Kidney Injury Molecule-1 and the Loss of Kidney Function in Diabetic Nephropathy: A Likely Causal Link in Patients With Type 1 Diabetes

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OBJECTIVE

We evaluated the predictive value and clinical benefit of urinary kidney injury molecule (KIM)-1 for progression of diabetic nephropathy (DN) in type 1 diabetes. We also investigated its causal role for the decrease of estimated glomerular filtration rate (eGFR) by a Mendelian randomization (MR) approach.

RESEARCH DESIGN AND METHODS

We followed 1,573 patients with type 1 diabetes for 6 years. KIM-1 was measured at baseline and normalized with urinary creatinine. KIM-1 predictive value was evaluated by Cox regression, while its added predictive benefit was evaluated using a panel of statistical indexes. The causality for the loss of renal function was evaluated with MR, utilizing the top signal from our genome-wide association study (GWAS) as the instrumental variable.

RESULTS

KIM-1 was not an independent predictor of progression of DN when adjusted for albumin excretion rate (AER) and added no prognostic benefit to AER or eGFR. In multiple regressions, KIM-1 was associated with lower eGFR independently of diabetes duration ($\beta = -4.066$; $P < 0.0001$) but not of AER. In our GWAS, rs2036402 in the *KIM1* gene was strongly associated with KIM-1 ($\beta = -0.51$; $P = 6.5 \times 10^{-38}$). In the MR, KIM-1 was associated with lower eGFR, independently of diabetes duration and AER ($\beta = -5.044$; $P = 0.040$), suggesting a causal relationship.

CONCLUSIONS

KIM-1 did not predict progression to end-stage renal disease independently of AER and added no prognostic benefit to current biomarkers. Nevertheless, the MR showed that the inverse association of increased KIM-1 levels with lower eGFR is likely to represent a causal link.

Diabetic nephropathy (DN) is a serious diabetes complication that may progress to end-stage renal disease (ESRD) and is associated with a high risk of premature death (1,2). DN screening is based on albumin excretion rate (AER) or estimated glomerular filtration rate (eGFR) that mainly reflect the glomerular damage. However, tubular damage may also play an important role in DN, and urinary biomarkers of tubular injury are already available (3–5).

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One such biomarker for tubular injury is the kidney injury molecule (KIM)-1, a type 1 transmembrane glycoprotein, mainly expressed at the apical membrane of the proximal tubular cells but also expressed in the glomerular epithelial cells (6–9). In animal studies, KIM-1 was a sensitive biomarker of kidney damage produced by different causes (10–14). Human studies confirmed KIM-1 as a biomarker of acute kidney injury or chronic kidney diseases (CKD) (11,15). Its predictive value for DN was studied, but data are contradictory (16–19). Furthermore, its potential causality regarding the loss of renal function has not been studied in patients with type 1 diabetes.

Therefore, we aimed to evaluate whether KIM-1 could serve as a clinically useful predictor of DN progression in patients with type 1 diabetes. We also investigated its possible causal role for the loss of renal function, using a Mendelian randomization (MR) approach. MR makes use of genetic factors associated with a biomarker in order to divide patients into genetic "treatment groups" to further assess the causality between the biomarker and the final outcome (20,21). The method has been used for example to study the causal role of HDL cholesterol for myocardial infarction (22). For the purpose of the MR, we performed a genome-wide association study (GWAS) on urinary KIM-1 levels to obtain genetic factors with strong effect on the urinary KIM-1 values.

RESEARCH DESIGN AND METHODS

Cohort Characteristics

This study was part of the prospective, ongoing Finnish Diabetic Nephropathy Study (FinnDiane), which was conducted in more than 80 hospitals or health centers across Finland and aimed to identify the risk factors for chronic diabetes complications in patients with type 1 diabetes. The full study protocol was approved by the local ethics committees and has previously been described (23). All patients signed a written consent, and the study was performed in accordance with the Declaration of Helsinki. Recruitment and baseline characterization took place between 1998 and 2006. Serum and 24-h urine collection samples taken at baseline were stored at −20°C until KIM-1 was measured.

Ascertainment of Outcomes

Renal status was based on AER measurements in two out of three consecutive urine collections and defined as normal AER (<30 mg/24 h or <20 μg/min), microalbuminuria (30–299 mg/24 h or 20–199 μg/min), or macroalbuminuria (≥300 mg/24 h or ≥200 μg/min). Presence of ESRD was defined as undergoing dialysis or having received a kidney transplant. Patients with ESRD at baseline were excluded. Glomerular filtration rate was estimated using the creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (24).

Patients were followed for a median of 6.0 years (95% CI 5.9–6.1). Progression of DN was defined as the passage from one AER category to the next or progression to ESRD. Dialysis and kidney transplantation were identified from patients' medical files and cross-checked with the Finnish Registry for Kidney Diseases and the Finnish Hospital Discharge Register.

Urinary KIM-1 Measurements

KIM-1 was quantified from single frozen 24-h urine samples, collected at the baseline visit, using a Cobas Elecsys 411 Immunoanalyzer with a DuoSet ELISA Development kit from R&D Systems (Abingdon, Oxon, U.K.). The KIM-1 levels were normalized by urinary creatinine. For all statistical tests, the KIM-1–to–creatinine ratio was further transformed to natural logarithm and presented as ln(KIM-1). We additionally measured KIM-1 concentrations in ~200 healthy individuals in order to test our assay but could not detect any KIM-1 in these samples.

Statistical Analysis

Statistical tests were performed with MedCalc 12.1.3.0 (MedCalc Software bvba, Mariakerke, Belgium) unless stated otherwise.

Descriptive Statistics

Normally distributed variables are presented as mean ± SD. Non–normally distributed variables are presented as median and interquartile range. Comparison between groups for normally distributed variables was done using one-way ANOVA and for non–normally distributed continuous variables using Mann-Whitney *U* test. The comparison of categorical variables was done by χ^2 test.

Prediction of DN Progression

We assessed whether KIM-1 predicted progression of DN using Cox proportional hazards models. Different Cox models were built for each stage of DN. Potential risk factors were first individually tested for prediction. Variables with a *P* value <0.25 after univariate testing were then considered in Cox models with backward selection of covariates. The variables retained in the models represented the basic models for progression at each stage of DN. KIM-1 was then added to these models to test its independence from the progression promoters. Finally, the independence of KIM-1 from AER was tested by addition of both AER and KIM-1 in the same models. All possible interactions between variables included in the models were tested. Cox model fit was assessed by cumulative Cox-Snell residuals to (−log) Kaplan-Meier estimates. The multico-linearity was evaluated using a common index that quantifies the severity of multicolinearity – the variance inflation factor, where variance inflation factor <10 was acceptable.

Clinical Benefit of KIM-1 as a Predictor of Progression to ESRD

The predictive value of KIM-1 was compared with AER and eGFR using receiver operating characteristic (ROC) curve analysis performed by the method described by DeLong et al. (1988) (25). We compared the area under the ROC curves (AUC) of models including KIM-1, eGFR, and AER alone and different combinations of the three. Finally, the improvement of prediction resulting from the addition of urinary KIM-1 to either AER alone, to the basic progression models together with AER, or to the basic progression models together with AER and eGFR was assessed by calculating category-less net reclassification improvement and integrated discrimination improvement indexes (26,27).

MR Analysis

The causality between the KIM-1 levels and eGFR was explored using the MR approach that uses genetic variants associated with KIM-1 levels to infer causality (20,21). MR can be considered a form of randomized controlled trial: the patients are randomly allocated before birth to different "treatment groups" (i.e., treatment with different levels of

KIM-1 concentrations based on their genetic variants) and followed up from birth until the study visit (>39 years' follow-up in our study) when the clinical end points were evaluated. The MR analysis was performed using the two-stage least squares (2SLS) method.

The MR analysis is based on three assumptions: *1*) the genetic variant ("instrumental variable" (IV) (a single nucleotide polymorphism [SNP] in the KIM-1 gene in this study) is strongly associated with the urinary KIM-1 levels ("modifiable exposure"), *2*) the SNP is independent of factors that may confound the association between the modifiable exposure and the outcome, and *3*) the SNP affects the outcome only through the modifiable exposure and not by other biological pathways (Fig. 1).

We selected the IV (SNP) based on the results from the GWAS. To fulfill the first assumption, we only used the strongest signal with genome-wide significance (*P* value $<5 \times 10^{-8}$). The IV was a SNP in the *KIM1* gene. The strength of the association was tested with *F* statistics, and values $\geq$10 were considered sufficient to ensure the validity of the MR analysis (28). Since genetic variants are assumed to be randomly distributed before birth, MR assumes that the IV (SNP genotype) is independent of confounders, fulfilling the second assumption. To test whether the SNP affects the outcome only through the modifiable exposure, we tested with multiple regression whether the association between the SNP and eGFR disappeared after adjustment for KIM-1 levels. To exclude the existence of other pathways, we explored whether any of the variables in our database were associated with the SNP by linear regressions or χ^2 test. The distributions of all residuals were tested for normality.

In addition using to the 2SLS method, we estimated the effect sizes of the association between KIM-1 levels and eGFR also with conventional linear regression. Both analyses were first performed without adjustments and then adjusted for diabetes duration, AER, and HbA_{1c}. Finally, we tested the endogeneity to see whether there is difference between the conventional estimate from the linear regression and the IV estimate from the MR analysis (29,30). The linear regression, 2SLS, and the endogeneity analysis were implemented with the Stata/MP2 software (version 13; StataCorp, College Station, TX) (21).

GWAS on KIM-1 Levels

In order to have a strong instrumental variable for the MR, we performed a GWAS on urinary KIM-1 levels. The data acquisition, quality control, and SNP imputation have previously been described (34). In short, the genotyping was performed in 3,651 samples using an Illumina 610Quad assay. Imputation was performed with the HapMapII CEU samples as reference panel and resulted in ~2.4 million SNPs. For this study, we extracted GWAS data for the 1,573 patients that were also tested for urinary KIM-1. The association analysis between ln(KIM-1) and the imputed allele dosage data were calculated using linear regression, assuming an additive association model, implemented in Plink (v1.07) (31). The models were adjusted for sex, diabetes duration, and the two first principal components, calculated with the EIGENSTRAT software (version 3.0; EIGENSOFT) (32). For the purpose of the MR analysis, the imputed SNP allele dosage data were converted to the most likely genotypes, accepting genotype calls with >0.9 genotype likelihood as estimated by the MACH imputation software (33).

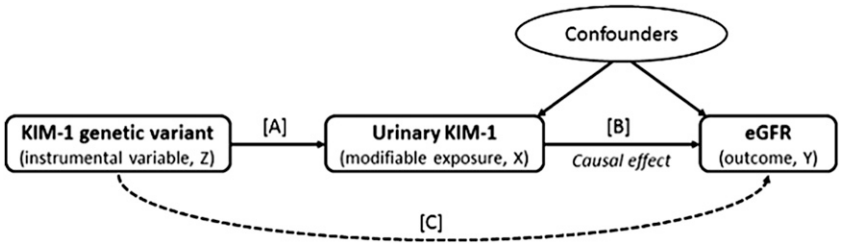

Figure 1—Investigation of the causal link between KIM-1 and eGFR: MR analysis. The main assumptions for MR studies are as follows: *1*) The IV (KIM-1 genotype) is strongly associated with the modifiable exposure (KIM-1 levels) (*A*). *2*) The IV (KIM-1 genotypes) is independent of confounding factors that may confound the association of the modifiable exposure, urinary KIM-1 levels, with the outcome, eGFR. This is considered a priori true, since all the confounding factors are considered to appear and act after the genotype random allocation at birth. *3*) The IV affects the outcome—eGFR, only through the modifiable exposure—KIM-1 levels and not by other biological pathways. It is expected that no other pathways are found if the possible direct association between KIM-1 and eGFR (*C*) disappears when adjusted for the modifiable exposure. The causal effect size (*B*) is obtained by 2SLS analysis where the endogenous variables are IV (Z), KIM-1 genetic variant; modifiable exposure (X), urinary KIM-1; and final outcome (Y), eGFR. Then, the causal effect size (*B*) is compared with the effect size from the normal regression having as dependent variable eGFR and as independent variable urinary KIM-1 levels.

RESULTS

Cohort Characteristics

At baseline, there were 1,573 patients divided into three groups (Supplementary Fig. 1). Baseline clinical characteristics are presented in Table 1 and KIM-1 levels across groups in Supplementary Fig. 2*A*.

After a median follow-up time of 6.0 years (interquartile range 5.7–6.4), 174 patients progressed to the next higher stage (Supplementary Fig. 1). Comparison of the baseline characteristics for the progressors and nonprogressors at different stages is provided in Supplementary Table 1. In patients with normal AER at baseline, there was no difference in the KIM-1 levels between progressors to microalbuminuria and nonprogressors (P = 0.62). However, KIM-1 levels were significantly higher in the patients who progressed from microalbuminuria to macroalbuminuria (P = 0.04) and from macroalbuminuria to ESRD ($P < 0.0001$) (Supplementary Fig. 2*B*).

Prediction of DN Progression

Progression From Normal AER to Microalbuminuria

KIM-1 did not predict progression to microalbuminuria in univariate analysis (hazard ratio [HR] 1.06; P = 0.86) or after adjustment for the basic progression model (HR 0.98; P = 0.96) or progression model and AER (HR 0.80; P = 0.54) (Table 2). The covariates of the basic progression model after backward selection were HbA_{1c}, serum triglycerides, and waist-to-hip ratio (WHR) (Supplementary Table 2).

Progression From Microalbuminuria to Macroalbuminuria

In univariate analysis, KIM-1 predicted progression to macroalbuminuria (HR

Table 1—Clinical baseline data for patients enrolled in the study

Variable	Normoalbuminuric patients	Microalbuminuric patients	Macroalbuminuric patients
Number of patients (male/female)	953 (407/546)	269 (163/106)	350 (190/160)
Age (years)	40.1 ± 12.1	39.2 ± 12.7	41.1 ± 10.5
Age of onset (years)	15.9 ± 9.1	12.8 ± 9.1	10.6 ± 8.5
Duration (years)	24.2 ± 9.9	26.4 ± 10.7	29.1 ± 8.0
BMI (kg/m^2)	25.2 ± 3.4	25.6 ± 3.5	26.0 ± 3.8
WHR			
Men	0.90 ± 0.07	0.92 ± 0.07	0.94 ± 0.07
Women	0.80 ± 0.06	0.83 ± 0.07	0.84 ± 0.07
History of smoking (%)	39.9	52.8	60.4
SBP (mmHg)	131 ± 16	137 ± 17	144 ± 20
DBP (mmHg)	78 ± 9	81 ± 10	83 ± 10
HbA_{1c} (mmol/mol)	66 ± 5	73 ± 8	76 ± 10
HbA_{1c} (%)	8.2 ± 1.2	8.8 ± 1.5	9.1 ± 1.6
Total cholesterol (mmol/L)	4.89 ± 0.87	4.99 ± 0.88	5.41 ± 1.11
HDL cholesterol (mmol/L)	1.37 ± 0.37	1.29 ± 0.37	1.21 ± 0.37
LDL cholesterol (mmol/L)	3.03 ± 0.79	3.11 ± 0.79	3.35 ± 0.89
Triglycerides (mmol/L)	0.92 (0.70–1.24)	1.08 (0.82–1.61)	1.36 (1.01–2.05)
AER (mg/24 h)	7 (5–11)	59 (29–110)	453 (168–1,210)
eGFR (mL/min/1.73 m^2)	88 ± 28	88 ± 38	50 ± 30
KIM-1 (ng/mmol)	27.8 (13.6–50.3)	33.1 (16.6–63.9)	49.5 (26.9–92.4)

Normally distributed variables are presented as means ± SD, and non–normally distributed variables are presented as median (interquartile range). Categorical data are presented as *n* or percentage. DBP, diastolic blood pressure; SBP, systolic blood pressure.

4.14; $P < 0.0001$). The basic model for progression to macroalbuminuria included HbA_{1c}, serum triglycerides, and WHR (Supplementary Table 1). KIM-1 no longer predicted progression after adjustment for this basic model (HR 1.61; $P = 0.27$) or after supplementary adjustment for AER (HR 1.07; $P = 0.89$) (Table 2).

Progression to ESRD in Patients With Macroalbuminuria

KIM-1 predicted progression to ESRD in unadjusted analysis (HR 2.08; $P < 0.0001$). The basic progression model for ESRD consisted of serum triglycerides and systolic blood pressure (Supplementary Table 1). Even after adjustment for this model, KIM-1 predicted progression to ESRD (HR 1.78; $P < 0.0001$). However, after addition of AER to the model, KIM-1 was no longer a predictor of progression to ESRD (HR 1.20; $P = 0.17$) (Table 3).

Clinical Benefit of KIM-1 as Predictor of ESRD

Comparison of the ROC curves for KIM-1 and AER, as single predictors of ESRD, revealed no difference between the two markers ($\Delta_{AUCs} = 0.062$; $P = 0.07$). However, when KIM-1 was compared with eGFR, there was a difference in favor of eGFR ($\Delta_{AUCs} = 0.126$; $P = 0.002$). Adding KIM-1 to either AER or eGFR did not improve the ROC curves compared with AER or eGFR alone (Supplementary Table 3). In addition, there was no added predictive benefit assessed by continuous net reclassification improvement and integrated discrimination improvement when KIM-1 was added to the basic progression models with or without AER or eGFR (Supplementary Table 4).

Identification of the Instrumental Variable: GWAS on KIM-1

The GWAS on ln(KIM-1) identified 49 SNPs on chromosome 5q33.3 with a genome-wide significant P value $< 5 \times 10^{-8}$. This region includes the *KIM1* gene (official name *HAVCR1* [hepatitis A virus cellular receptor 1]) (Supplementary Fig. 3). The strongest association was observed for rs2036402 with $P = 6.5 \times 10^{-38}$ ($\beta = -0.51$; i.e., each copy of the minor G allele decreases ln(KIM-1) by 0.51 [95% CI −0.47 to −0.54]) (Supplementary Fig. 2*C*). After conditional analysis on rs2036402, no other SNP reached genome-wide significance, suggesting that rs2036402 explains the majority of the association seen on the locus (Supplementary Table 5).

Causal Link Between KIM-1 and eGFR

The causality between KIM-1 and eGFR was assessed using the MR analysis. MR analysis requires that the IV (rs2036402) affect the outcome (eGFR) through the modifiable exposure (KIM-1 levels) without pleiotropic effects. In un adjusted cross-sectional analysis, rs2036402 was associated with eGFR ($P = 0.02$). This association vanished after adjustment for KIM-1 ($P = 0.48$), confirming that rs2036402 acts on eGFR mainly through urinary KIM-1. To exclude other pathways between rs2036402 and eGFR, we tested all the variables in the database for association with rs2036402, but no other variable except KIM-1 was associated with the IV.

Table 2—Prediction of progression using Cox regression analysis with baseline data for KIM-1

		Unadjusted (univariate)			Adjusted for basic model			Adjusted for basic model and AER		
Initial stage	Predictive variable	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Normoalbuminuria	ln(KIM-1)	1.06	0.54–2.08	0.86	0.98	0.49–1.97	0.96	0.80	0.39–1.64	0.54
Microalbuminuria	ln(KIM-1)	4.14	2.75–6.23	<0.0001	1.61	0.69–3.74	0.27	1.07	0.43–2.64	0.89
Macroalbuminuria	ln(KIM-1)	2.08	1.66–2.62	<0.0001	1.78	1.41–2.56	<0.0001	1.20	0.92–1.57	0.17

Since KIM-1 had a nonnormal distribution, its values have been logarithmic transformed to ln(KIM-1). Basic models for progression for every stage are described in RESEARCH DESIGN AND METHODS (Supplementary Table 1). In unadjusted analysis, ln(KIM-1) predicted progression from microalbuminuria to macroalbuminuria and from macroalbuminuria to ESRD. When Cox regressions were adjusted for basic models for progression, ln(KIM-1) was an independent predictor of progression to ESRD. Finally, when AER was added to the models, ln(KIM-1) was no longer an independent predictor of progression.

Table 3—Comparison of estimates from instrumental analysis and observed association between KIM-1 and eGFR

IV	*F* (first-stage regression)	IV estimate			Observational estimate			Endogeneity *P*
		β	95% CI	*P*	β	95% CI	*P*	
Unadjusted					−4.522	−6.238 to −2.807	<0.0001	
rs2036402	93.66	−6.786	−12.027 to −1.546	0.011				0.368
Adjusted for duration					−4.066	−5.699 to −2.434	<0.0001	
rs2036402	63.05	−5.654	−10.647 to −0.661	0.026				0.507
Adjusted for duration and HbA_{1c}					−3.827	−5.493 to −2.161	<0.0001	
rs2036402	64.67	−5.667	−10.681 to −0.655	0.027				0.445
Adjusted for duration and AER					0.325	−1.305 to 1.956	0.696	
rs2036402	113.93	−5.044	−9.865 to −0.224	0.040				0.019
Adjusted for duration, HbA_{1c}, and AER					0.114	−1.525 to 1.755	0.891	
rs2036402	103.23	−5.106	−9.920 to −0.292	0.038				0.022

Instrumental analysis (2SLS) was performed for calculation of the two-stage estimator for the causal effect of the modifiable exposure (urinary KIM-1) on the final outcome (eGFR), using rs2036402 in *KIM-1* gene as the IV. Association between KIM-1 levels and eGFR was evaluated with a multiple linear regression. The differences between the IV estimators and the conventional regression-based estimators (endogeneity) were tested by a Durbin-Wu test to see whether the differences were statistically significant. The covariates used for adjustments have been described in Table 3. Duration, duration of type 1 diabetes; *F* (first-stage regression), *F* statistics from the first-stage regression of 2SLS; β, causal effect on eGFR per 1 SD of ln(KIM-1); SE, SE of coefficient β; *P*, statistical significance (values <0.05).

In linear regression analysis, performed for the comparison purpose, KIM-1 was strongly and inversely associated with lower eGFR (β = −4.52; $P <$ 0.0001). This association remained significant after adjustment for diabetes duration (β = −4.07; $P <$ 0.0001) but disappeared after adjustment for AER (P = 0.70) (Table 3).

When we performed MR analysis using the 2SLS method with rs2036402 as the IV, the KIM-1 levels were associated with lower eGFR in the unadjusted analysis (β = −6.79 [95% CI −12.03 to −1.55]; P = 0.011). KIM-1 remained associated with lower eGFR after adjustment for diabetes duration (β = −5.65 [95% CI −10.65 to −0.66]; P = 0.026), HbA_{1c} (β = −5.67 [95% CI −10.68 to −0.66]; P = 0.027), AER (β = −5.04 [95% CI −9.87 to −0.22]; P = 0.040), or all of them (β = −5.11 [95% CI −9.92 to −0.29]; P = 0.038). The F statistics from the first regression, describing the strength of the IV, were by far >10 ($F_{\text{unadjusted}}$ = 93.66, F_{duration} = 63.05, $F_{\text{duration\&HbA1c}}$ = 64.67, $F_{\text{duration\&AER}}$ = 113.93, and $F_{\text{duration\&HbA1c\&AER}}$ = 103.23), which confirmed that the strength of the IV was sufficient for this study (Table 3).

The endogeneity was nonsignificant in the unadjusted analysis (P = 0.368) and also after adjustment for diabetes duration (P = 0.507) or HbA_{1c} (P = 0.445) indicating no difference between the observed estimator (from linear regression) and the IV estimator. However, when the models were adjusted for AER, significant endogeneity was found ($P <$ 0.05), indicating a difference between the two estimators (Table 3).

CONCLUSIONS

This study showed that urinary KIM-1 did not predict progression to ESRD independently of AER in patients with type 1 diabetes and macroalbuminuria. Also, KIM-1 showed no prognostic benefit beyond AER or eGFR. However, this is the first report to show the strong genetic determination of the KIM-1 levels by SNPs in the KIM-1 gene, identified through a GWAS on KIM-1 levels. Importantly, our subsequent MR analysis showed a causal association of increased KIM-1 levels with low eGFR, independent of glycemic control or AER and possibly explaining the lack of prediction in the observational studies.

This is the first well-powered study to assess the predictive value and the clinical benefit of KIM-1 in patients with type 1 diabetes. Two small studies of 63 and 124 patients evaluated KIM-1 in urine and plasma in regard to DN progression in type 1 diabetes, but the data were contradictory (17,35). Another study in type 2 diabetes showed that KIM-1 predicted the decline of eGFR in unadjusted analysis but not progression to macroalbuminuria, whereas progression to ESRD was not evaluated (16). Therefore, our finding that KIM-1 did not predict progression to ESRD in macroalbuminuric patients with type 1 diabetes independently of AER is important. While tubulo-interstitial damage was proposed as one final pathway leading to ESRD and KIM-1 was shown to be a sensitive and specific marker of proximal tubular damage in other studies, our results showed its lack of independence from AER (36). In addition, the association between KIM-1 and eGFR seen in our linear regression analysis was also not independent of AER. In the context of this strong interaction with AER (correlation between AER and KIM-1 $r = 0.35$, $P < 0.001$), the lack of added predictive benefit for DN progression seen in our study is not unexpected.

To further study the relationship between KIM-1 and renal function, we performed an MR analysis, which showed a causal impact of KIM-1 on the decrease of eGFR. Unlike the Cox regression analysis or the linear regression, in the MR analysis one unit change of ln(KIM-1) was associated with a 5.0 to 6.8 mL/min/1.73 m^2 decrease in eGFR and remained significant after adjustment for duration of diabetes, HbA_{1c}, or AER (Table 3). This consistent finding strongly suggested a causal relationship between KIM-1 and the eGFR loss.

Although the MR approach is a useful tool to show potential causality, it does not describe the mechanism behind the pathology. While the exact causal mechanism remains unknown, previous studies have implicated KIM-1 both in promoting interstitial fibrosis and in tubular cell repairing because of its involvement in the cell-cell or cell-matrix interactions, development of interstitial fibrosis, internalization of oxidized LDL, removal of necrotic cells and other debris from the tubular lumen, and ciliary chemo-receptor sensing (6).

The effect sizes obtained in the MR analysis are robust and should pro vide a true estimate of the causal effect of exposure (KIM-1) on the final outcome (eGFR), even in the presence of confounders. This is because the genotype randomization is accomplished already before birth, much earlier than the potential actions of confounders (e.g., AER, HbA_{1c}, etc.) (20,21). Since AER was confounding the linear regression, the MR is better suited for this situation. Indeed, the effect size estimates from the MR remained robust and significant even after adjustment for AER, unlike the observational estimates.

There are three possible explanations for the significant difference between the estimates from the MR and from the linear regression. First, the lifetime effect of KIM-1 on eGFR may be more evident in the MR analysis and may be attenuated in the standard regression using a single measurement of KIM-1, AER, and eGFR, which may not capture the total effect of KIM-1 on eGFR. The second explanation may be a potential negative confounding, since when we further adjusted for renin-angiotensin inhibitors treatment or other antihypertensive treatments, the endogeneity decreased (data not shown). The third possible explanation may be a more complex relationship with a potential reverse causality between eGFR and KIM-1 mediated through AER, as suggested by the tubulo-glomerular feedback theory (36).

Previous studies supported this complex relationship between KIM-1, AER, and eGFR (18,37,38). Preclinical studies showed an increase of KIM-1 in protein overload nephropathy (15). In addition, KIM-1 expression was increased in cultured glomerular epithelial cells in response to albumin and attenuated by antiproteinuric treatment. Further, elevated KIM-1 levels were associated with podocytopenia and proteinuria (7). KIM-1 levels increased also in patients with nondiabetic proteinuric CKD and decreased in parallel with the decline of proteinuria after treatment (39). All these results support a complex relationship between KIM-1, eGFR, and AER. This complex relationship is particularly unclear in patients with type 1 diabetes, where the role of KIM-1 as a predictor is debated, while the concept of nonalbuminuric DN is also present (17,18,40).

Our study partially clarifies this relationship in patients with type 1 diabetes and explains also the previous contradictory results from other studies, since this is the first study to show the genetic determination of urinary KIM-1. We hypothesize that the increase of KIM-1 was driven by two factors in our study: a strong genetic determination and acquired factors such as diabetes and glomerular damage (through HbA_{1c}, diabetes duration, or AER). While the genetic causal effect is independent of the later events (AER, elevated HbA_{1c}, or diabetes duration), the reverse causality of AER on KIM-1 could explain why AER is a better predictor of DN progression than KIM-1. This could also explain why in the observational study, KIM-1 predicted progression only to ESRD, independently of other clinical risk factors than AER. Probably, at that stage, its levels were high enough to become predictive, although even then the strong interaction with AER canceled its predictive independence.

From the clinical standpoint, these results are important, since they show that despite the complex relationship between KIM-1, AER, and eGFR, the association of KIM-1 with lower eGFR was a causal relationship. In addition, this study is an excellent example of why a causal biomarker may not be useful in today's clinical practice for the prediction of DN progression, where AER and eGFR—the two factors used to define the severity of DN—remain the best markers for the progression. However, in the future it could be important to consider also the genetic factors affecting such biomarkers. The complex relation between KIM-1, AER, and eGFR seen in this study is another argument that DN is a multifactorial disease and represents more than just glomerular damage.

The strengths of this study are the comprehensive clinical characterization, the long follow-up, the availability of GWAS data, and a strong IV given by the genetic determination of the KIM-1 levels in our cohort. One potential limitation could be that the biochemical measurements are only based on a single baseline measurement. However, to our knowledge, no relevant variability of KIM-1 or eGFR has been described. Even if there would be large variability, this would only dilute our results. Another

possible limitation is the number of patients. Although this probably represents the largest study regarding the progression analysis, it may be considered small for an MR analysis. This is compensated for by accurate clinical measurements together with a strong IV, more than sufficient for our sample size, as proved by the large *F* statistic values.

We would like to emphasize the fact that prediction is not the same as causality. While for a biomarker the predictive value can be inferred using a prospective study (Cox regression in our study), the causality can be demonstrated either in a randomized controlled trial or by using an MR approach. In summary, we show that urinary KIM-1 did not predict progression to ESRD independently of AER and added no prognostic benefit to currently used biomarkers. Nevertheless, the MR analysis showed that the inverse association of increased KIM-1 levels with lower eGFR is independent of AER and very likely to represent a causal link.

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Author Contributions. N.M.P. and N.S. researched data, performed statistical analyses, and wrote the manuscript. N.M.P., C.F., M.S., A.B., P.M.H., and P.-H.G. designed the study. C.F., M.S., E.H.D., L.M.T., D.G., N.T., J.W., and V.H. researched data, contributed to discussion, and reviewed and edited the manuscript. P.M.H. and P.-H.G. contributed to discussion and reviewed and edited the manuscript. P.-H.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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