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Cardiovascular Disease Biomarkers and suPAR in Predicting Decline in Renal Function: A Prospective Cohort Study

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Introduction: Soluble urokinase-type plasminogen activator receptor (suPAR) strongly predicts outcomes and incident chronic kidney disease (CKD) in patients with cardiovascular disease (CVD). Whether the association between suPAR and CKD is a reflection of its overall association with chronic inflammation and poor CVD outcomes is unclear. We examined whether CVD biomarkers, including high-sensitivity C-reactive protein (hs-CRP), fibrin-degradation products (FDPs), heat-shock protein 70 (HSP-70), and high-sensitivity troponin I (hs-TnI) were associated with a decline in kidney function in the Emory Cardiovascular Biobank cohort, in which suPAR levels were shown to be predictive of both incident CKD and CVD outcomes.

Methods: We measured suPAR, hs-CRP, HSP-70, FDP, and hs-TnI plasma levels in 3282 adults (mean age 63 years, 64% male, 75% estimated glomerular filtration rate [eGFR] >60 ml/min per 1.73 m^2). Glomerular filtration rate was estimated using Chronic Kidney Disease–Epidemiology Collaboration (eGFR) at enrollment (n = 3282) and follow-up (n = 2672; median 3.5 years). Urine protein by dipstick at baseline was available for 1335 subjects.

Results: There was a weak correlation among biomarkers (r range: 0.17−0.28). hs-CRP, FDPs, hs-TnI, and suPAR were independently associated with baseline eGFR and proteinuria. The median yearly decline in eGFR was −0.6 ml/min per 1.73 m^2. hs-CRP (β: −0.04; $P = 0.46$), FDPs (β: −0.13; $P = 0.08$), HSP-70 (β: 0.05; $P = 0.84$), or hs-TnI (β: −0.01; $P = 0.76$) were associated with eGFR decline. suPAR remained predictive of eGFR decline even after adjusting for all biomarkers.

Discussion: hs-CRP, FDP, HSP-70, and hs-TnI were not associated with eGFR decline. The specific association of suPAR with eGFR decline supported its involvement in pathways specific to the pathogenesis of kidney disease.

Kidney Int Rep (2017) **2,** 425–432; http://dx.doi.org/10.1016/j.ekir.2017.02.001
KEYWORDS: CKD; creatinine; CRP; eGFR; FDP; HSP-70; proteinuria; troponin; urokinase

Chronic kidney disease (CKD), which is defined as a reduced glomerular filtration rate (GFR), affects >14% of the US population and has been steadily increasing in incidence and prevalence.[1] Patients with CKD are at high risk of cardiovascular disease (CVD) and mortality.[1,2] Despite the overall improvement in cardiovascular outcomes over the past few decades, there has been negligible progress in identifying patients at risk of CKD.[1] Current methods for screening for kidney disease are limited, and rely on the measurement of proteinuria and estimation of GFR, which are both reflective of active kidney injury rather than risk.[3–6]

Recently, we identified soluble urokinase-type plasminogen activator receptor (suPAR) as an important predictor of incident CKD in patients with CVD.[7] suPAR is the circulating form of a glycosyl-phosphatidylinositol–anchored 3-domain membrane protein expressed on a variety of cells, including immunologically active cells, endothelial cells, and podocytes[8–10]; it has been implicated in the

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Received 2 November 2016; revised 2 November 2016; accepted 2 February 2017; published online 10 February 2017

pathogenesis of various forms of kidney disease.[9,11–15] Elevated suPAR levels are strongly predictive of poor cardiovascular outcomes and are associated with endothelial dysfunction, increased vascular stiffness, and atherosclerosis.[16–20]

Other biomarkers of CVD and inflammation have been previously associated with kidney disease.[3,21] High-sensitivity C-reactive protein (hs-CRP) is elevated in patients with CKD[22] and is associated with worse outcomes in this population.[23] Studies that have examined the association between hs-CRP and progression of kidney disease have been conflicting.[24,25] Heat shock protein-70 (HSP-70), a marker of cellular stress, is believed to be involved in the regulation of oxidative stress and pathogenesis of CKD.[26] Fibrin degradation products (FDPs) are elevated in patients with CKD and reflect a hypercoagulable state associated with increased cardiovascular risk.[27] Lastly, high-sensitivity troponin-I (hs-TnI), despite being higher in patients with CKD due to reduced clearance, remains predictive of CVD outcomes.[28] Whether these markers are predictive of incident decline in renal function and whether the association between suPAR and estimated GFR (eGFR) decline is independent of the aforementioned CVD biomarkers is unclear. We examined whether hs-CRP, FDPs, HSP-70, and hs-TnI are associated with eGFR decline in the Emory Cardiovascular Biobank, the cohort in which suPAR levels were shown to be predictive of incident CKD and CVD outcomes.[7,17,29] We hypothesized that only suPAR would be associated with future eGFR decline and that the association would be independent of hs-CRP, HSP-70, FDP, and hs-TnI levels.

METHODS

The study is presented following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement checklist for cohort studies (www.strobe-statement.org).[30]

Study Design and Population

We measured suPAR, hs-CRP, HSP-70, FDP, and hs-TnI plasma levels in 3282 adult patients who underwent left heart catheterization for suspected or confirmed coronary artery disease (CAD) at 3 Emory Healthcare sites from 2003 to 2014, and who were enrolled in the Emory Cardiovascular Biobank.[17] Exclusion criteria included congenital heart disease, severe valvular heart disease, severe anemia, recent blood transfusion, myocarditis, or history of active inflammatory disease and cancer. Patients were interviewed to collect demographic characteristics, medical history, and medication use. Medical records were reviewed to confirm self-reported medical history. The average discrepancy across variables between self-reported medical history and electronic medical record review was 6.6%. In the event of a discrepancy between self-reported history of electronic medical record documentation, we adopted the version denoting the presence of disease. All available measures of eGFR and urine protein performed at Emory Healthcare sites were collected. The study was approved by the Institutional Review Board at Emory University (Atlanta, GA), and conducted according to the Declaration of Helsinki. All patients provided written informed consent at the time of enrollment.

We first examined the association between baseline biomarker levels and measures of kidney function (eGFR and semi-quantitative assessment of proteinuria). We then investigated the association between suPAR, hs-CRP, HSP-70, FDP, and hs-TnI plasma levels and change in eGFR during follow-up in 2672 (81%) patients with at least 1 additional measure of eGFR (median number of measurements: 7) during a median follow-up of 3.5 years (Figure S1).

Sample Collection and Biomarker Measurements

Fasting arterial blood samples were collected and serum and plasma stored at −80°C for a mean duration of 4.9 years. Serum hs-CRP concentrations were determined using a particle-enhanced immunoturbidimetry assay with a lower detection limit of 0.03 mg/L (FirstMark, Division of GenWay Biotech Inc, San Diego, CA).[31] Plasma levels of suPAR were measured by Virogates (suPARnostic kit; Copenhagen, Denmark). FDP levels were determined using a sandwich immunoassay. FDP components included fragments D and E, D-dimer, and additional intermediate cleavage products. HSP-70 was measured with a sandwich enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota) and optimized by FirstMark. Minimum detectable suPAR, hs-CRP, FDP, HSP70, and hs-TnI concentrations were 100 pg/ml, 0.1 mg/L, 0.06 μg/ml, 0.625 ng/ml, and 0.3 pg/ml, respectively.

Measures of Kidney Function

Serum creatinine measurements at enrollment and all subsequent values acquired during routine follow-up clinic visits or hospitalizations within the Emory Healthcare system were collected. eGFR was calculated using the chronic kidney disease-EPI equation.[32] Semi-quantitative random urine protein excretion by dipstick testing was available for 1355 patients at the time of enrollment.

Statistical Analysis

Continuous variables are summarized as means ± SD or as median (interquartile range), and categorical

variables as proportions (percent). Independent *t*-tests or Wilcoxon rank-sum tests and χ^2 tests were used to compare continuous and categorical variables, respectively. Proteinuria data were available in a subset of patients (n = 1355) and were dichotomized as "no proteinuria," which included negative or trace, and "proteinuria" (n = 109), which included grades ≥1+. eGFR values >120 ml/min per 1.73 m^2 (<1% of measurements) were set at 120 ml/min per 1.73 m^2. The associations between each biomarker and eGFR at baseline were initially evaluated using Spearman's correlation. Logistic regression was used to examine the association between each biomarker and proteinuria. The association between baseline biomarker levels and change in eGFR over time was investigated using linear regression in 2672 patients with follow-up eGFR measurements. We regressed the follow-up eGFR values on baseline biomarker levels, follow-up time (years since baseline), and interactions between biomarkers and follow-up time. suPAR, hs-CRP, FDPs, and hs-TnI were log-transformed (base 2) in all regression models, such that the interpretation was eGFR decline per 100% increase in the biomarker, whereas HSP-70 was examined as a categorical variable (HSP-70 ≥1 ng/ml). All models included the following covariates: age, sex, race (blacks vs. others), body mass index, history of smoking, hypertension, diabetes, low-density lipoprotein, high-density lipoprotein, history of myocardial infarction, history of revascularization, presence of obstructive coronary artery disease, heart failure, and use of renin-angiotensin system inhibitors. The covariates were chosen *a priori* due to potential confounding effects on the relationship between suPAR and eGFR, based on the known association between the chosen variables and suPAR, the other biomarkers, or renal function.[7,17,18,29] Missing eGFR data were assumed to be missing at random, and were handled via maximum likelihood estimation. The fixed-effects models with autoregressive-1 correlation structure (chosen based on smallest Akaike information criterion value) were used to account for within-subject correlations in repeated eGFR measurements. Two-tailed *P* values ≤0.05 were considered statistically significant. All analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

RESULTS

Cohort Characteristics

Demographic and clinical characteristics of the total cohort, stratified according to baseline eGFRs are shown in Table 1. Overall, the cohort consisted of a majority of men (64%) and Caucasians (82%), with at least two-thirds having obstructive coronary artery disease at enrollment. Seventy-five percent of subjects had an eGFR >60 ml/min per 1.73 m^2. Less than 10% had at least 1+ proteinuria by dipstick testing. In multivariable analyses, a lower eGFR at baseline was independently associated with increasing age, male gender, hypertension, diabetes mellitus, higher low-density lipoprotein levels, lower high-density lipoprotein levels, heart failure, and use of renin-angiotensin system inhibitors (Table 2). Proteinuria was independently associated with African American race, diabetes mellitus, and heart failure (Table 2).

CVD Biomarkers and Kidney Function at Baseline

Decreasing eGFR was associated with increasing levels of suPAR, hs-CRP, FDPs, and hs-TnI (Tables 1 and 3). Patients with lower eGFRs were more likely to have HSP-70 levels ≥1 ng/ml. We found a significant negative correlation between all 5 biomarkers and eGFR, with suPAR levels having the strongest correlation with eGFR (r = −0.42; $P < 0.001$), and hs-CRP and HSP-70 having the weakest correlations (r = −0.05 and r = −0.07; $P < 0.001$, respectively) (Table 3). The correlation between the biomarkers was weak (r range: 0.07−0.27). After adjusting for CVD and CVD risk factors, suPAR (β: −13.55; $P < 0.001$), hs-CRP (β: −0.72; $P < 0.001$), FDP (β: −0.97; $P < 0.001$), and hs-TnI (β: −0.77; $P < 0.001$) were independently associated with baseline eGFR. A 100% higher suPAR (odds ratio [OR]: 3.00; $P < 0.001$), hs-CRP (OR: 1.18; $P = 0.009$), FDP (OR: 1.15; $P = 0.023$), and hs-TnI (OR: 1.12; $P = 0.004$) level was associated with at least +1 proteinuria on dipstick testing (Table 3).

CVD Biomarker Levels and eGFR Decline

We sought to determine whether hs-CRP, FDPs, HSP-70, and hs-TnI were associated with eGFR decline, and whether suPAR remained predictive of eGFR decline after adjusting for all biomarkers. Overall, in 2672 patients in whom eGFR was measured during follow-up, the median yearly decline in eGFR was −0.6 ml/min per 1.73 m^2. Of 1935 subjects with baseline eGFR ≥60 ml/min per 1.73 m^2, 406 (21%) developed CKD stage III (eGFR <60 ml/min per 1.73 m^2).

In unadjusted analyses, HSP-70 (β: 0.35; 95% confidence interval [CI]: 0.20−0.49) or hs-TnI (β: −0.02; 95% CI: − 0.05 to −0.0002) were significantly associated with eGFR decline, whereas hs-CRP (β: −0.03; 95% CI: −0.07 to 0.003), and FDP (β: −0.04; 95% CI: −0.07 to 0.002) were not.

Table 4 shows the multivariable analysis results of the associations between each of the biomarkers and eGFR decline. Even when adding 1 biomarker to the base model at a time, suPAR levels remained associated

Table 1. Clinical characteristics and biomarker levels stratified by estimated glomerular filtration rate

Variables	Entire cohort (n = 3282)	eGFR (ml/min per 1.73 m^2)			*P* value
		>90 (n = 782)[a]	60−89 (n = 1670)[b]	<15−59 (n = 830)[c]	
Age, yr	63±12	55±10	64±10[a]	70±11[a,b]	**<0.001**
Male	2108 (64)	492 (63)	1132 (68)[c]	484 (58)	**<0.001**
African American	604 (18)	217 (28)[b,c]	253 (15)	134 (16)	**<0.001**
Body mass index, kg/m^2	30±6	31±7[b,c]	30±6	29±6	**0.001**
Clinical characteristics					
Smoking history	2165 (66)	494 (63)	1122 (67)	549 (66)	0.15
Hypertension	2366 (72)	515 (66)	1169 (70)	682 (83)[a,b]	**<0.001**
Diabetes mellitus	1013 (31)	222 (28)	471 (28)	320 (39)[a,b]	**<0.001**
Low-density lipoprotein, mg/dl	97±37	103±39)[b,c]	98±36[c]	91±36	**<0.001**
High-density lipoprotein, mg/dl	42±13	42±13	42±12	42±14	0.93
Myocardial infarction history	923 (28)	202 (26)	448 (27)	232 (28)[a,b]	**0.005**
Revascularization history	2039 (63)	427 (55)	1034 (62)	518 (62)[a,b]	**<0.001**
Obstructive coronary artery disease	2149 (69)	439 (60)	1096 (69)	614 (79)[a,b]	**<0.001**
Heart failure	513 (16)	80 (10)	228 (14)	205 (25)[a,b]	**<0.001**
eGFR, ml/min per 1.73 m^2	74±22	101±8[b,c]	75±9	45±14	**<0.001**
Proteinuria ≥1+[d]	109 (8)	13 (5)	41 (6)	55 (7)[a,b]	**<0.001**
ACEi/ARB use	1931 (59)	434 (56)	996 (60)	501 (60)	0.09
Biomarkers					
SuPAR, pg/ml	3019 (2359, 3974)	2610 (2090, 3287)	2853 (2299, 3553)	4070 (3191, 5392)	**<0.001**
Hs-CRP, mg/dl	3.05 (1.2, 7.6)	3.4 (1.3, 7.8)	2.5 (1.1, 6.4)	3.8 (1.5, 9.9)	**<0.001**
FDP, μg/ml	0.54 (0.36, 0.84)	0.46 (0.32, 0.70)	0.52 (0.36, 0.78)	0.68 (0.45, 1.10)	**<0.001**
HSP-70 ≥1	622 (19)	139 (18)	283 (17)	200 (24)[a,b]	**<0.001**
Hs-Tn I, pg/ml	5.4 (2.9, 14.5)	3.8 (2.3, 10.2)	5.1 (2.8, 12.0)	9.1 (1.6, 26.4)	**<0.001**

Obstructive coronary artery disease denotes the presence of at least 50% obstruction in any of the coronary arteries on angiogram.
Values are reported as mean±SD or n (%). Biomarker levels are reported as median (25th, 7th percentiles). Statistically significant values at $P < 0.05$ are highlighted in bold.
ACEi, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; FDP, fibrin degradation product; Hs-CRP, high-sensitivity C-reactive protein; HSP-70, heat shock protein 70; hs-TnI, high sensitivity troponin I; suPAR, soluble urokinase-type plasminogen activator receptor.
[a,b,c]Results of pairwise comparisons using the Bonferroni correction are denoted as follows: for each significant pair, the key of the category ([a], [b], or [c] for each eGFR category) with the smallest value appears in the category with the larger value.
[d]Proteinuria data was available for 1335 patients.

with eGFR decline ($P < 0.001$). Specifically, eGFR was estimated to decrease by 0.42 (95% CI: −0.63 to −0.20) a year per 100% increase in baseline suPAR level, even after adjusting for clinical characteristics and hs-CRP, FDP, HSP-70, and hs-TnI.

DISCUSSION

In this study, we characterized the association between CVD biomarkers and kidney function in a prospective cohort of adults with CVD. Although all 5 biomarkers, suPAR, hs-CRP, FDPs, HSP-70, and hs-TnI, correlated with measures of renal function cross sectionally, only suPAR was associated with future decline in eGFR. The importance of these findings is 2-fold: first, we showed that well-established biomarkers associated with CVD and CKD did not predict future decline in eGFR, which suggested that they were unlikely to be reflective of pathways related to kidney disease, and thus, were not useful in predicting incident renal dysfunction. Second, suPAR, which we previously showed to be predictive of eGFR decline and outcomes in the same cohort, remained associated with incident renal dysfunction, ever after adjusting for clinical characteristics, hs-CRP, FDPs, HSP-70, and hs-TnI, which are all biomarkers that are independently and highly predictive of CVD outcomes.[7,17,29,33] Thus, the relation between suPAR and eGFR decline goes beyond reflecting overall worse clinical status and CVD outcomes.

CKD and CVD are tightly linked and share common risk factors and underlying pathophysiologic mechanisms, including inflammation, oxidative stress, and a pro-coagulant state.[2,3] hs-CRP, as a measure of inflammation, rises significantly with declining renal function, and although it is strongly predictive of adverse CVD outcomes in patients with CKD, the association with incident renal disease has been inconsistent.[24,25,34] In a substudy of the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial, statins reduced CRP levels but did not improve renal outcomes despite better survival.[35] FDPs are markers of hemostasis that were associated with CKD and CVD mortality.[27,36] Previous studies also did not find an association between eGFR decline and FDPs.[37,38] Elevations in HSP-70 typically result as a counter-regulatory mechanism to cellular stress, and are increased in several clinical conditions, including CKD.[26,39] Inhibition of HSP slowed renal parenchymal fibrosis in rats with obstructive nephropathy.[40] Although it was predictive of mortality

Table 2. Independent predictors of glomerular filtration rate and proteinuria at enrollment

	eGFR (ml/min per kg/m^2)			≥+1 Proteinuria		
Variables	**β**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
Model 1: Clinical characteristics						
Age, per 10 yr	**−0.40**	**−8.12** to **−6.65**	**<0.001**	0.86	0.69 to 1.09	0.22
Male	**0.09**	**2.39** to **5.89**	**<0.001**	1.23	0.71 to 2.14	0.46
African American	0.00	−1.99 to 2.21	**0.92**	**2.93**	**1.72** to **4.98**	**<0.001**
Body mass index, per 5 kg/m^2 increase	−0.01	−0.83 to 0.51	0.64	0.98	0.81 to 1.19	0.84
Smoking history	−0.01	−2.10 to 1.21	0.60	0.98	0.58 to 1.67	0.95
Hypertension	**−0.08**	**−5.79** to **−2.20**	**<0.001**	1.74	0.87 to 3.50	0.12
Diabetes mellitus	**−0.04**	**−3.55** to **−0.10**	**0.038**	**3.60**	**2.13** to **6.09**	**<0.001**
Low-density lipoprotein, per 10 mg/dl	**0.07**	**0.18** to **0.61**	**<0.001**	0.99	0.93 to 1.06	0.8
High-density lipoprotein, per 10 mg/dl	**0.04**	**0.04, 1.33**	**0.038**	1.06	0.87 to 1.28	0.59
Myocardial infarction history	0.00	−1.83 to 1.79	0.98	0.71	0.38 to 1.32	0.28
Revascularization history	0.02	−1.72 to 3.08	0.58	0.67	0.35 to 1.29	0.23
Obstructive coronary artery disease	−0.03	−3.91, 1.16	0.29	1.39	0.67 to 2.87	0.38
Heart failure	**−0.12**	**−8.68** to **−4.51**	**<0.001**	**1.90**	**1.10** to **3.31**	**0.023**
ACEi/ARB use	**0.07**	**1.32** to **4.61**	**<0.001**	0.67	0.40 to 1.12	0.12
Model 2−7: Clinical characteristics + individual biomarkers						
SuPAR, per 100% increase	**−13.55**	**−14.83** to **−12.27**	**<0.001**	**3.00**	**2.03** to **4.44**	**<0.001**
Hs-CRP, per 100% increase	**−0.72**	**−1.14** to **−0.30**	**<0.001**	**1.18**	**1.04** to **1.34**	**0.009**
FDP, per 100% increase	**−0.97**	**−1.44** to **−0.51**	**<0.001**	**1.15**	**1.02** to **1.29**	**0.023**
HSP-70 ≥ 1 ng/ml	−1.36	−3.65 to 0.40	0.12	1.71	0.96 to 3.03	0.07
Hs-Tn I, per 100% increase	**−0.77**	**−1.08** to **−0.46**	**<0.001**	**1.12**	**1.04** to **1.21**	**0.004**
Model 8: Clinical characteristics + all biomarkers						
SuPAR, per 100% increase	**−13.57**	**−14.89 to −12.24**	**<0.001**	**2.67**	**1.79** to **3.99**	**<0.001**
Hs-CRP, per 100% increase	0.36	−0.05 to 0.76	0.09	1.04	0.91 to 1.20	0.54
FDP, per 100% increase	**−0.55**	**−0.99** to **−0.11**	**0.015**	1.07	0.94 to 1.23	0.31
HSP-70 > 1 ng/ml	1.36	−0.54 to 3.26	0.16	1.43	0.77 to 2.65	0.26
Hs-Tn I, per 100% increase	**−0.38**	**−0.68** to **−0.08**	**0.014**	**1.10**	**1.01** to **1.20**	**0.038**

Biomarkers were each entered into separate models incorporating demographics and risk factors. The estimate, OR, and CI reported for the demographics and clinical characteristics are derived from the model not incorporating any biomarkers. Statistically significant values at $P < 0.05$ are highlighted in bold.
ACEi, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CI, confidence interval; eGFR, estimated glomerular filtration rate; FDP, fibrin degradation product; Hs-CRP, high-sensitivity C-reactive protein; HSP-70, heat shock protein 70; hs-TnI, high-sensitivity troponin I; OR, odds ratio; suPAR, soluble urokinase-type plasminogen activator receptor.

and CVD outcomes, we were the first to show that elevation in HSP-70 was not associated with future eGFR decline in humans.[29] Similarly, although hs-TnI levels correlated with both eGFR and proteinuria and were associated with adverse outcomes in both patients with and without CKD, we found that its levels did not predict eGFR decline.[28] Various additional markers of inflammation, such as interleukin-6 and intercellular adhesion molecule-1 were also found not to be predictive of eGFR decline.[37,38] These findings suggested that conventional markers of inflammation that are typically associated with the atherosclerotic process and CVD outcomes might not represent a major driver of kidney disease progression. Increased production, decreased renal clearance, or a combination of both mechanisms likely contributed to elevations of the aforementioned biomarkers, including suPAR, in renal insufficiency.[12,41–43]

The association between suPAR and kidney disease was first described in focal segmental glomerulosclerosis.[13] Although the debate is still ongoing as to whether suPAR is merely a biomarker of the disease rather than a causative agent in humans,[12] there is increasing evidence from mouse models that overexpress certain forms of suPAR, that a direct pathological effect, induced by binding and activation of

Table 3. Spearman-Rank correlations between biomarkers and estimated glomerular filtration rate

	eGFR		SuPAR		Hs-CRP		FDP		HSP-70		Hs-TnI	
Characteristics	**R**	***P* value**	**r**	***P* value**	**r**	***P* value**	**R**	***P* value**	**r**	***P* value**	**r**	***P* value**
SuPAR	**−0.42**	**<0.001**			**0.27**	**<0.001**	**0.28**	**<0.001**	**0.17**	**<0.001**	**0.26**	**<0.001**
Hs-CRP	**−0.05**	**0.003**	**0.27**	**<0.001**			**0.22**	**<0.001**	**0.07**	**<0.001**	**0.20**	**<0.001**
FDP	**−0.23**	**<0.001**	**0.28**	**<0.001**	**0.22**	**<0.001**			**0.19,**	**<0.001**	**0.24**	**<0.001**
HSP-70	**−0.07**	**<0.001**	**0.17**	**<0.001**	**0.07**	**<0.001**	**0.19**	**<0.001**			0.04	0.30
Hs-TnI	**−0.24**	**<0.001**	**0.26**	**<0.001**	**0.20**	**<0.001**	**0.24**	**<0.001**	0.04, 0.30	0.30		

Statistically significant values at $P < 0.05$ are highlighted in bold.
FDP, fibrin degradation product; Hs-CRP, high-sensitivity C-reactive protein; HSP-70, heat shock protein 70; hs-TnI, high sensitivity troponin I; suPAR, soluble urokinase-type plasminogen activator receptor.

Table 4. Independent predictors of glomerular filtration rate decline

	eGFR (ml/min per kg/m^2)		
Variables	**β**	**95% CI**	***P* value**
Model 1: Clinical characteristics			
Age, per 10 yr	**−0.89**	**−1.77** to **−0.01**	**0.050**
Male	0.21	−2.02 to 2.44	0.85
African American	−1.24	−4.64 to 2.16	0.48
Body mass index, per 5 kg/m^2 increase	0.31	−0.52 to 1.13	0.47
Smoking history	0.36	−1.58 to 2.30	0.72
Hypertension	−0.32	−2.65 to 2.02	0.79
Diabetes mellitus	**−3.87**	**−5.72** to **−2.01**	**<0.001**
Low-density lipoprotein, per 10 mg/dl	−0.03	−0.28 to 0.23	0.84
High-density lipoprotein, per 10 mg/dl	0.46	−0.38 to 1.30	0.29
Myocardial infarction history	−0.93	−2.87 to 1.00	0.35
Revascularization history	**2.28**	**0.06** to **4.49**	**0.040**
Obstructive coronary artery disease	−1.00	−3.81 to 1.80	0.48
Heart failure	**−3.89**	**−5.91** to **−1.87**	**0.002**
ACEi/ARB use	**2.57**	**0.75** to **4.40**	**0.01**
Baseline eGFR, per 10 ml/min per 1.73 m^2	**7.18**	**6.73** to **7.64**	**<0.001**
Follow-up time, per year	**−1.25**	**−1.46** to **−1.04**	**<0.001**
Model 2−6: Clinical characteristics + individual biomarkers			
SuPAR, per 100% increase×follow-up time	**−0.46**	**−0.84** to **−0.08**	**0.02**
Hs-CRP, per 100% increase×follow-up time	−0.04	−0.15 to 0.07	0.46
FDP, per 100% increase×follow-up time	−0.13	−0.27 to 0.01	0.08
HSP-70 > 1 ng/ml×follow-up time	0.05	−0.47 to 0.58	0.84
Hs-TnI, per 100% increase×follow-up time	−0.01	−0.09 to 0.06	0.76
Model 7: Clinical characteristics + suPAR adjusting for other biomarker levels			
SuPAR, per 100% increase×follow-up time	**−0.44**	**−0.83** to **−0.07**	**0.02**

Biomarkers were each entered into separate models incorporating demographics and risk factors. The estimate and CIs reported for the demographics and clinical characteristics are derived from the model not incorporating any biomarkers. Statistically significant values at $P < 0.05$ are highlighted in bold.
ACEi, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CI, confidence interval; eGFR, estimated glomerular filtration rate; FDP, fibrin degradation product; Hs-CRP, high-sensitivity C-reactive protein; HSP-70, heat shock protein 70; hs-TnI, high-sensitivity troponin I; suPAR, soluble urokinase-type plasminogen activator receptor.

podocyte αvβ3 integrin, in turn leads to activation of the small GTPase Ras-related C3 botulinum toxin substrate 1. Subsequently, podocyte effacement and proteinuric disease is responsible.[9,11,44] Recently, we showed in this cohort that suPAR levels were highly predictive of decline in kidney function and incident CKD, even in patients with normal kidney function at baseline.[7] The role of suPAR in renal disease thus appears to go beyond focal segmental glomerulosclerosis, although it might be related to different pathophysiologic mechanisms involving its different isoforms and potentially both its proteolytic and signaling functions.[8,13,15,44,45] Further studies are needed in humans to elucidate these mechanisms and identify potential therapies modulating the suPAR pathway.

Our study was strengthened by the large sample size and a well-characterized cohort with long follow-up duration and availability of multiple eGFR measurements. Unfortunately, follow-up proteinuria data were lacking, and specific diagnoses of kidney disease were not available. Thus, although we did not identify an association between hs-TnI, hs-CRP, FDPs, and HSP-70 and a decline in renal function, we were unable to make definite conclusions on associations with specific kidney diseases nor exclude confounding by the occurrence of contrast-induced nephropathy. Moreover, the cohort consisted of a highly select population with CVD that underwent cardiac catheterization; therefore, conclusions could not be generalized. Nevertheless, the present study complemented our previous finding of the association of suPAR with incident kidney disease, and highlighted that the association is independent of other markers of inflammation and CVD.

DISCLOSURE

AAQ is equity holder in GenWay Biotech and received consulting fees. SSH, YK, and AAQ had full access to the data and take responsibility for the integrity and accuracy of the data analysis. CW has a pending patent application on suPAR in diabetic kidney disease. JR and SS are cofounders of TRISAQ, a biopharmaceutical company aimed to develop new therapies for kidney disease. They stand to gain royalties from commercialization of these therapies. All the other authors declared no competing interests.

ACKNOWLEDGMENTS

We would like to acknowledge the members of the Emory Biobank Team, Emory Clinical Cardiovascular Research Institute (ECCRI), and Atlanta Clinical and Translational Science Institute for recruitment of participants, compilation of data, and preparation of samples.

AAQ is supported by grants 5P01HL101398-02, 1P20HL113451-01, 1R56HL126558-01, 1RF1AG051633-01, R01 NS064162-01, R01 HL89650-01, HL095479-01, 1U10HL110302-01, 1DP3DK094346-01, 2P01HL086773-06A1. J.R. and S.S. were supported by grant 5R01DK101350-03. SSH is supported by the Abraham J. & Phyllis Katz Foundation (Atlanta, GA). Funding for collection and management of samples was received from the Robert W. Woodruff Health Sciences Center Fund (Atlanta, GA), Emory Heart and Vascular Center (Atlanta, GA), Katz Family Foundation Preventive Cardiology Grant (Atlanta, GA), and National Institutes of Health (NIH) Grants UL1 RR025008 from the Clinical and Translational Science Award program. suPAR sample kits were provided by ViroGates (Denmark). hs-CRP, FDP, and HSP-70 measurements were conducted by FirstMark, Division of GenWay Biotech Inc (San Diego, CA). hs-TnI was measured by Abbott Laboratories (Abbott Park, IL).

SUPPLEMENTARY MATERIAL

Figure S1. Association between soluble urokinase-type plasminogen activator receptor (suPAR), high-sensitivity C-reactive protein (hs-CRP), heat shock protein 70 (HSP-70), fibrin-degradation products (FDPs), and high-sensitivity troponin I (hs-TnI) plasma levels and change in estimated glomerular filtration rate (eGFR0 during follow-up in patients with at least 1 additional measure of eGFR.

Supplementary material is linked to the online version of the paper at http://www.kireports.org.

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