration.

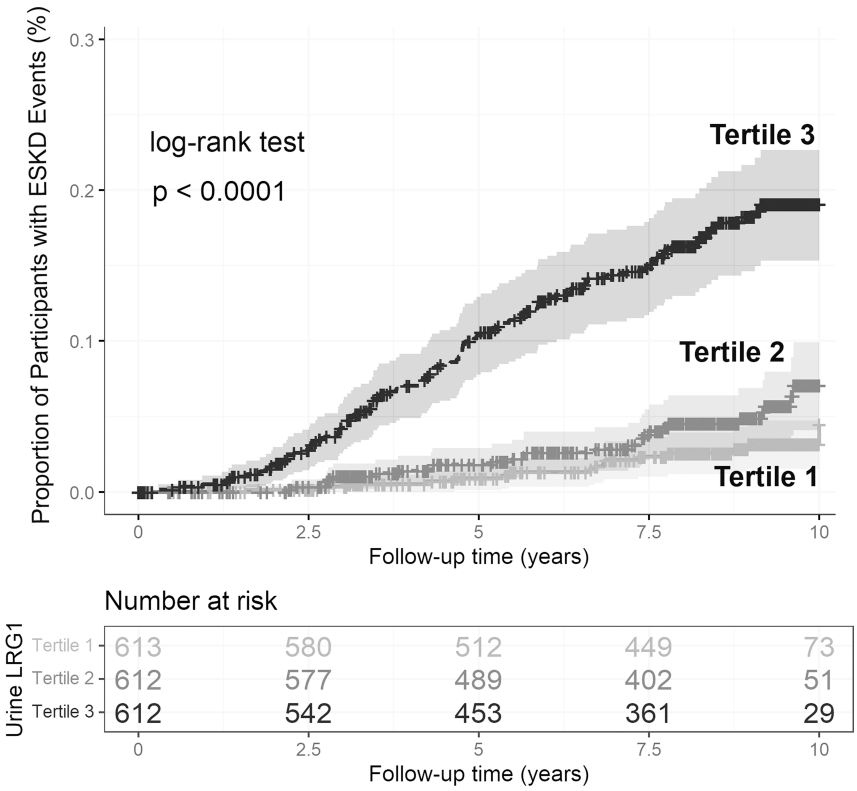

Figure 1—Proportion of participants with ESKD events (%) stratified by urine LRG1 tertile. Shaded area indicates 95% CI.

also a surrogate renal outcome in clinical trials (4,17). A median of four (IQR three to eight) urine ACR measurements were recorded for each participant during follow-up. Among 1,560 participants with baseline urine ACR <300 μg/mg, 209 experienced progression to macroalbuminuria. Urine LRG1 was significantly associated with risk of incident macroalbuminuria after adjustment for demographic and cardiometabolic risk factors and eGFR (adjusted HR [95% CI] 1.47 [1.23–1.76] per 1 SD urine LRG1). However, the strength of the association was markedly attenuated (adjusted HR [95% CI] 1.11 [0.94–1.32]) after further adjustment for baseline urine ACR. A similar outcome was obtained when urine LRG1 was analyzed as a categorical variable (Table 3). We identified 111 participants with persistent macroalbuminuria. Urine LRG1 was significantly associated with persistent macroalbuminuria after adjustment for cardiometabolic risk factors and eGFR, but the association was markedly attenuated after further adjustment for baseline ACR (Supplementary Table 6).

Table 2—Association of urine LRG1 with risk of progression to ESKD

	Model			
	Unadjusted		Multivariable	
Urine LRG1	HR (95% CI)	*P*	HR (95% CI)	*P*
Continuous				
1 SD increment	3.33 (2.69–4.11)	<0.001	1.53 (1.19–1.98)	0.001
Categorical, tertile				
1	Reference		Reference	
2	1.81 (0.98–3.37)	0.06	1.18 (0.63–2.21)	0.60
3	6.71 (3.94–11.42)	<0.001	1.91 (1.04–3.50)	0.04

Cox PH regression model: time to ESKD as outcome; urine LRG1 was normalized to urine creatinine level and modeled as a continuous variable (1 SD log-transformed LRG1) and categorical variable (tertiles), respectively. Multivariable model adjusted for age, sex, ethnicity (Chinese as reference), smoking (active vs. others), CVD history (yes vs. no), BMI, diabetes duration, HbA_{1c}, systolic blood pressure, diastolic blood pressure, baseline eGFR, and urine ACR (log transformed).

Association of Urine LRG1 With Risk of ESKD Is Independent of Plasma LRG1

Urine LRG1 and plasma LRG1 were only modestly correlated at the cross-sectional level (Spearman ρ = 0.15). Plasma LRG1 was independently associated with ESKD after adjustment for multiple clinical risk factors including eGFR and urine ACR (adjusted HR [95% CI] 1.33 [1.09–1.61]). Of note, both urine LRG1 and plasma LRG1 were independently associated with incident ESKD after mutual adjustment for each other in the same multivariable model (Supplementary Table 7).

Sensitivity Analyses

A total of 164 deaths resulting from nonrenal causes were identified. The multivariable competing risk regression model showed that urine LRG1 was significantly associated with risk of ESKD after considering death resulting from nonrenal causes a competing risk (adjusted subdistribution HR [95% CI] 1.35 [1.00–1.82]; *P* = 0.05; per 1 SD urine LRG1).

The linear mixed model suggested that eGFR in participants with urine LRG1 in tertile 2 declined 0.44 mL/min/1.73 m^2 faster per year (*P* = 0.03), and for those in tertile 3, it declined 2.21 mL/min/1.73 m^2 faster per year (*P* = 2e-16) compared with those in tertile 1 (Supplementary Fig. 3). An estimated 33.9% variance in eGFR might be explained by a fixed effect (marginal R^2) and a 59.2% variance in eGFR might be explained by both random and fixed effects (conditional R^2) in the model.

CONCLUSIONS

In this prospective cohort study, we found that a high level of urine LRG1 was associated with an increased risk of progression to ESKD independent of traditional cardiorenal risk factors and plasma LRG1. Our data suggest that urine LRG1, a modulator of the TGF-β signaling pathway, may be a novel biomarker for risk of ESKD. Together with the early mechanistic studies in cellular and animal models, this clinical study adds new evidence to support that LRG1 may be a potential novel factor in the pathophysiological network driving the progression of DKD.

Table 3—Association of urine LRG1 with risk of progression to macroalbuminuria

	Model					
			Multivariable			
	Unadjusted		1		2	
Urine LRG1	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Continuous						
1 SD increment	1.51 (1.28–1.78)	<0.001	1.47 (1.23–1.76)	<0.001	1.11 (0.94–1.32)	0.23
Categorical, tertile						
1	Reference		Reference		Reference	
2	1.73 (1.22–2.46)	0.002	1.69 (1.18–2.40)	0.004	1.48 (1.03–2.12)	0.03
3	2.27 (1.59–2.35)	<0.001	2.12 (1.45–3.10)	<0.001	1.22 (0.82–1.82)	0.32

Cox PH regression model: time to macroalbuminuria as outcome; urine LRG1 was modeled as a continuous variable (1 SD log-transformed LRG1) and categorical variable (tertiles), respectively. Model 1 adjusted for age, sex, ethnicity (Chinese as reference), smoking (active vs. others), CVD history (yes vs. no), use of renin-angiotensin system blocker, BMI, diabetes duration, HbA_{1c}, systolic blood pressure, diastolic blood pressure, and baseline eGFR. Model 2 further adjusted for baseline urine ACR (natural log transformed) above model 1.

To our knowledge, the finding of a strong association of urine LRG1 with incident ESKD is novel. In our earlier study, we observed that urine LRG1 did not independently predict rapid kidney function decline after adjustment for urine ACR (14). However, that study used eGFR decline between two measurements over 3 years as a surrogate for DKD progression. With a much longer follow-up and using ESKD as the hard outcome, we now demonstrate a strong association of urine LRG1 with risk of ESKD. Moreover, baseline urine LRG1 was predictive of rapid kidney function decline defined by eGFR slope and was associated with risk of progression to macroalbuminuria, further supporting the robustness of the association between urine LRG1 and progressive DKD.

An increased expression of LRG1 in the kidney may contribute to DKD progression in several pathways. First, TGF-β is the master regulator of fibrosis, and tubulointerstitial fibrosis is often considered the final pathway in progression to ESKD (20,21). LRG1 has been established as an amplifier of TGF-β signaling in several mouse models of kidney injury (10,22). An in vitro coculture study showed that tubular epithelial cell–derived LRG1 potentiated TGF-β–mediated Smad3 activation in kidney fibroblasts (13), suggesting that LRG1 might act in autocrine and paracrine manners to enhance TGF-β signaling in epithelial cells and myofibroblasts. Thus, increased expression and secretion of LRG1 in the kidney may amplify TGF-β signaling, drive the fibrotic pathways, and lead to progressive loss of kidney function. Second, metabolic burden, loss of microvasculature, and resultant intrarenal hypoxia in the diabetic kidney may be compensated for by abnormal angiogenesis characterized by growth of fragile small blood vessels, at least in the early stage of DKD (23,24). LRG1 has been identified as a proangiogenic factor in the presence of TGF-β (9). Therefore, overexpression of LRG1 in the diabetic kidney may enhance angiogenesis through ALK1-Smad1/5/8 signaling in the TGF-β pathway and promote DKD progression (10). Third, chronic inflammation is a known driver of progressive loss of kidney function (25). LRG1 expression may be activated under various inflammatory conditions (8,13,26). It is reasonable to postulate that LRG1 may act as a mediator of inflammation to drive fibrosis and DKD progression under a proinflammatory milieu. Fourth, the TGF-β accessory receptor endoglin is a regulator of endothelial nitric oxide synthase stability (27). Future study is warranted to examine whether the binding of LRG1 to endoglin may disrupt the homeostasis of nitric oxide and lead to disruption of the glomerular filtration barrier (28,29). Nevertheless, we would like to highlight that, although several preclinical studies have shown the profibrotic effect of LRG1 (8,13,30), a recent study reported that LRG1 might have an antifibrotic effect in a mouse model (31). Therefore, additional studies are needed to reconcile the discrepancy and ascertain the role of LRG1 in kidney disease.

Because of the nature of the study design, we cannot ascertain the cellular source of urine LRG1. LRG1 expression was markedly increased in the tubulointerstitial compartment in kidney tissue of diabetic mice and patients with diabetic nephropathy (13). It was also detectable in urine of a mouse model after albumin overload–induced renal injury (11). Therefore, it is reasonable to postulate that urine LRG1 is at least partly secreted from tubular epithelial cells. On the other hand, LRG1 is abundantly expressed in glomerular endothelial cells (10). Therefore, urine LRG1 may also originate from endothelial cells and leak from the injured glomerulus. This postulation is partly supported by the following: *1*) urine LRG1 and ACR were moderately correlated (Spearman $\rho = 0.46$) and, *2*) the strength of the association of urine LRG1 with incident macroalbuminuria, a marker for overt glomerulopathy (32), was attenuated after adjustment for urine ACR (Table 3).

We have extended our early finding by showing that plasma LRG1 independently predicted the hard renal outcome ESKD even after mutual adjustment for urine LRG1. We reasoned that urine and plasma LRG1 may be secreted from different cellular sources and organs. This is because *1*) in unilateral ureteral obstruction and aristolochic acid–induced nephropathy mouse models, increased LRG1 expression in renal tubular epithelial cells markedly promoted extracellular matrix deposition, but the plasma LRG1 level in these animal models remained largely unchanged (13); *2*) urine and plasma LRG1 were only modestly correlated (Spearman $\rho = 0.15$); and *3*) both urine and plasma LRG1 were independently associated with ESKD even after mutual adjustment. These data suggest that plasma and urine LRG1 may have different biological implications in the pathophysiological process of DKD progression.

Data from the current work may have potential translational implications.

Targeting the profibrotic pathway by inhibiting TGF-β signaling for treatment of DKD has been considered in past years. However, total blockage of TGF-β signaling was ineffective for DKD treatment (33), partly because the TGF-β pathway has multifaceted effects in multiple cellular processes. Of note, global LRG1 deficiency in mouse models does not seem to result in overt kidney abnormality or other physiological deficits (9,10). Therefore, it is worthwhile to explore whether LRG1 may be taken as a target to specifically manipulate the TGF-β/ALK1 signaling pathway for DKD prevention or treatment, given that the function-blocking LRG1 antibody is now available (34). Additionally, although fibrosis has been considered an important cellular process and a potential target for kidney disease progression, biomarkers for fibrosis in the kidney are still scarce (35,36). Given the close relationship between urine LRG1, activation of TGF-β, and deposit of the extracellular matrix in the tubulointerstitial compartment, future studies are warranted to assess whether urine LGR1 may be explored as a potential biomarker for intrarenal fibrosis.

Strengths of the current study include a large sample size, a long duration of follow-up, and a large number of ESKD events, enabling us to designate ESKD the outcome in our primary analysis. Nevertheless, several weaknesses should be highlighted. First, this is an observational study, residual confounding is inevitable, and we are unable to infer causality between urine LRG1 and kidney disease progression. Second, we do not have urine biomarkers for inflammation to elucidate the potential relationship between inflammation and expression of LRG1 in the kidney. Third, we measured urine LRG1 at baseline. The change in urine LRG1 over time may provide additional evidence to better suggest the involvement of LRG1 in DKD progression. Finally, our study was performed in Southeast Asian individuals with type 2 diabetes. Our findings may not be readily extrapolated to other ethnic groups or the population without diabetes.

In conclusion, a high level of urine LRG1 predicted an increased risk of progression to ESKD independently of traditional risk factors in patients with type 2 diabetes. Our data may pave the way for further characterization of the underlying pathophysiological mechanisms linking urine LRG1 and progressive DKD and prompt additional studies to examine whether urine LRG1 may be considered a novel biomarker for intrarenal fibrosis and a potential intervention target to slow the progression of kidney disease in patients with type 2 diabetes.

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