Kidney Medicine

Matrix Metalloproteinase-2 and CKD Progression: The Chronic Renal Insufficiency Cohort (CRIC) Study

Robin L. Baudier, Paula F. Orlandi, Wei Yang, Hsiang-Yu Chen, Nisha Bansal, J. Walker Blackston, Jing Chen, Rajat Deo, Mirela Dobre, Hua He, Jiang He, Ana C. Ricardo, Tariq Shafi, Anand Srivastava, Dawei Xie, Katalin Susztak, Harold I. Feldman, Amanda H. Anderson, and the CRIC Study Investigators

Rationale & Objective: Matrix metalloproteinase 2 (MMP-2) plays an important role in the development of fibrosis, the final common pathway of chronic kidney disease (CKD). This study aimed to assess the relationship between repeated measures of MMP-2 and CKD progression in a large, diverse prospective cohort.

Study Design: In a prospective cohort of Chronic Renal Insufficiency Cohort (CRIC) participants (N = 3,827), MMP-2 was measured at baseline. In a case-cohort design, MMP-2 was additionally measured at year 2 in a randomly selected subcohort and cases of estimated glomerular filtration rate (eGFR) halving or kidney replacement therapy (KRT) (N = 1,439).

Setting & Participants: CRIC is a multicenter prospective cohort of adults with CKD.

Exposure: MMP-2 measured in plasma at baseline and at year 2.

Outcomes: A composite kidney endpoint (KRT/eGFR halving).

Analytical Approach: Weighted Cox proportional hazards models for case-cohort participants.

Results: Participants were followed for a median of 4.6 years from year 2 and 6.9 years from the baseline. Persistently elevated MMP-2 (≥300 ng/mL at both baseline and year 2) increased the hazard of the composite kidney endpoint (HR, 1.61; 95% CI, 1.07-2.42; $P = 0.09$) after adjusting for covariates. The relationship of persistently elevated MMP-2 was modified by levels of inflammation, with a 2.6 times higher rate of the composite kidney endpoint in those with high-sensitivity C-reactive protein < 2.5 g/dL at study entry. Heterogeneity of effect was found with proteinuria, with a baseline MMP-2 level of ≥300 ng/mL associated with an increased risk of the composite kidney endpoint (HR, 1.30; 95% CI, 1.09-1.54) only with proteinuria ≥ 442 mg/g.

Limitations: The observational study design limits causal interpretation.

Conclusions: Elevated MMP-2 is associated with CKD progression, particularly among those with low inflammation and those with proteinuria. Future investigations are warranted to confirm the reduction in risk of CKD progression among these subgroups of patients with CKD.

Complete author and article information provided before references.

Correspondence to A.H. Anderson (ahanders@uab.edu)

Kidney Med. 6(8):100850. Published online June 6, 2024.

doi: 10.1016/j.xkme.2024.100850

Chronic kidney disease (CKD) is a leading cause of death in the United States and a public health burden affecting ~15% of American adults.[1] CKD is typically characterized by progressive loss of kidney function and structural kidney damage and is associated with high morbidity and mortality and an increased risk of cardiovascular disease (CVD).[2] Many heterogeneous disease pathways result in CKD; however, the final common manifestation of nearly all chronic and progressive nephropathies is kidney fibrosis.[2,3] This fibrosis is perpetuated by chronic injury resulting in unchecked fibrotic matrix deposition that results in loss of kidney function and progression toward the need for kidney replacement therapy (KRT).[3]

Matrix metalloproteinase 2 (MMP-2), or gelatinase A, is a zinc-containing, matrix-degrading protease that regulates key cellular events relating to fibrosis.[4] Normally found at low levels in the kidney, MMP-2 is rapidly upregulated during the progression of fibrosis.[4] MMP-2 has been identified as a biomarker that is prognostic of fibrosis on biopsy,[5] and higher levels of MMP-2 have been found in those with CKD.[6-9] MMP-2 has also been found to be associated with longitudinal decline in kidney function as measured by estimated glomerular filtration rate (eGFR) in nondiabetic, nonproteinuric patients with coronary artery disease and CKD[10] and in prospective kidney disease interventional studies of patients with type 2 diabetes.[11]

Proteinuria is associated with increased risk of KRT and death, and early decline in proteinuria has been found to be associated with decreased risk of KRT or death, particularly in those with a high baseline proteinuria.[12] It has been shown that MMPs play a role in the development of proteinuric kidney diseases[13] and MMP-2 has been shown to independently correlate with proteinuria in humans.[14] In animal models of kidney disease, including aldosterone and sodium induced kidney damage,[15] mesangial cell-mediated forms of glomerulonephritis,[16] Alport syndrome,[17] and type 1 diabetes,[18] development of proteinuria or albuminuria was blocked or reversed through inhibition of MMP-2. This suggests that there may be a causal relationship between MMP-2 and proteinuria in some CKD etiologies.

The objective of this study was to investigate whether baseline or 2-year patterns of MMP-2 levels were

PLAIN LANGUAGE SUMMARY

Matrix metalloproteinase 2 (MMP-2) is a matrix-degrading protease involved in fibrosis and elevated in chronic kidney disease (CKD). Longitudinal patterns of MMP-2 have not previously been assessed as a predictor of CKD progression in a large prospective cohort. Here, we found that a higher baseline level and an increasing or persistently elevated 2-year pattern of MMP-2 were associated with CKD progression, independent of all covariates except proteinuria. The association of baseline MMP-2 with CKD progression differed by level of proteinuria, whereas levels of inflammation modified the associations of 2-year MMP-2 patterns with CKD progression.

independently associated with CKD progression among men and women enrolled in the Chronic Renal Insufficiency Cohort (CRIC) Study.

METHODS

Study Design and Population

From June 2003 to August 2008, 7 clinical centers across the United States enrolled 3,939 adults with moderate to advanced CKD in the CRIC Study. Baseline analyses used the entire cohort with available plasma. To investigate the 2-year patterns of MMP-2 with CKD outcomes, a case-cohort study design was used for its combination of efficiency and ability to minimize bias in estimates through inverse probability weighting of cases and a representative subcohort.[19] In this study design, a subcohort of 1,300 participants was randomly selected from the 3,939 original CRIC participants. This subcohort, combined with cases of the primary outcome, a composite kidney endpoint (eGFR halving or KRT), or secondary outcome KRT, are the populations eligible to be included in the 2-year pattern case-cohort analyses. The subcohort alone was used to estimate the eGFR slope. Because outcomes were assessed in 2-year pattern analyses beginning at year 2, those who withdrew, died, did not have a year 2 visit, or did not have plasma available at both baseline and year 2 were excluded, resulting in a maximum of 990 subcohort members that were included in analyses (Fig 1). Further study design information and baseline analysis methods are included in Item S1: Supplementary Methods.

Primary Exposure

Plasma was available for assessment of MMP-2 for 3,827 participants at baseline. For 2-year-pattern analyses, baseline, and year 2 ethylenediaminetetraacetic acid (EDTA) plasma was available for assessment in 990 participants in the randomly selected subcohort and 449 nonsubcohort cases who initiated KRT or had eGFR halving after their year 2 visit (analysis flow chart in Fig 1).

Two-year patterns of MMP-2 were defined as Low/Low (MMP-2 < 300 ng/mL at baseline and year 2), Low/High (baseline < 300 ng/mL and year 2 ≥ 300 ng/mL), High/Low (baseline ≥ 300 ng/mL and year 2 < 300 ng/mL), and High/High (≥300 ng/mL at both time points). The cut point of 300 ng/mL was selected after examining the shape of a multivariable adjusted restricted cubic spline of baseline MMP-2 and outcome that showed an increased

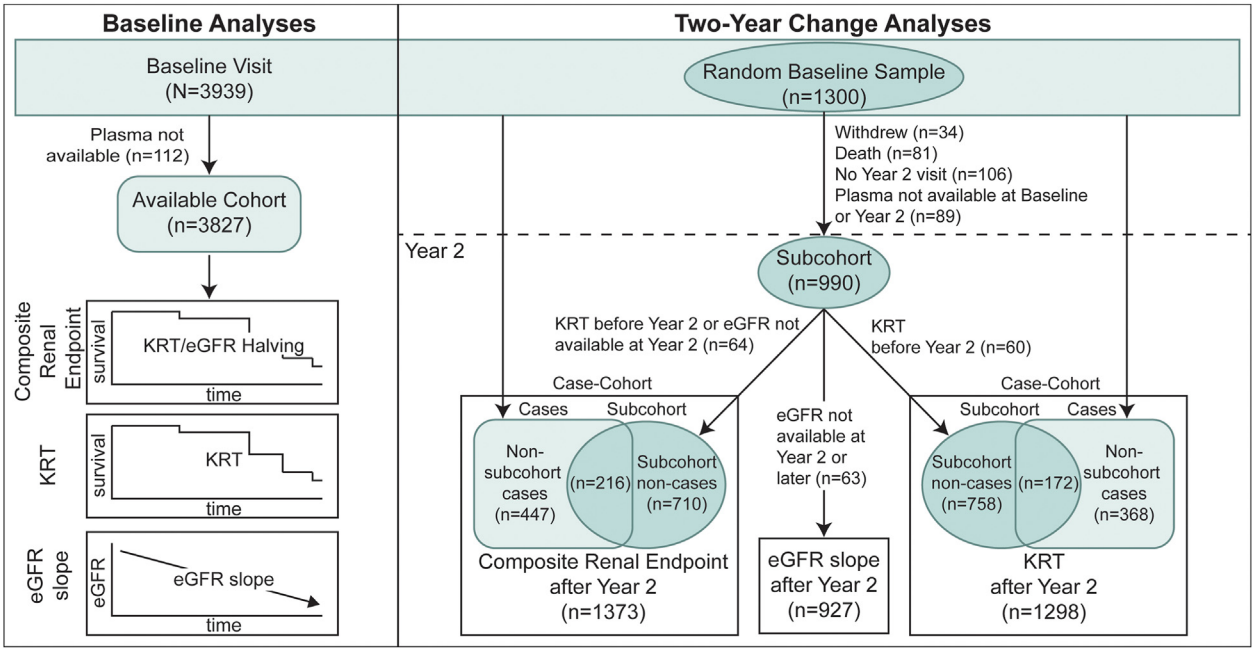

Figure 1. Baseline and 2-year change in MMP-2 analysis flow chart.

hazard with an increasing level of MMP-2 until reaching the median (309 ng/mL), at which point the hazard plateaued. Given this nonlinear relationship, continuous 2-year change was only assessed as a secondary outcome.[20]

MMP-2 was measured using an enzyme-linked immunosorbent assay (R & D Systems), run in duplicate and averaged for each sample, at the CRIC Central Laboratory at the University of Pennsylvania. The coefficients of variation for control samples with concentrations of 4.02 ng/mL and 20.9 ng/mL were 9.4% and 9.7%, respectively.

Outcomes and Censoring Events

The primary CKD progression outcome was time to initiation of KRT or eGFR halving (composite kidney endpoint). Time to KRT and change in eGFR over time (eGFR slope) were assessed as secondary outcomes. Outcomes were ascertained as described in Item S1. Participant follow-up was censored at loss to follow up, death, or the end of the follow-up period. For baseline analyses, outcomes were assessed after study entry for a median of 6.9 years of follow-up. For 2-year pattern analyses, outcomes were assessed beginning at year 2 with a median of 4.6 years of follow-up.

Covariates

Covariates in a series of nested models were age, sex, race or ethnicity (non-Hispanic White, non-Hispanic African American with low-risk APOL1 genotype [0 or 1 copies of APOL1 G1 and G2 risk alleles], non-Hispanic African American with high-risk APOL1 genotype [2 copies of APOL1 G1 and G2 risk alleles], Hispanic, and other), level of education, eGFR, body mass index, systolic blood pressure (SBP), current smoking, diabetes, history of CVD, urine neutrophil gelatinase-associated lipocalin to creatinine ratio (NGAL/cr), serum phosphate, high-sensitivity C-reactive protein (hsCRP), high-sensitivity Troponin T (hsTnT), N-terminal pro-B-type natriuretic peptide (NTproBNP), and urine protein to creatinine ratio (UPCR).

For baseline analyses, covariates were measured at baseline. For 2-year-pattern analyses, year 2 covariate measurements were used with the exception of UPCR and variables not measured at year 2 (hsCRP, phosphate, hsTNT, NTproBNP, and NGAL/cr), for which baseline measurements were used. The baseline measurements of UPCR were used to avoid potential overadjustment of MMP-2 through the inclusion of contemporaneous measurements of an intermediate variable hypothesized to be on the causal pathway to outcomes.[21] Further covariate information and data collection methods are described in Item S1.

Statistical Analysis

Summary statistics were assessed using χ^2, analysis of variance, or Kruskal-Wallis, where appropriate. To reduce the potential bias introduced by missing data, multiple imputations were performed to replace missing covariate values, as described in Item S1.

Cox proportional hazards models were used to model the hazards of the composite kidney endpoint or secondary outcome, initiation of KRT. For case-cohort analyses assessing associations of 2-year patterns of MMP-2, participants in the randomly selected subcohort and all cases with both baseline and year 2 MMP-2 measurements who experienced the event of interest after their second annual visit were included in the case-cohort analyses (composite kidney endpoint N = 1,373; KRT N = 1,298). A flow chart of participant inclusion in analyses is depicted in Fig 1. For these case-cohort analyses, the Barlow method of weighting was used,[22] which is described further in Item S1.

A priori determined models stratified by study site were used to assess the relationship between MMP-2 and the composite kidney outcome, or KRT, as follows: unadjusted (Model 1), adjusted for age, sex, race or ethnicity, education, baseline eGFR, body mass index, SBP, current smoking, history of diabetes, and CVD (Model 2), adjusted for variables from Model 2 and urine NGAL/cr, serum phosphate, hsCRP, hsTNT, and NTproBNP (Model 3), and adjusted for variables from Model 3 and UPCR (Model 4). Covariates were included in continuous form unless otherwise indicated with right-skewed variables (UPCR, NGAL/cr, hsCRP, hsTNT, and NTproBNP) log transformed. The UPCR and eGFR were modeled as restricted cubic splines with knots placed at the 25th, 50th, and 75th percentiles. Effect modification in Cox proportional hazards models was explored by an a priori selected set of covariates, including UPCR (<132.6, 132.6-441.9, or ≥442 mg/g), age (< or ≥ 60 years), sex, and race or ethnicity (non-Hispanic White or other), SBP (< or ≥120 mm Hg), hsCRP (< or ≥2.5 g/dL), and eGFR (< or ≥30 mL/min/1.73m^2). Multiple comparisons were controlled for in effect modification testing using the false discovery rate.

Linear mixed effects models with random intercepts and random slopes and an unstructured covariance structure were used to model the change in eGFR over time (eGFR slope). The same a priori determined covariates are used in Cox proportional hazard. Models 1 through 4 were used in eGFR slope models, with the difference that study site and baseline eGFR were included as additional covariates in all models. Two-year-change eGFR slope analyses included only members of the randomly selected subcohort of 1,300 with both baseline and year 2 MMP-2 measurements and 1 or more eGFR measurements from their year 2 annual visit or later (N = 927). All participants with baseline MMP-2 measurements were included in the baseline eGFR slope analyses (N = 3,827).

Statistical analyses were performed using SAS software v9.4 (Cary). An α of .05 was used to determine the significance. All hypothesis tests were 2-sided. Statistical analyses are described in further detail in Item S1.

RESULTS

Baseline Analyses

Characteristics of the cohort participants (N = 3,827) are shown by the baseline level of MMP-2 in Table 1. The baseline characteristics and results are described further in

Table 1. Baseline Demographic and Clinical Characteristics of Participants According to Level of Baseline MMP-2

Characteristic	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Total	
Range (ng/mL)	**0.4-241.4**	**242.0-308.8**	**309.0-396.5**	**397.0-1379.0**	**0.4-1379.0**	
N	**951**	**958**	**961**	**957**	**3,827**	***P***
MMP-2, ng/mL						
Median (IQR)	205.0 (179.9-222.0)	275.0 (259.0-290.2)	348.0 (327.0-369.0)	477.0 (433.0-538.2)	309.0 (242.0-397.0)	
Demography						
Age (y), mean ± SD	55.2 ± 11.7	58.2 ± 10.6	58.9 ± 10.4	58.4 ± 11.0	57.7 ± 11.0	<0.001
Female sex, n (%)	465 (48.9)	422 (44.1)	431 (44.9)	407 (42.5)	1,725 (45.1)	0.04
Racial/ethnicity, n (%)						
NH-White	428 (47.2)	439 (47.7)	386 (42.0)	345 (37.8)	1,598 (43.7)	<0.001
NH-African American, APOL1 low-risk	274 (30.2)	276 (30.0)	301 (32.8)	295 (32.3)	1,146 (31.3)	
NH-African American APOL1 high-risk	74 (8.2)	76 (8.3)	61 (6.6)	65 (7.1)	276 (7.5)	
Hispanic	88 (9.7)	94 (10.2)	136 (14.8)	170 (18.6)	488 (13.3)	
Other	43 (4.7)	36 (3.9)	35 (3.8)	39 (4.3)	153 (4.2)	
Education level, n (%)						
Less than high school	135 (14.2)	193 (20.2)	214 (22.3)	260 (27.2)	802 (21.0)	<0.001
High school graduate	179 (18.8)	180 (18.8)	186 (19.4)	177 (18.5)	722 (18.9)	
Some college	292 (30.7)	287 (30.0)	251 (26.1)	276 (28.8)	1,106 (28.9)	
College graduate or higher	344 (36.2)	298 (31.1)	310 (32.3)	244 (25.5)	1,196 (31.3)	
Anthropometry						
BMI, kg/m^2						
Mean ± SD	32.1 ± 7.3	32.0 ± 7.4	32.1 ± 8.0	32.3 ± 8.5	32.1 ± 7.8	0.88
Blood pressure						
Systolic, mmHg						
Mean ± SD	122.2 ± 18.6	125.8 ± 20.9	130.6 ± 22.2	135.6 ± 24.4	128.6 ± 22.2	<0.001
Medical history, n (%)						
Diabetes	303 (31.9)	393 (41.0)	510 (53.1)	655 (68.4)	1,861 (48.6)	<0.001
Current smoker	157 (16.5)	120 (12.5)	114 (11.9)	102 (10.7)	493 (12.9)	0.001
History of CVD	222 (23.3)	312 (32.6)	339 (35.3)	404 (42.2)	1,277 (33.4)	<0.001
Kidney function measure						
eGFR, mL/min/1.73m^2						
Mean ± SD	50.1 ± 18.5	45.8 ± 16.0	44.0 ± 16.0	39.5 ± 14.6	44.8 ± 16.8	<0.001
Kidney injury markers						
UPCR, mg/g						
Median (IQR)	93.7 (46.9-324.4)	124.6 (52.2-483.5)	165.3 (61.9-907.0)	393.4 (84.9-2167.6)	152.9 (57.5-777.0)	<0.001
NGAL/Cr, ng/mg						
Median (IQR)	19.2 (8.7-47.9)	20.1 (8.7-49.1)	23.7 (10.1-60.7)	33.1 (12.2-96.5)	23.4 (9.8-61.0)	<0.001
Metabolic marker						
Phosphate, mg/dL						

(Continued)

Table 1 (Cont'd). Baseline Demographic and Clinical Characteristics of Participants According to Level of Baseline MMP-2

Characteristic	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Total	
Range (ng/mL)	0.4-241.4	242.0-308.8	309.0-396.5	397.0-1379.0	0.4-1379.0	
N	951	958	961	957	3,827	*P*
Mean ± SD	3.6 ± 0.6	3.7 ± 0.7	3.7 ± 0.6	3.9 ± 0.7	3.7 ± 0.7	<0.001
Inflammatory marker						
hsCRP, mg/L						
Median (IQR)	3.3 (1.3-7.6)	2.7 (1.1-7.0)	2.4 (1.0-5.5)	2.0 (0.8-5.3)	2.6 (1.1-6.4)	<0.001
Cardiovascular risk markers						
hsTNT, pg/mL						
Median (IQR)	7.7 (3.4-14.8)	10.9 (5.6-19.7)	13.8 (6.8-26.1)	20.0 (10.4-38.1)	12.3 (5.8-23.9)	<0.001
NTproBNP, pg/mL						
Median (IQR)	81.3 (38.2-196.0)	129.8 (58.5-309.2)	171.2 (75.4-418.7)	309.6 (124.1-820.2)	152.5 (63.3-413.3)	<0.001

Note: SI conversion: To convert serum phosphate to mmol/L, multiply by 0.323.
Abbreviations: BMI, body mass index; CI, confidence interval; Cr, creatinine; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; hsTNT, high-sensitivity Troponin T; IQR, interquartile range; MMP-2, matrix metalloproteinase-2; NGAL, neutrophil gelatinase-associated lipocalin; NH, non-Hispanic; NTproBNP, N-terminal pro-B-type natriuretic peptide; SD, standard deviation; UPCR, urine protein to creatinine ratio; yr, year.

Item S2: Supplementary Results. Crude incidence by quartile is shown in Table S1.

In adjusted analyses, baseline MMP-2 was found to be independent of all covariates except proteinuria (Table 2). Proteinuria was found to be a significant effect modifier, with elevated MMP-2 levels (≥300 ng/mL) related to increased hazard of the composite kidney endpoint (HR, 1.30; 95% CI, 1.09-1.54) only in those with UPCR ≥ 442 mg/g (Fig 2). A similar relationship was found with KRT (Table S2 and Fig S1). In models including baseline MMP-2 as a restricted cubic spline without adjustment for UPCR or only including those with UPCR ≥ 442 mg/g, a plateauing of hazard was found, in which the hazard of time-to-event CKD outcomes increased until median levels of MMP-2 (309 ng/mL), at which point the hazard did not increase further (Fig S2). This plateauing of hazards informed the decision to investigate 2-year patterns of MMP-2 as above or below 300 ng/mL.

Two-year Change Analyses

Two-year change analyses were performed, including a categorical predictor indicating whether participants had above or below 300 ng/mL MMP-2 at baseline or at year 2. The baseline characteristics of the 1,439 participants included in the 2-year primary or secondary analyses are shown in Table 3 and described further in Item S2. The crude rates of outcomes for the subcohort are shown in Table S3.

Compared with those in the Low/Low MMP-2 group and after adjustment for Model 3 covariates, the Low/High MMP-2 two-year pattern is associated with over one and a half-fold rates of the composite kidney endpoint (HR, 1.65; 95% CI, 1.09-2.50) and the High/High 2-year pattern is associated with nearly 2-fold rates of the composite kidney endpoint (HR, 1.86; 95% CI, 1.26-2.75; Table 4). With adjustment for baseline UPCR in Model 4, the hazard ratio for High/High 2-year pattern was still significantly different from the Low/Low pattern (HR, 1.61; 95% CI, 1.07-2.42) though the overall type 3 P value testing for differences between all levels of the variable was marginally nonsignificant at $P = 0.09$.

Adjusted for Model 4 covariates, a significant interaction was detected by level of inflammation, with those with hsCRP < 2.5 mg/L having over two and a half times the rates of the composite kidney endpoint (HR, 2.60; 95% CI, 1.40-4.82) with the High/High 2-year pattern compared with those with the Low/Low pattern, whereas those with hsCRP ≥ 2.5 mg/L did not have a significantly elevated hazard (HR, 1.18; 95% CI, 0.63-2.21; Fig 3).

Two-year patterns in MMP-2 were also tested for associations with secondary outcomes, initiation of KRT, and change in the eGFR over time (eGFR slope), described further in Item S2. Associations with these outcomes were similar to those with the composite kidney outcome, with the highest associations found in those with persistently elevated MMP-2 and significant effect modification with low inflammation at the initiation of KRT (Tables S3 and S4 and Fig S3).

Table 2. Hazard Ratios of the Composite Kidney Endpoint by Quartile of Baseline MMP-2

	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Composite kidney endpoint (KRT/eGFR halving)								
Quartile 1	(Reference)	<0.001	(Reference)	<.001	(Reference)	0.007	(Reference)	0.41
Quartile 2	1.26 (1.04-1.51)		1.23 (1.01-1.48)		1.19 (0.98-1.45)		1.11 (0.92-1.35)	
Quartile 3	1.69 (1.41-2.02)		1.39 (1.15-1.67)		1.31 (1.08-1.58)		1.18 (0.97-1.43)	
Quartile 4	2.67 (2.25-3.16)		1.66 (1.39-1.99)		1.38 (1.14-1.67)		1.13 (0.93-1.37)	

Model 1: Unadjusted. All Cox proportional hazard models were stratified by study site.
Model 2: Adjusted for age, sex, race/ethnicity, level of education, eGFR, body mass index, systolic blood pressure, current smoking, diabetes, and history of cardiovascular disease.
Model 3: Adjusted for variables from Model 2 and urine neutrophil gelatinase-associated lipocalin to creatinine ratio, serum phosphate, high-sensitivity C-reactive protein, high-sensitivity Troponin T, and N-terminal pro-B-type natriuretic peptide.
Model 4: Adjusted for variables from Model 3 and urine protein to creatinine ratio.
Results are pooled from 20 imputed datasets and shown by quartile of MMP-2 (ranges, ng/mL: Quartile 1 [0.4-241.4]; Quartile 2 [242.0-308.8]; Quartile 3 [309.0-396.5]; Quartile 4 [397.0-1379.0]. Analyses included 3,827 participants with 1,192 composite kidney endpoint events.
Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; KRT, kidney replacement therapy; MMP-2, matrix metalloproteinase-2; SE, standard error.

Change in the MMP-2 from baseline to year 2 was also explored as a continuous predictor and is characterized in Table S5. A significant increase in hazard of the composite kidney endpoint was found with 1 standard deviation (136 ng/mL) increase in MMP-2 from baseline to year 2 with adjustment for baseline MMP-2 and all covariates

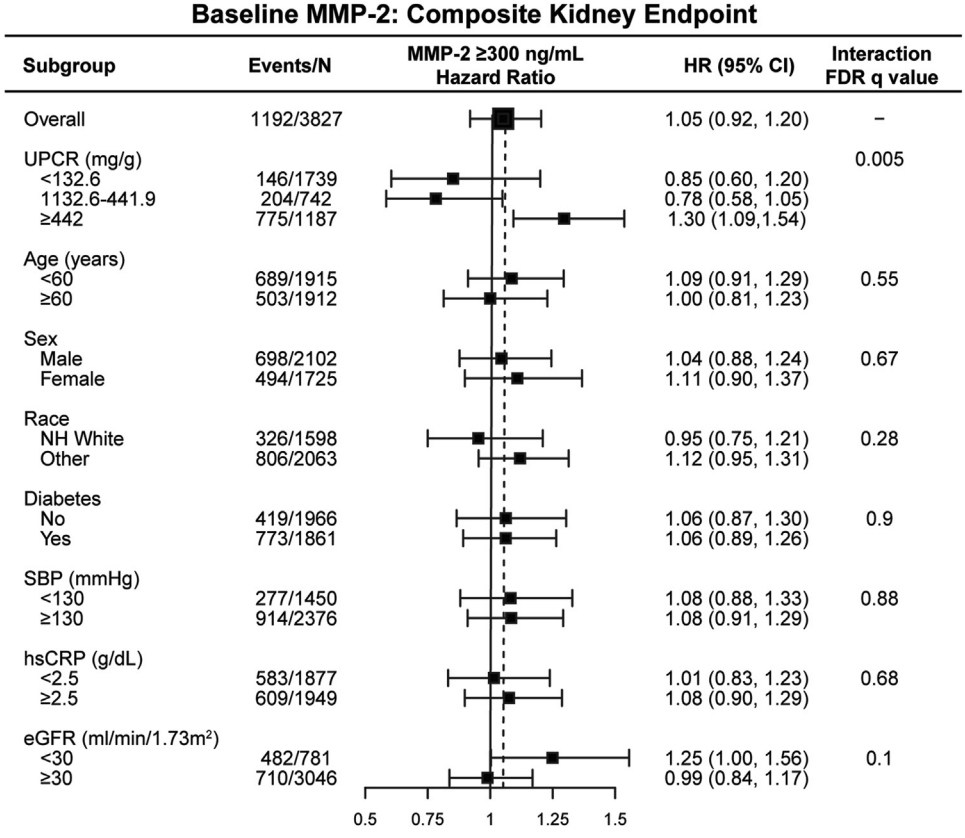

Figure 2. Effect modification of elevated baseline MMP-2 on the composite kidney endpoint Heterogeneity of the effect of elevated matrix metalloproteinase-2 (MMP-2; ≥300 ng/mL) on the composite kidney endpoint of initiation of kidney replacement therapy (KRT) and estimated glomerular filtration rate (eGFR) halving across subgroups was tested through inclusion of interaction terms. Models were stratified by study site and adjusted for Model 4 covariates: age, sex, race or ethnicity, level of education, eGFR, body mass index, systolic blood pressure (SBP), current smoking, diabetes, history of cardiovascular disease, urine neutrophil gelatinase-associated lipocalin to creatinine ratio, serum phosphate, high-sensitivity C-reactive protein (hsCRP), high-sensitivity Troponin T, and N-terminal pro-B-type natriuretic peptide, and urine protein to creatinine ratio (UPCR). The UPCR, age, race or ethnicity, SBP, hsCRP, eGFR, and baseline MMP-2 were categorized as shown for effect modification testing and included as main effects and interactions with all model variables. A false discovery rate correction (FDR q value) was used to correct for multiple comparisons using the *P* values from the interaction term between elevated MMP-2 and the indicated subgroup variables. Results are pooled estimates of 20 imputed datasets.

Table 3. Baseline Demographic and Clinical Characteristics of Participants According to Level of Baseline and Year 2 MMP-2

MMP-2 (ng/mL) Low <300, High (≥300)	Baseline: Low Year 2: Low	Baseline: Low Year 2: High	Baseline: High Year 2: Low	Baseline: High Year 2: High	Total	*P*
N	322	307	187	623	1,439	
MMP-2 at baseline, ng/mL						
Median (IQR)	230.5 (193.0-266.0)	250.0 (215.0-275.8)	371.0 (333.0-436.0)	409.0 (349.1-493.0)	322.0 (252.0-416.0)	
MMP-2 at year 2, ng/mL						
Median (IQR)	244.5 (218.0-274.0)	385.0 (338.0-443.0)	250.0 (228.0-275.0)	427.0 (361.0-514.0)	347.0 (270.0-442.0)	
MMP-2 Δ, ng/mL						
Mean ± SD	13.8 ± 58.9	159.9 ± 91.2	−146.1 ± 90.7	13.6 ± 127.1	24.1 ± 135.5	
Demography						
Age (y), mean ± SD	55.5 ± 11.3	57.2 ± 11.0	56.8 ± 11.8	58.4 ± 10.3	57.3 ± 10.9	0.001
Female sex, n (%)	158 (49.1)	122 (39.7)	082 (43.9)	260 (41.7)	622 (43.2)	0.09
Racial/ethnicity, n (%)						
NH-White	145 (46.6)	133 (44.9)	081 (44.0)	202 (33.9)	0561 (40.5)	<0.001
NH-African American, APOL1 low-risk	087 (28.0)	096 (32.4)	054 (29.3)	210 (35.3)	0447 (32.3)	
NH-African American, APOL1 high-risk	030 (9.6)	025 (8.4)	018 (9.8)	044 (7.4)	0117 (8.4)	
Hispanic	027 (8.7)	034 (11.5)	023 (12.5)	109 (18.3)	0193 (13.9)	
Other	022 (7.1)	008 (2.7)	008 (4.3)	030 (5.0)	0068 (4.9)	
Education level, n (%)						
Less than high school	040 (12.5)	059 (19.2)	036 (19.3)	162 (26.0)	0297 (20.7)	<0.001
High school graduate	058 (18.1)	058 (18.9)	033 (17.6)	112 (18.0)	0261 (18.2)	
Some college	096 (29.9)	089 (29.0)	053 (28.3)	171 (27.4)	0409 (28.4)	
College graduate higher	127 (39.6)	101 (32.9)	065 (34.8)	178 (28.6)	0471 (32.8)	
Anthropometry						
BMI, kg/m^2						
Mean ± SD	32.1 ± 7.2	32.1 ± 7.6	31.5 ± 7.5	32.5 ± 8.2	32.2 ± 7.8	0.45
Blood pressure						
Systolic, mm Hg						
Mean ± SD	122.2 ± 18.6	127.4 ± 19.4	126.0 ± 21.6	136.3 ± 23.9	129.9 ± 22.3	<0.001
Medical history, n (%)						
Diabetes	112 (34.8)	140 (45.6)	085 (45.5)	422 (67.7)	0759 (52.7)	<0.001
Current smoker	046 (14.3)	034 (11.1)	025 (13.4)	066 (10.6)	0171 (11.9)	0.34
History of CVD	086 (26.7)	108 (35.2)	055 (29.4)	242 (38.8)	0491 (34.1)	0.001
Kidney function measure						
eGFR, mL/min/1.73m^2						
Mean ± SD	48.2 ± 17.8	43.0 ± 15.4	46.1 ± 17.6	39.3 ± 13.6	43.0 ± 15.9	<0.001
Kidney injury markers						
UPCR, mg/g						
Median (IQR)	118.5 (53.0-445.5)	243.1 (57.5-978.6)	253.7 (64.5-905.2)	480.0 (105.2-2135.7)	267.9 (68.1-1186.3)	<0.001

(Continued)

Table 3 (Cont'd). Baseline Demographic and Clinical Characteristics of Participants According to Level of Baseline and Year 2 MMP-2

MMP-2 (ng/mL) Low <300, High (≥300)	Baseline: Low Year 2: Low	Baseline: Low Year 2: High	Baseline: High Year 2: Low	Baseline: High Year 2: High	Total	*P*
N	322	307	187	623	1,439	
NGAL/Cr, ng/mg						
Median (IQR)	22.4 (8.3-54.2)	19.5 (9.0-56.5)	20.7 (10.2-53.6)	32.7 (12.0-88.8)	25.4 (10.2-67.0)	<0.001
Metabolic Marker						
Phosphate, mg/dL						
Mean ± SD	3.6 ± 0.6	3.7 ± 0.7	3.7 ± 0.6	3.8 ± 0.7	3.7 ± 0.7	<0.001
Inflammatory marker						
hsCRP, mg/L						
Median (IQR)	3.0 (1.3-7.2)	2.7 (1.1-6.2)	2.0 (0.9-5.0)	2.0 (0.8-4.9)	2.4 (1.0-5.6)	<0.001
Cardiovascular risk markers						
hsTNT, pg/mL						
Median (IQR)	8.0 (4.1-15.1)	12.5 (5.9-22.6)	10.2 (5.1-17.9)	19.5 (9.9-37.5)	12.9 (6.3-26.4)	<0.001
NTproBNP, pg/mL						
Median (IQR)	85.6 (43.4-206.3)	139.1 (64.4-334.7)	157.9 (62.2-376.7)	264.2 (120.4-597.4)	173.5 (71.7-430.8)	<0.001

Note: SI conversion: To convert serum phosphate to mmol/L, multiply by 0.323.
Abbreviations: BMI, body mass index; CI, confidence interval; Cr, creatinine; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; hsTNT, high-sensitivity Troponin T; IQR, interquartile range; MMP-2, matrix metalloproteinase-2; MMP-2 Δ, Change in MMP-2 from baseline to year 2; NGAL, neutrophil gelatinase-associated lipocalin; NH, non-Hispanic; NTproBNP, N-terminal pro-B-type natriuretic peptide; SD, standard deviation; UPCR, urine protein to creatinine ratio; yr, year.

except UPCR, which attenuated the effect enough to become marginally nonsignificant (Table S6; HR, 1.17; 95% CI, 0.98-1.40). KRT followed a similar pattern to the composite kidney endpoint, but the eGFR slope was not associated with a continuous 2-year change in MMP-2 after adjusting for baseline eGFR (Table S6).

DISCUSSION

In this large, well-characterized, and diverse prospective cohort of adults with CKD, we report associations between baseline and persistent (2-year) elevation of MMP-2 ≥ 300 ng/mL with an increase in the hazard of the composite kidney endpoint and KRT, independent of key demographic, kidney function, medical history, anthropometric, and inflammation covariates, with the exception of proteinuria. Proteinuria significantly modified the effect of elevated MMP-2 (≥300 ng/mL), with an increased risk of the composite kidney endpoint and KRT found only in participants with UPCR ≥ 442 mg/g. Effects of persistent elevation were significantly modified by level of inflammation, with a 2.6-fold higher rate of both CKD progression outcomes in those with a high-sensitivity C-reactive protein of <2.5 g/dL at study entry. We report associations between a single measurement of MMP-2 with CKD progression, independent of all tested covariates except proteinuria.

Potential reasons for the increased hazard of outcomes with a 2-year pattern of elevated MMP-2 among those with a C-reactive protein of <2.5 g/dL at study entry are manyfold and would require a complex analysis approach that is beyond the scope of this observational study. First, the relationship between MMP-2 and inflammation is complex. MMP-2 has previously been found to positively correlate with CRP in those with angina[23,24] and coronary artery disease.[25] However, CRP has not been found to have a substantive correlation with MMP-2 in multiple studies in the setting of CKD,[26,27] including this one (Spearman $\rho = -0.1$). A potential cause of the association or lack thereof between MMP-2 and CRP among different populations could be that MMP-2 synthesis can be regulated by different factors such as proinflammatory cytokines or oxidative stress, which has previously been shown in multiple disease settings, including CKD.[28-31] Medication usage could potentially affect inflammation or MMP-2 expression. A recent publication by Kopanko et al,[32] found differential expression of MMP-2 and inflammatory biomarkers and the relationship between those markers in subgroups with different medication usage; β-blocker usage was associated with lower MMP-2 and inflammation markers interleukin 6 (IL-6) and tumor necrosis factor-α, whereas calcium channel antagonists were associated with higher levels of these factors. In the group not using β-blockers, variations in MMP-2 levels were associated with the oxidative stress marker Cu/Zn superoxide dismutase (Cu/Zn SOD) and CKD etiology, neither of which are available in the CRIC cohort. In

Table 4. Hazard Ratios of the Composite Kidney Endpoint by MMP-2 Baseline and Year 2 Levels

MMP-2 (ng/mL) Low <300, High ≥300		Model 1		Model 2		Model 3		Model 4	
Baseline	**Year 2**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Composite kidney endpoint (KRT/eGFR halving)									
Low	Low	(Reference)	<0.001	(Reference)	0.004	(Reference)	0.01	(Reference)	0.09
High	Low	1.34 (0.93-1.93)		1.45 (0.90-2.35)		1.37 (0.84-2.25)		1.10 (0.65-1.86)	
Low	High	2.11 (1.55-2.90)		1.71 (1.13-2.60)		1.65 (1.09-2.50)		1.34 (0.87-2.06)	
High	High	3.12 (2.38-4.10)		1.94 (1.34-2.82)		1.86 (1.26-2.75)		1.61 (1.07-2.42)	

Model 1: Unadjusted. All models were stratified by study site.
Model 2: Adjusted for age, sex, race/ethnicity, level of education, eGFR, body mass index, systolic blood pressure, current smoking, diabetes, and history of cardiovascular disease.
Model 3: Adjusted for variables from Model 2 and urine neutrophil gelatinase-associated lipocalin to creatinine ratio, serum phosphate, high-sensitivity C-reactive protein, high-sensitivity Troponin T, and N-terminal pro-B-type natriuretic peptide.
Model 4: Adjusted for variables from Model 3 and urine protein to creatinine ratio.
Results are pooled from 30 imputed datasets and included 1,373 subcohort members and incident cases after year 2 with 663 events.
Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; KRT, kidney replacement therapy; MMP-2, matrix metalloproteinase-2; SE, standard error.

addition, though inflammation can be elevated at various stages of CKD progression and vary depending on the underlying cause, it can be particularly pronounced in the early stages of kidney fibrosis when injury triggers an inflammatory response in the injured cells and inflammatory cells in the kidney, which can in turn promote the activity and expression of MMP-2 and the development of renal interstitial fibrosis.[33] It is possible that for some participants, the co-occurrence of elevated CRP and MMP-2 was a result of early stages of fibrosis and thus less likely to be associated with the hazard of outcomes during the follow-up period of this study. Last, MMP-2 is involved in multiple mechanistic pathways of multiple diseases, with some resulting in kidney damage and others in vascular damage.[13] There is the potential that for some participants, co-elevation of CRP and MMP-2 was caused by a comorbid condition rather than kidney fibrosis. These complex relationships between inflammation and MMP-2 warrant further mechanistic studies to fully elucidate the relationship between inflammation and MMP-2 within specific CKD etiologies and subpopulations.

MMP-2 has also been shown to have an increasing trend with increasing CKD stages;[34] however, we did not find effect modification by eGFR, suggesting the interaction between MMP-2 and proteinuria cannot fully be explained by CKD stage. MMP-2 has previously been found to not be associated with eGFR decline in a general nondiabetic population.[35] Of the outcomes assessed in our study, the association of MMP-2 with eGFR slope, ie, rate of eGFR decline, was the weakest.

MMPs, including MMP-2, effect CKD and CVD through multiple pathophysiological mechanisms[13] and are involved in healthy vascular remodeling and acute kidney injury repair, and inappropriate vascular remodeling and kidney fibrosis.[36,37] Dimas et al[38] suggested an approach of considering MMPs as beneficial regarding angiogenesis before the development of proteinuria and harmful after the early stages of atherosclerosis, nephritis, and glomerular basement membrane structural defects. Although potentially useful, this approach does not explain 2 previous prospective cohort studies finding MMP-2 to be an independent predictor of CKD progression in specific nonproteinuric or combined proteinuric and nonproteinuric subpopulations, in which cohorts were limited to those without diabetes with coronary artery disease[10] or those with diabetes,[11] respectively. Proteinuria is considered a marker of systemic vascular damage[13] and also occurs consistently with diabetic kidney disease.[2] A possible explanation of these seemingly inconsistent findings with our study and previous reports may be that these high-risk or "pro-proteinuric" populations have already traversed a threshold of disease progression after which increased MMP-2 expression is more likely to be due to upregulation of MMP-2 during the progression of fibrosis than to angiogenesis.

Reductions in proteinuria, especially in those with high baseline proteinuria, have previously been associated with a decreased risk of KRT or death.[12] In multiple animal models of kidney disease, development of proteinuria or albuminuria was blocked or reversed through inhibition of MMP-2,[15-18] which has previously suggested MMP-2 could be a potential target for intervention in specific proteinuric CKDs. Here, we show participants that reduced their MMP-2 levels from above 300 ng/mL at baseline to below 300 ng/mL at year 2 had a similar risk of kidney endpoints as those with persistently low levels after 7 years of follow-up, which lends further support for MMP-2 as a potential promising target for intervention in established CKD. Specific and broad spectrum synthetic MMP inhibitors and inhibitory antibodies have been developed and used in animal models.[13,39] Renin-angiotensin-aldosterone system inhibitors, which have been shown to reduce the risk of CKD progression and to reduce proteinuria,[40] have been shown to inhibit MMP gelatinases.[41]

Strengths of this study include the large size and diversity of the CRIC cohort, with follow-up for as long as 9.8 years from the baseline. Inclusion of covariates informed by previous research reduces the likelihood of unmeasured confounders. Effect modification testing elucidated the relationship between single measurements

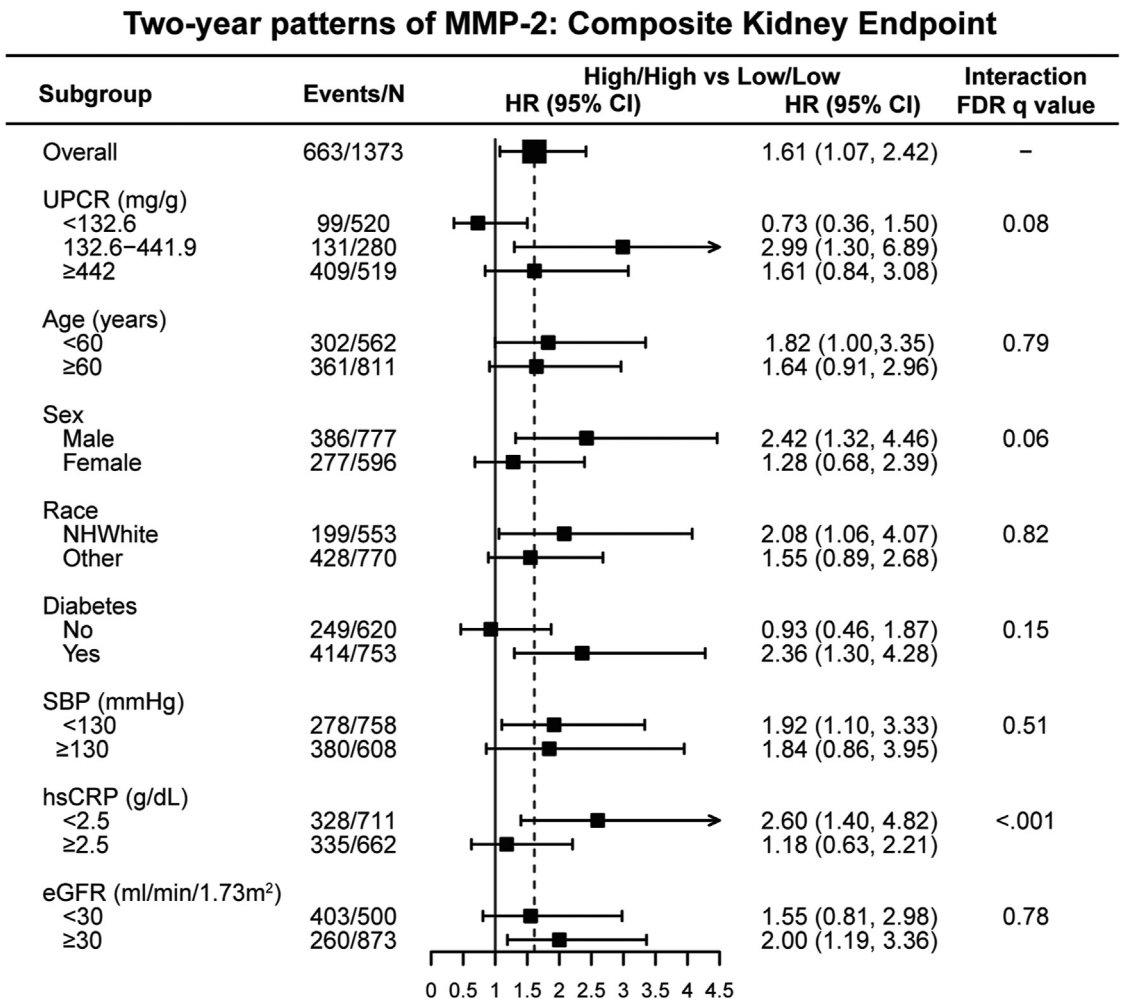

Figure 3. Effect modification of baseline and year 2 levels of MMP-2 on the composite kidney endpoint. Heterogeneity of the effect of high Matrix metalloproteinase-2 levels (MMP-2; ≥300 ng/mL) at baseline or year 2 on a composite kidney endpoint of initiation of kidney replacement therapy (KRT) and estimated glomerular filtration rate (eGFR) halving across subgroups was tested through inclusion of interaction terms. Hazard ratios (HRs) are shown for participants with high MMP-2 levels at both baseline and year 2 (High/High) in comparison to participants with low levels of MMP-2 at both baseline and year 2 (Low/Low). A false discovery rate correction (FDR q value) was used to correct for multiple comparisons using the overall *P* values from the interaction term between the MMP-2 two-year pattern (4-level variable) and indicated subgroup variables. All models were stratified by study site and adjusted for Model 4 covariates: age, sex, race or ethnicity, level of education, eGFR, body mass index, systolic blood pressure (SBP), current smoking, diabetes, history of cardiovascular disease, urine neutrophil gelatinase-associated lipocalin to creatinine ratio, serum phosphate, high-sensitivity C-reactive protein (hsCRP), high-sensitivity Troponin T, N-terminal pro-B-type natriuretic peptide, and urine protein to creatinine ratio (UPCR). The UPCR, age, race or ethnicity, SBP, hsCRP, and eGFR were categorized as shown for effect modification testing and included as main effects and interactions with all model variables. Results are pooled estimates of 30 imputed datasets. Participants included in above models were members of the subcohort and cases that experienced the event of interest after year 2.

of MMP-2 and proteinuria and 2-year patterns of MMP-2 and hsCRP. Multiple imputations of missing data (Table S7) reduced the potential bias in estimates and preserved sample size and statistical power. This study is the first to report associations between MMP-2 and CKD progression within a large prospective cohort containing a diverse CKD population or to report the relationship between 2-year patterns in MMP-2 and CKD outcomes, which were confirmed in sensitivity competing risk analyses (Table S8). This is also the first to report a higher risk associated with persistent elevation of MMP-2 in those with lower levels of inflammation. To our knowledge, the observation that a single measurement of MMP-2 is predictive of CKD progression in those with proteinuric CKD has not been reported elsewhere.

There are several limitations of this study. A major limitation is that we are unable to differentiate whether increased hazard with elevated MMP-2 is a direct effect of circulating MMP-2 in the kidney or whether the leakage of kidney-derived MMP-2 is largely responsible for the increase in MMP-2 levels that is associated with CKD progression. The CRIC Study population is not entirely

representative of all patients with CKD in the United States, and it is possible that important covariates have not been considered.

Single elevated measurements of MMP-2 are associated with CKD progression in those with proteinuria. Persistently elevated MMP-2 over 2 years above 300 ng/mL is associated with CKD progression, with a 2.6-fold higher rate of the composite kidney outcome in those with C-reactive protein <2.5 g/dL at study entry. Future investigations should confirm if elevation of the fibrosis marker MMP-2 relates to an increased risk of CKD progression among these subgroups of patients with CKD. Further mechanistic studies identifying MMP-2's role in specific proteinuric CKD pathways are warranted.

SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Figure S1: Effect modification of elevated baseline MMP-2 on initiation of KRT.

Figure S2: Adjusted hazard ratio for the composite kidney endpoint and KRT by level of baseline MMP-2 for participants overall or with protein to creatinine ratio above or below 50 mg/mmol.

Figure S3: Effect modification of baseline and year 2 levels of MMP-2 on kidney replacement therapy (KRT)

Item S1: Supplementary Methods.

Item S2: Supplementary Results.

Table S1: Crude Event Rates of the Composite Kidney Endpoint and KRT Endpoints by Quartile of Baseline MMP-2

Table S2: Hazard Ratios of KRT and Slopes of Estimated Glomerular Filtration Rate (mL/min/1.73m^2/y) by Quartile of Baseline MMP-2.

Table S3: Post Year 2 Crude Event Rates of the Composite Kidney Endpoint and Initiation of KRT by MMP-2 Baseline and Year 2 levels.

Table S4: Hazard Ratios of Initiation of KRT and Slopes of Estimated Glomerular Filtration Rate (mL/min/1.73m^2/y) by MMP-2 Baseline and Year 2 levels.

Table S5: Baseline Demographic and Clinical Characteristics by Change in MMP-2 from Baseline to Follow up.

Table S6: Hazard Ratios of the Composite Kidney Endpoint and KRT with Decrease in MMP-2 from Baseline to Year 2.

Table S7: Missingness by Variable and Timepoint.

Table S8: Hazard Ratios of the Composite Kidney Endpoint by Quartile of Baseline MMP-2 by Cause-Specific (primary analysis) and Competing Risks Fine and Gray analysis.

ARTICLE INFORMATION

The CRIC Study Investigators include Lawrence J. Appel, MD, Debbie Cohen, MD, Laura Dember, MD, Alan S. Go, MD, James P. Lash, MD, Robert G. Nelson, MD, PhD, Mahboob Rahman, MD, Panduranga S. Rao, MD, Vallabh O. Shah, PhD, and Mark L. Unruh, MD

Authors' Full Names and Academic Degrees: Robin L. Baudier, MSPH, Paula F. Orlandi, MD, MSCE Wei Yang, PhD, Hsiang-Yu Chen, MS, Nisha Bansal, MD, J. Walker Blackston, MSPH, Jing Chen, MD, Rajat Deo, MD, MSCE, Mirela Dobre, MD, Hua He, PhD, Jiang He, MD, PhD, Ana C. Ricardo, MD, Tariq Shafi, MD, MHS, Anand Srivastava, MD, Dawei Xie, PhD, Katalin Susztak, MD, PhD, Harold I. Feldman, MD, MSCE, Amanda H. Anderson, PhD, and the CRIC Study Investigators

Authors' Affiliations: Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA (RLB, HH, JH, AHA); Biostatistics and Design Program, Oregon Health & Science University, Portland, OR (RLB); Bristol Myers Squibb, Pennington, NJ (PFO); Department of Biostatistics, Epidemiology and Informatics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA (WY, H-YC, DX, HIF, AHA); Renal Division, University of Washington, Seattle, WA (NB); DeepIntent, New York, NY (JWB); Department of Medicine, Tulane University, New Orleans, LA (JC); Cardiovascular Institute, University of Pennsylvania, Philadelphia, PA (RD); Department of Medicine, Case Western Reserve University, Cleveland, OH (AS); Department of Medicine, University of Illinois College of Medicine, Chicago, IL (ACR); Division of Nephrology, Houston Methodist Hospital, Houston, TX (TS); Department of Medicine, Perelman School of Medicine, University of Pennsylvania, PA (KS); Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL (AHA).

Address for Correspondence: Amanda H. Anderson, PhD, MPH, Department of Epidemiology, University of Alabama at Birmingham School of Public Health, 1720 Second Avenue S, RPHB 217J, Birmingham, AL 35294. Email: ahanders@uab.edu

Authors' Contributions: AHA designed the study; CRIC Study personnel carried out data and specimen collection; RLB and AHA analyzed the data; RLB made the figures; RLB and AHA drafted and revised the article; all authors either contributed to the conception or design of the work or the acquisition, analysis, or interpretation of data as well as gave input on revisions and approved the final version of the article.

Support: The CRIC Study is funded under cooperative agreements between the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK; U01DK060990, U01DK060984, U01DK061022, U01DK061021, U01DK061028, U01DK060980, U01DK060963, U01DK060902, and U24DK060990). Funding for this ancillary study of MMP-2 was provided by funding from the NIDDK (R01DK104730). In addition, this work was supported in part by: the Perelman School of Medicine at the University of Pennsylvania Clinical and Translational Science Award NIH/NCATS UL1TR000003, Johns Hopkins University UL1TR000424, University of Maryland GCRC M01RR16500, Clinical and Translational Science Collaborative of Cleveland, UL1TR000439 from the National Center for Advancing Translational Sciences (NCATS) component of the National Institutes of Health and NIH roadmap for Medical Research, Michigan Institute for Clinical and Health Research (MICHR) UL1TR000433, University of Illinois at Chicago CTSA UL1RR029879, Tulane COBRE for Clinical and Translational Research in Cardiometabolic Diseases P20GM109036, Kaiser Permanente NIH/NCRR UCSF-CTSI UL1RR024131, Department of Internal Medicine, University of New Mexico School of Medicine Albuquerque, NM R01DK119199. The views expressed in this article are those of the authors and do not reflect those of the National Institutes of Health (NIH). The NIH was not involved in the design, data collection, data analysis, interpretation of the data, writing of the report, or the decision to submit for publication. Dr Feldman is currently an employee of the Patient-Centered Outcomes Research Institute (PCORI) but the views presented in this article are solely the responsibility of the authors and do not necessarily represent the views of PCORI, its Board of Governors, or its Methodology Committee.

Financial Disclosure: The authors declare they have no relevant financial interests.

Acknowledgements: The authors would like to thank the CRIC Study participants, personnel, and investigators.

Peer Review: Received December 13, 2023, as a submission to the expedited consideration track with 5 external peer reviews. Direct editorial input from the Statistical Editor and the Editor-in-Chief. Accepted in revised form May 6, 2024.

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