Circulating Proteins and Mortality in CKD: A Proteomics Study of the AASK and ARIC Cohorts

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Rationale & Objective: Proteomics could provide pathophysiologic insight into the increased risk of mortality in patients with chronic kidney disease (CKD). This study aimed to investigate associations between the circulating proteome and all-cause mortality among patients with CKD.

Study Design: Observational cohort study.

Setting & Participants: Primary analysis in 703 participants in the African American Study of Kidney Disease and Hypertension (AASK) and validation in 1,628 participants with CKD in the Atherosclerosis Risk in Communities (ARIC) study who attended visit 5.

Exposure: Circulating proteins.

Outcome: All-cause mortality.

Analytical Approach: Among AASK participants, we evaluated the associations of 6,790 circulating proteins with all-cause mortality using multivariable Cox proportional hazards models. Proteins with significant associations were further studied in ARIC Visit 5 participants with CKD.

Results: In the AASK cohort, the mean age was 54.5 years, 271 (38.5%) were women, and the mean measured glomerular filtration rate (GFR) was 46 mL/min/1.73 m^2. The median follow-up was 9.6 years, and 7 distinct proteins were associated with all-cause mortality at the Bonferroni-level threshold ($P < 0.05$ of the 6,790) after adjustment for demographics and clinical factors, including baseline measured estimated GFR and proteinuria. In the ARIC visit 5 cohort, the mean age was 77.2 years, 903 (55.5%) were women, the mean estimated GFR was 54 mL/min/1.73 m^2 and median follow-up was 6.9 years. Of the 7 proteins found in AASK, 3 (β_2-microglobulin, spondin-1, and N-terminal pro-brain natriuretic peptide) were available in the ARIC data, with all 3 significantly associated with death in ARIC.

Limitations: Possibility of unmeasured confounding. Cause of death was not known.

Conclusions: Using large-scale proteomic analysis, proteins were reproducibly associated with mortality in 2 cohorts of participants with CKD.

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Chronic kidney disease (CKD) is a major public health concern around the world with a prevalence of 9% globally and 15% in the United States.[1,2] Patients with CKD are at a high risk of premature death, and the mortality risk attributable to CKD increased by 41.5% globally between 1990 and 2017.[1] Among patients with kidney disease, various pathophysiological processes may contribute to the elevated risk of death. Knowledge of the underlying mechanisms and identification of circulating biomarkers reflecting these processes may allow for enhanced risk stratification, prognostication, and targeted strategies of prevention or early intervention.

Aptamer-based technologies are increasingly used to discover biomarkers. For example, the slow off-rate modified aptamers (SOMAmers) scan can identify proteins through simultaneous, highly multiplexed quantitative measurements of a vast range of protein targets using small sample volumes.[3] Studies have used this technology to evaluate the plasma proteome and the risk of CKD progression and incident cardiovascular disease.[4,5] The few studies that have examined the relationship between the proteome and death in various populations—general population, participants with CKD, kidney failure, or dialysis-dependent acute kidney injury (AKI)—have discovered that biomarkers of inflammation, increased coagulation, and endothelial injury are associated with early mortality.[6-10]

To gain a better understanding of the pathophysiological processes associated with increased mortality in CKD, we used proteomic profiling to investigate associations between circulating proteins and the risk of mortality in a cohort of patients with CKD from the African American Study of Kidney Disease and Hypertension (AASK). We then replicated our findings among participants with CKD in an independent, population-based cohort.

METHODS

Study Population

For the primary discovery analysis, associations were evaluated in the AASK, which consisted of a trial and a cohort phase. The AASK trial was a multicenter, randomized, and 3×2 factorial trial from February 1995 to September 2001, with the goal of investigating different blood pressure (BP) goals and BP medications on progression of kidney disease in African American adults with CKD.[11] Participants aged 18-70 years with a diastolic BP of >95mmHg and a measured glomerular filtration rate (GFR) of 20-65mL/min/1.73 m^2 were included. Excluded participants were those with a history of diabetes mellitus,

PLAIN-LANGUAGE SUMMARY

Patients with chronic kidney disease (CKD) have a high risk of premature death, with various pathophysiological processes contributing to this increased risk of mortality. This observational cohort study aimed to investigate the associations between circulating proteins and all-cause mortality in patients with CKD using large-scale proteomic analysis. The study analyzed data from the African American Study of Kidney Disease and Hypertension (AASK) study and validated the findings in the Atherosclerosis Risk in Communities (ARIC) Study. A total of 6,790 circulating proteins were evaluated in AASK, and 7 proteins were significantly associated with all-cause mortality. Three of these proteins (β_2-microglobulin, spondin-1, and N-terminal pro-brain natriuretic peptide (BNP)) were also measured in ARIC and were significantly associated with death. Additional studies assessing biomarkers associated with mortality among patients with CKD are needed to evaluate their use in clinical practice.

urine protein to creatinine ratio (UPCR) of >25 mg/g, malignant or secondary hypertension, serious systemic disease, congestive heart failure, or a contraindication to the study drugs. Participants who did not reach kidney failure at the end of the trial were invited to participate in the AASK cohort study, which ran from April 2002 to June 2007.[12] During this second phase, all participants were placed on ramipril with a target BP of <140/90mmHg (<130/80mmHg after 2004). Participants with available proteomic profiling and non-missing covariates at the baseline visit (n=703) were included in the current study.

For validation, associations were evaluated in the Atherosclerosis Risk in Communities (ARIC) Study, which is an ongoing prospective cohort with 15,792 participants (ages 45-64 years) recruited from 4 US communities (Minneapolis, MN; Forsyth County, NC; Washington County, MD; and Jackson, MS).[13] The first study visit occurred in 1987-1989 with 7 follow-up visits to date. Participants who attended visit 5 (2011-2013), did not have kidney failure, with non-missing covariates, available proteomic data, and CKD ($eGFR_{Cr-Cys}$ <60 mL/min/1.73 m^2 or UACR of >30 mg/g) were included (n=1,628). The institutional review boards of each participating center approved the study protocols for both cohorts, and all participants provided written informed consent.

Circulating Protein Measurements

Proteomic profiling was performed on blood samples collected from participants at the baseline visit of AASK and Visit 5 of ARIC. The AASK serum samples were collected using serum separator tubes and frozen at −70 °C, and the ARIC blood samples were collected in EDTA tubes, centrifuged, and the resulting plasma was frozen at −80 °C. SOMAscan (SomaLogic, Inc) was used for measurements, with the v4 platform (∼5,000 proteins) in 2018 for ARIC plasma samples and the v4.1 platform (∼7,000 proteins) in 2021 for AASK serum samples. In AASK, only proteins with a Bland Altman coefficient of variation (BACV) of <50% were included, leaving 6,790 proteins in 18 blind duplicate samples with a mean BACV of < 5.1%. The %. BACV is calculated as the standard deviation of each blind duplicate pair divided by the mean of the pair, averaged over all blind duplicate pairs. In ARIC (visit 5), the mean BACV from 5,284 proteins from 26 samples in blind triplicate was 6.6%. Briefly, SOMAscan assay relies on SOMAmers, which are highly selective single-stranded deoxyoligonucleotides that bind to proteins or protein complexes with high affinity.[3,14] Proteins are quantified based on their fluorescence intensity, expressed in relative fluorescence units, and reflect relative rather than absolute protein concentrations. Data quality control, normalization, and calibration were performed as previously described.[15] For this study, 6,790 proteins that passed quality control in AASK were included for analysis. In ARIC, we only evaluated those proteins that were significantly associated with AASK and available on the v4 platform.

Outcomes and Covariates

The primary study outcome was all-cause mortality, which was determined in AASK through active follow-up during the trial and cohort phases. Time to death was defined from the time of the baseline study visit until the event or administrative censoring. Covariates, which were assessed at the baseline visit, included age, sex, group of trial assignment, systolic blood pressure (SBP), history of atherosclerotic cardiovascular disease (ASCVD), smoking status (current smoker, former smoker, and never smoker), measured GFR, high-density lipoprotein (HDL), total cholesterol, UPCR, and body mass index (BMI). History of ASCVD and smoking was self-reported. SBP was measured in a standardized fashion using the Tycos classic handheld aneroid device. The average of 2 BP measurements was taken as the systolic BP. The UPCR was obtained using one 24-hour urine collection. The GFR was ascertained through direct measurement of kidney ^{125}I-iothalamate clearance and was the average of 2 measurements.

In ARIC, covariates included age, sex, self-reported race or ethnicity, BMI, SBP, HDL, total cholesterol, UACR, smoking status, history of ASCVD, diabetes (fasting glucose of ≥126 mg/dL or non-fasting glucose level of ≥200 mg/dL, self-reported history of diabetes diagnosed by a physician, or use of medications for diabetes or high blood sugar), hypertension (SBP ≥140 mm Hg and DBP ≥90 mm Hg, or use of medication for high BP), and eGFR (calculated using the CKD-EPI 2021 equation, which takes both serum creatinine and cystatin C into account).[16] BP was measured with an automatic sphygmomanometer in visit 5 by a certified trained technician. Three BP measurements were obtained and the reported BP was an average of the second and third measurements. Serum

creatinine was measured using a Roche enzymatic method (visit 5). Serum cystatin C was measured using the Roche Cobas 6000 chemistry analyzer. The UACR was calculated using urine albumin (measured using an immunoturbidometric method on the ProSpec nephelometric analyzer) and urine creatinine (measured using the Roche enzymatic method).

Statistical Analysis

Baseline characteristics of the study population were described using the mean and standard deviation (SD) for variables with approximate normal distributions and number (percentage) for categorical variables. All proteins were $\log_2$-transformed to normalize distributions, and outliers outside of 5 SDs were winsorized. In our discovery analysis in AASK, Cox proportional hazards models were used to evaluate the association between each protein measured at the baseline visit and the risk of death. Two primary adjustment models were used: a minimally adjusted Model 1 accounting for age, sex, and trial group assignment; and a fully adjusted Model 2 additionally accounting for SBP, history of ASCVD, smoking, GFR, BMI, total cholesterol, HDL, and log-transformed UPCR. Bonferroni adjustment was used to account for multiple comparisons with a threshold of 7.36×10^{-6} (0.05 of the6,790) to assess statistical significance. For validation, associations among the top protein hits in AASK were then evaluated in ARIC. Similar sequential regression models were constructed in ARIC to relate each protein to the risk of death. Model 1 adjusted for age, sex, and race-study center; Model 2 adjusted for prevalent cardiovascular disease, hypertension, diabetes, smoking, SBP, BMI, total cholesterol, HDL, eGFRCr-Cys, and log-transformed UACR. Follow-up time was determined from baseline until the event or administrative censoring (end of study date for AASK and December 31, 2019 for ARIC). The threshold for statistical significance reflected the number of protein lookups. We tested for interactions in ARIC by presence or absence of hypertension, diabetes, and ASCVD by including a product term of protein level and comorbidity. All analyses were performed using STATA version 17 (StataCorp, College Station).

RESULTS

Participant Characteristics

The AASK cohort included 703 participants with a mean age of 54.5 years, 271 (38.5%) were female, all 703 (100%) were Black, the mean SBP was 151 mm Hg, median UPCR was 81 mg/g, and mean baseline measured GFR was 46 mL/min/1.73 m^2 (Table 1). Among all participants, 358 participants (50.9%) reported a history of cardiovascular disease, 205 (29.2%) were current smokers, and the mean BMI was 30.5 kg/m^2. The ARIC Visit 5 cohort included 1,628 participants with a mean age of 77.2 years, 903 (55.5%) were female, 321 (19.7%) were Black, the mean SBP was 132 mm Hg, median UACR was 26 mg/g, and mean eGFR was 54 mL/min/1.73 m^2 (Table 1). A total of 405 participants (24.9%) reported a history of cardiovascular disease, 1,359 (83.5%) had hypertension, 648 (39.8%) had diabetes, 104 (6.4%) were current smokers, and the mean BMI was 29.3 kg/m^2.

Table 1. Characteristics of Participants From the AASK Cohort and Those With CKD in the ARIC Visit 5 (2011-2013) Cohort Study

Characteristic	AASK	ARIC Visit 5
n	703	1,628
Age, y	54.5 (10.7)	77.2 (5.4)
Female Sex, %	271 (38.5)	903 (55.5)
Self-reported Black race, %	703 (100)	321 (19.7)
History of ASCVD, %	358 (50.9)	405 (24.9)
Antihypertensive medication, %	703 (100)	1,259 (77.3)
Hypertension, %	703 (100)	1,359 (83.5)
Diabetes, %	0 (0)	648 (39.8)
Systolic blood pressure, mm Hg	151.1 (24.8)	132.1 (19.5)
Body mass index, kg/m^2	30.5 (6.4)	29.3 (5.9)
GFR[a], mL/min /1.73 m^2	45.7 (13.0)	53.8 (16.7)
Albuminuria[b], mg/g	81.4 (27.75-372.8)	26.0 (8.5-62.3)
Total cholesterol, mg/dL	211.9 (45.7)	174.0 (42.5)
HDL, mg/dL	48.3 (16.0)	50.3 (15.5)
Current smoker, %	205 (29.2)	104 (6.4)
Former Smoker, %	212 (30.2)	857 (52.6)
Follow-up variables		
Number of events, %	150 (21.3)	572 (35.1)
Median follow-up time, y	9.6 (6.7-11.0)	6.9 (5.2-7.7)

Note: Mean ± SD or median (25th percentile-75th percentile) was obtained for continuous variables and n (%) was obtained for categorical variables.
Abbreviations: AASK, African American study of kidney disease and hypertension; ARIC, Atherosclerosis Risk in Communities; ASCVD, atherosclerotic cardiovascular disease; GFR, glomerular filtration rate; HDL, high-density lipoprotein
[a]Iothalamate measured in AASK and calculated in ARIC using 2021 CKD-Epi equation.
[b]Urine protein to creatinine ratio in AASK and urine albumin to creatinine ratio in ARIC.

Discovery in AASK

In total, there were 150 deaths over a median follow-up time of 9.6 years in the AASK cohort. A total of 7 proteins were significantly associated with death at the Bonferroni threshold ($P < 0.05$ of the 6790) in the fully adjusted model with all exhibiting a higher risk of death (Fig 1; Table 2). These proteins were lamin-B2 (HR, 1.64; 95% CI, 1.42-1.89), spondin-1 (HR, 1.50; 95% CI, 1.29-1.75), somatostatin receptor type 1 (HR, 1.37; 95% CI,

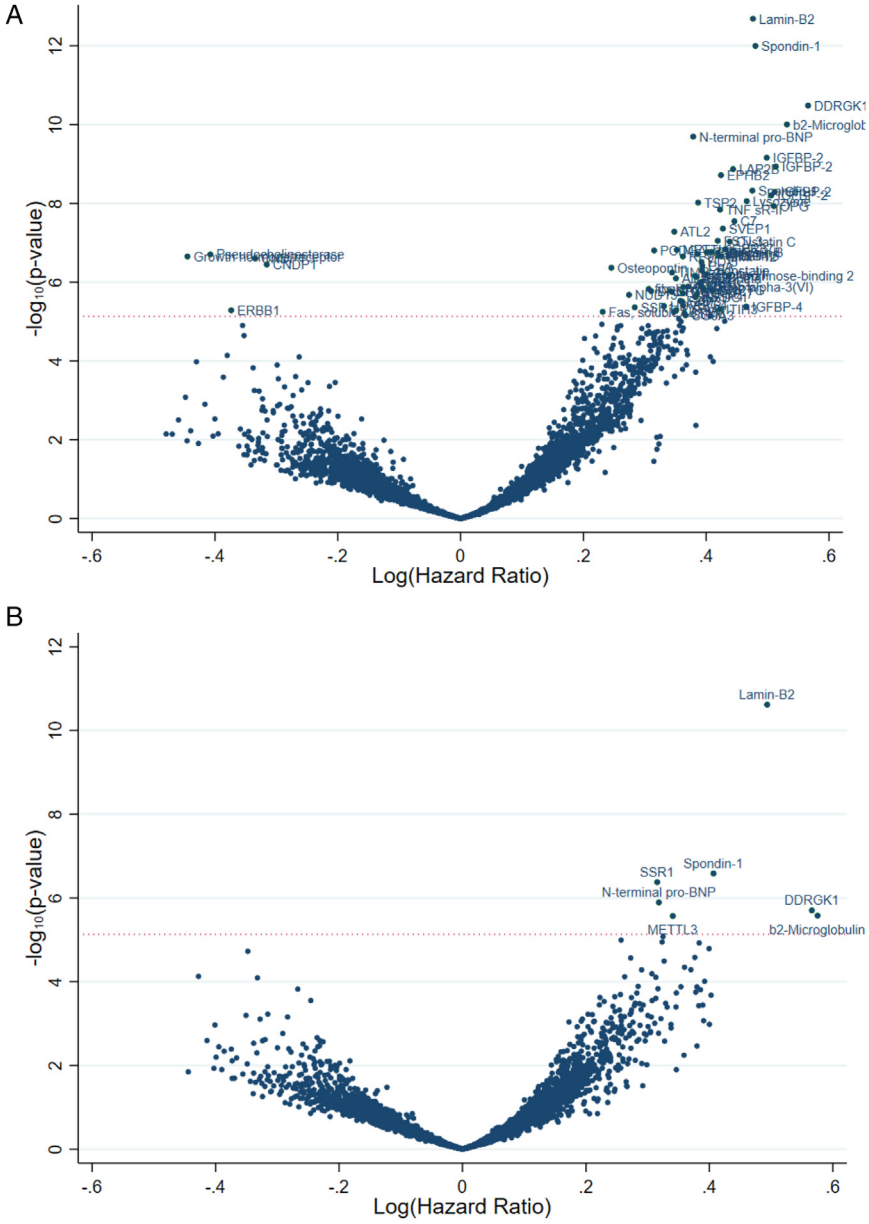

Figure 1. Volcano plots of proteins associated with death in AASK. (A) Model 1. (B) Model 2. SBP, systolic blood pressure; ASCVD, atherosclerotic cardiovascular disease; GFR, glomerular filtration rate; HDL, high-density lipoprotein; UPCR, urine protein to creatine ratio. [a]Model 1: age, sex, and trial group assignment. Model 2: additionally, adjusted for SBP, history of ASCVD, smoking, measured GFR, Total cholesterol, HDL, and log-transformed UPCR. [b]Proteins with statistically significant associations are labeled in the volcano plot. Dotted line reflects Bonferroni threshold.

1.21-1.55), N-terminal pro-BNP (HR, 1.37; 95% CI, 1.21-1.56), DDRGK domain-containing protein 1 (HR, 1.76; 95% CI, 1.39-2.22), β_2-microglobulin (HR, 1.78; 95% CI, 1.40-2.26) and N6-adenosine-methyltransferase 70 kDa subunit (HR, 1.41; 95% CI, 1.22-1.62).

Validation in ARIC Visit 5

Overall, there were 572 deaths over a median follow-up time of 6.9 years in the ARIC validation cohort. Out of the 7 proteins identified in AASK, 3 were available on the v4 platform in ARIC, and all 3 of these proteins were significantly associated with death (Table 2). These proteins were spondin-1 (HR, 1.33; 95% CI, 1.24-1.43), N-terminal pro-BNP (HR, 1.16; 95% CI, 1.10-1.22), and β_2-microglobulin (HR, 1.46; 95% CI, 1.24-1.72). Magnitudes of associations were similar to those in AASK. All proteins remained positively associated with increased risk of death regardless of hypertension, diabetes, or ASCVD status (Table 3). In ARIC, risk of mortality with spondin-1 was significantly higher in participants with

Table 2. Proteins Associated With Death in AASK Cohort and ARIC Visit 5

			AASK Cohort		ARIC Visit 5	
UniProt ID	Symbol	Protein Name	HR (95% CI)[a]	*P*	HR (95% CI)[b]	*P*
Q03252	Lamin-B2	Lamin-B2	1.64 (1.42-1.89)	< 0.001	NA	NA
Q9HCB6	Spondin-1	Spondin-1	1.50 (1.29-1.75)	< 0.001	1.33 (1.24-1.43)	< 0.001
P30872	SSR1	Somatostatin receptor type 1	1.37 (1.21-1.55)	< 0.001	NA	NA
P16860	N-terminal pro-BNP	N-terminal pro-BNP	1.37 (1.21-1.56)	< 0.001	1.16 (1.10-1.22)	< 0.001
Q96HY6	DDRGK1	DDRGK domain-containing protein 1	1.76 (1.39-2.22)	< 0.001	NA	NA
P61769	B2M	β_2-microglobulin	1.78 (1.40-2.26)	< 0.001	1.46 (1.24-1.72)	< 0.001
Q86U44	METTL3	N6-adenosine-methyltransferase 70 kDa subunit	1.41 (1.22-1.62)	< 0.001	NA	NA

Abbreviation: AASK, African American study of kidney disease and hypertension; ARIC, Atherosclerosis Risk in Communities; HR, hazard ratio; NA, not available
[a]AASK fully adjusted model. Adjusted for age, sex and trial assignment group, systolic blood pressure, history of ASCVD, smoking, measured GFR, total cholesterol, HDL, and log-transformed UPCR. Bonferroni threshold of $P <$ of 7.36 × 10-6 (0.05/6790) was applied to identify statistical significance.
[b]ARIC fully adjusted model. Adjusted for age, sex, race-study center, systolic blood pressure, history of ASCVD, smoking, GFR, hypertension, diabetes, total cholesterol, HDL, and log-transformed urine albumin creatinine ratio.

diabetes than those without diabetes ($P > 0.03$ for interaction) and in participants with ASCVD than those without ASCVD ($P > 0.05$ for interaction).

Discussion

In this study of patients with CKD, using one of the largest available platforms, we identified 7 proteins robustly associated with mortality among African American adults with CKD attributed to hypertension after adjustment for kidney function and albuminuria. Furthermore, we successfully replicated the associations for 3 proteins that were available in a separate cohort of patients with CKD. Some of the proteins, such as β_2-microglobulin and N-terminal pro-BNP, have been extensively studied and are well-known biomarkers of adverse outcomes in CKD, providing proof of concept for the approach. Others, such as spondin-1, DDRGK domain-containing protein 1, and N6-adenosine-methyltransferase 70 kDa subunit (the latter 2 of which were available in only one cohort), are lesser known and could provide further insight into the pathophysiological processes leading to mortality in this population.

Beta-2-microglobulin is a light chain subunit of major histocompatibility (MHC) class I molecule that is expressed on the surface of all nucleated cells. Previous work has showed that β_2-microglobulin accumulation occurs as kidney function declines, and higher serum β_2-microglobulin levels are independently associated with increased risk of cardiovascular disease events and mortality in both dialysis and non–dialysis-dependent CKD.[17-22] N-terminal pro-BNP is a 76 amino acid prohormone which, when cleaved, releases BNP. Studies have shown this marker to be independently associated with all-cause mortality in both the general population and those with cardiovascular disease.[23,24] N-terminal pro-BNP was also identified in a cluster of proteins and metabolites that were jointly associated with cardiovascular disease mortality and was particularly strongly

Table 3. Subgroup Analysis of Proteins Associated With Death in ARIC Visit 5 Stratified by Presence and Absence of Hypertension, Diabetes, and ASCVD.

	Proteins					
	Spondin-1		N-Terminal Pro-BNP		β_2-Microglobulin	
Subgroup	HR (95% CI)	*P* for Interaction	HR (95% CI)	*P* for Interaction	HR (95% CI)	*P* for Interaction
Hypertension		0.63	NA	0.30	NA	0.73
No	1.26 (1.03-1.54)	NA	1.10 (0.96-1.26)	NA	1.67 (1.00-2.80)	NA
Yes	1.35 (1.25-1.46)	NA	1.19 (1.12-1.25)	NA	1.54 (1.29-1.84)	NA
Diabetes	NA	0.03	NA	0.89	NA	0.10
No	1.27 (1.16-1.39)	NA	1.17 (1.10-1.25)	NA	1.39 (1.11-1.73)	NA
Yes	1.53 (1.34-1.75)	NA	1.17 (1.08-1.25)	NA	1.88 (1.44-2.44)	NA
ASCVD	NA	0.05	NA	0.42	NA	0.50
No	1.29 (1.18-1.40)	NA	1.18 (1.11-1.25)	NA	1.61 (1.29-2.02)	NA
Yes	1.52 (1.31-1.77)	NA	1.12 (1.03-1.22)	NA	1.42 (1.10-1.83)	NA

Abbreviation: ASCVD, atherosclerotic cardiovascular disease; NA, not available

linked to the protein Sushi, von Willebrand factor type A, EGF, and pentraxin domain-containing 1 (SVEP1).[25] Overall, our study adds to the existing literature by providing further evidence on the potential use of these 2 markers for future prognostication in the setting of CKD.

Although β_2-microglobulin and N-terminal pro-BNP are well-known predictors of poor outcomes, this study discovered several novel biomarkers that are all independently associated with increased mortality. The notable proteins (spondin-1, DDRGK domain-containing protein 1, and N6-adenosine-methyltransferase 70 kDa subunit) are involved in biochemical processes such as apoptosis, angiogenesis, inflammation, and thrombosis that are important to highlight in the context of CKD. Spondin-1 is an extracellular matrix cell adhesion glycoprotein of the thrombospondin family and has been shown to have prognostic value for heart failure. More specifically, higher spondin-1 levels were associated with an increased risk of heart failure hospitalization among both CKD and non-CKD participants in a proteomic analysis.[26] Spondin-1 was also significantly associated with an increased risk of cardiovascular events (heart failure hospitalization, stroke, myocardial infarction, and sudden cardiac death) among patients with coronary artery disease and heart failure with reduced ejection.[27] Increases in plasma spondin-1 levels have been shown to precede adverse cardiac outcomes and have been associated with cardiovascular mortality particularly in patients with coronary heart disease.[28,29] Murine models have suggested that higher spondin-1 protein expression in developing kidney vasculature could potentially contribute to hypertension in rats, with the postulation that spondin-1 may regulate BP by modulating vascular smooth muscle signaling.[30] This study further highlights the increased risk of mortality associated with high spondin-1 levels among the population with CKD. In addition, a higher risk was noted among those with diabetes and ASCVD, suggesting that diabetes and ASCVD independently may modify the association between spondin-1 and mortality.

Epigenetic modifications play a role in the development of many disease processes, with the most common post-transcriptional modification being methylation at the N6-methyladenosine (m6A) by a methyltransferase complex consisting of an m6A-METTL complex (MAC) and an m6A-METTL associated complex.[31] The MAC includes the N6 adenosine methyltransferase 70 kDa catalytic subunit (*METTL3* or *MTA70*) and the noncatalytic methyltransferase 14 (*METTL14*). *METTL3* modulates various cellular processes and plays a role in cancer, inflammatory diseases, metabolic diseases, ischemic heart disease, and angiogenesis. A growing body of evidence suggests that m6A methylation is a dynamic and reversible event involved in kidney injury and repair. Recent work in mouse models has shown increased *METTL3* expression in response to various acute kidney injury (AKI) stimuli, including tumor necrosis factor-α and lipopolysaccharide, suggesting that *METTL3* promotes renal inflammation and injury.[32] *METTL3* has been shown to positively regulate *MALAT1*, which plays an important role in transforming growth factor-β1 induced renal fibrosis in obstructive nephropathy through the miR-145/FAK pathway.[33] *METTL3* has been found to be upregulated across both mouse and human autosomal dominant polycystic kidney disease models, with increased *METTL3* levels linked to cyst growth.[34] Outside of the kidney, numerous studies have demonstrated that *METTL3* levels are elevated in cardiovascular disease, including cardiac hypertrophy and myocardial infarction.[35] *METTL3* inhibitors are currently under investigation for cancer treatment, though further research is needed to understand the role of their inhibition in patients with AKI and CKD.

DDRGK domain-containing protein 1 (DDRGK1) is encoded by the *DDRGK1* gene and regulates nuclear factor -κB activity. It also interacts with components of the ubiquitin fold modifier 1 conjugation pathway and helps prevent apoptosis in endoplasmic reticulum stressed secretory tissues.[36] The protein has been found to be widely expressed in the central nervous system, liver, heart, lumen of kidney tubules, and urinary tract. DDRGK1 has been detected in the urine of 6 patients in the intensive care unit who reported pre-existing kidney disease and AKI requiring kidney replacement therapy, out of whom 2 had died.[37] Higher urinary levels of DDRGK1 were found in 10 patients with AKI requiring kidney replacement therapy, of whom 9 had died, raising the possibility that its secretion plays a role in predicting organ failure. Thus, our study extends the literature by illustrating a significant association between DDRGK1 and an increased risk of mortality.

The findings of this study should be interpreted in the context of its strengths and limitations. Strengths of this study include the longitudinal study design, large sample sizes, replication in an independent cohort, and the use of advanced proteomic profiling technology with the inclusion of a large number of protein biomarkers in the analyses. In addition, GFR was measured in AASK, which allowed us to assess whether the associations were independent of the gold standard measurement for kidney function. There are a few limitations that are important to note. Proteins were measured at a single time point, and not all identified proteins were available in the second cohort, which used an earlier version of the proteomics platform. Furthermore, the discovery was limited to the 6,790 proteins present in the v4.1 platform which does not represent all human circulating proteins. Moreover, cause of death was not known. The AASK trial enrolled primarily self-identified Black adults with CKD with mild to moderately increased albuminuria that was attributed because of hypertension, and excluded adults with diabetes, limiting the generalizability of our findings to other populations with CKD. However, the replication of all available proteins in a second cohort allowed us to provide additional support to our findings.

In conclusion, high-throughput proteomic profiling identified 7 circulating proteins independently associated

with mortality in a CKD cohort. Out of these 7 proteins, 3 were available in an external CKD cohort, and all 3 showed significant associations with death. Future studies are warranted to evaluate the biomarkers identified in this study for their clinical use in risk prediction purposes.

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REFERENCES

1. Bikbov B, Purcell CA, Levey AS. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2020;395(10225):709-733. doi:10.1016/S0140-6736(20)30045-3
2. Chronic kidney disease in the United States, 2023. Centers for Disease Control and prevention. Accessed January 24, 2023. https://www.cdc.gov/kidneydisease/publications-resources/ckd-national-facts.html
3. Gold L, Ayers D, Bertino J, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One*. 2010;5(12):e15004. doi:10.1371/journal.pone.0015004
4. Grams ME, Surapaneni A, Chen J, et al. Proteins associated with risk of kidney function decline in the general population. *J Am Soc Nephrol*. 2021;32(9):2291-2302. doi:10.1681/ASN.2020111607
5. Ganz P, Heidecker B, Hveem K, et al. Development and validation of a protein-based risk score for cardiovascular outcomes among patients with stable coronary heart disease. *JAMA*. 2016;315(23):2532-2541. doi:10.1001/jama.2016.5951
6. Eiriksdottir T, Ardal S, Jonsson BA, et al. Predicting the probability of death using proteomics. *Commun Biol*. 2021;4(1):758. doi:10.1038/s42003-021-02289-6
7. Yu LR, Sun J, Daniels JR, et al. Aptamer-based proteomics identifies mortality-associated serum biomarkers in dialysis-dependent AKI patients. *Kidney Int Rep*. 2018;3(5):1202-1213. doi:10.1016/j.ekir.2018.04.012
8. Schmidt IM, Sarvode Mothi S, Wilson PC, et al. Circulating plasma biomarkers in biopsy-confirmed kidney disease. *Clin J Am Soc Nephrol*. 2022;17(1):27-37. doi:10.2215/CJN.09380721
9. Feldreich T, Nowak C, Fall T, et al. Circulating proteins as predictors of cardiovascular mortality in end-stage renal disease. *J Nephrol*. 2019;32(1):111-119. doi:10.1007/s40620-018-0556-5
10. Glorieux G, Mullen W, Duranton F, et al. New insights in molecular mechanisms involved in chronic kidney disease using high-resolution plasma proteome analysis. *Nephrol Dial Transplant*. 2015;30(11):1842-1852. doi:10.1093/ndt/gfv254
11. Appel LJ, Wright JT, Greene T, et al. Intensive blood-pressure control in hypertensive chronic kidney disease. *N Engl J Med*. 2010;363(10):918-929. doi:10.1056/NEJMoa0910975
12. Appel LJ, Middleton J, Miller ER, et al. The rationale and design of the AASK cohort study. *J Am Soc Nephrol*. 2003;14(7)(suppl 2):S166-S172. doi:10.1097/01.asn.0000070081.15137.c0
13. Wright JD, Folsom AR, Coresh J, et al. The ARIC (atherosclerosis risk in communities) study: JACC focus seminar 3/8. *J Am Coll Cardiol*. 2021;77(23):2939-2959. doi:10.1016/j.jacc.2021.04.035
14. Kraemer S, Vaught JD, Bock C, et al. From SOMAmer-based biomarker discovery to diagnostic and clinical applications: a SOMAmer-based, streamlined multiplex proteomic assay. *PLoS One*. 2011;6(10):e26332. doi:10.1371/journal.pone.0026332

15. Hathout Y, Brody E, Clemens PR, et al. Large-scale serum protein biomarker discovery in Duchenne muscular dystrophy. *Proc Natl Acad Sci U S A*. 2015;112(23):7153-7158. doi:10.1073/pnas.1507719112
16. Inker LA, Eneanya ND, Coresh J, et al. New creatinine- and cystatin c-based equations to estimate GFR without race. *N Engl J Med*. 2021;385(19):1737-1749. doi:10.1056/NEJMoa2102953
17. Cheung AK, Rocco MV, Yan G, et al. Serum beta-2 microglobulin levels predict mortality in dialysis patients: results of the HEMO study. *J Am Soc Nephrol*. 2006;17(2):546-555. doi:10.1681/ASN.2005020132
18. Foster MC, Coresh J, Hsu CY, et al. Serum β-trace protein and β2-Microglobulin as predictors of ESRD, mortality, and cardiovascular disease in adults with CKD in the chronic renal insufficiency cohort (CRIC) study. *Am J Kidney Dis*. 2016;68(1):68-76. doi:10.1053/j.ajkd.2016.01.015
19. Astor BC, Shafi T, Hoogeveen RC, et al. Novel markers of kidney function as predictors of ESRD, cardiovascular disease, and mortality in the general population. *Am J Kidney Dis*. 2012;59(5):653-662. doi:10.1053/j.ajkd.2011.11.042
20. Foster MC, Inker LA, Levey AS, et al. Novel filtration markers as predictors of all-cause and cardiovascular mortality in US adults. *Am J Kidney Dis*. 2013;62(1):42-51. doi:10.1053/j.ajkd.2013.01.016
21. Foster MC, Inker LA, Hsu CY, et al. Filtration markers as predictors of ESRD and mortality in southwestern American Indians with type 2 diabetes. *Am J Kidney Dis*. 2015;66(1):75-83. doi:10.1053/j.ajkd.2015.01.013
22. Juraschek SP, Coresh J, Inker LA, et al. Comparison of serum concentrations of β-trace protein, β2-microglobulin, cystatin C, and creatinine in the US population. *Clin J Am Soc Nephrol*. 2013;8(4):584-592. doi:10.2215/CJN.08700812
23. Ho JE, Lyass A, Courchesne P, et al. Protein biomarkers of cardiovascular disease and mortality in the community. *J Am Heart Assoc*. 2018;7(14):e008108. doi:10.1161/JAHA.117.008108
24. Geng Z, Huang L, Song M, Song Y. N-terminal pro-brain natriuretic peptide and cardiovascular or all-cause mortality in the general population: a meta-analysis. *Sci Rep*. 2017;7:41504. doi:10.1038/srep41504
25. Zhou L, Surapaneni A, Rhee EP, et al. Integrated proteomic and metabolomic modules identified as biomarkers of mortality in the atherosclerosis risk in communities study and the African American study of kidney disease and hypertension. *Hum Genomics*. 2022;16(1):53. doi:10.1186/s40246-022-00425-9
26. Dubin RF, Whooley M, Pico A, Ganz P, Schiller NB, Meyer C. Proteomic analysis of heart failure hospitalization among patients with chronic kidney disease: the heart and soul study. *PLoS One*. 2018;13(12):e0208042. doi:10.1371/journal.pone.0208042
27. Girerd N, Cleland J, Anker SD, et al. Inflammation and remodeling pathways and risk of cardiovascular events in patients with ischemic heart failure and reduced ejection fraction. *Sci Rep*. 2022;12(1):8574. doi:10.1038/s41598-022-12385-0
28. Wallentin L, Eriksson N, Olszowka M, et al. Plasma proteins associated with cardiovascular death in patients with chronic coronary heart disease: A retrospective study. *PLoS Med*. 2021;18(1):e1003513. doi:10.1371/journal.pmed.1003513
29. Klimczak-Tomaniak D, Bouwens E, Schuurman AS, et al. Temporal patterns of macrophage- and neutrophil-related markers are associated with clinical outcome in heart failure patients. *ESC Heart Fail*. 2020;7(3):1190-1200. doi:10.1002/ehf2.12678
30. Clemitson JR, Dixon RJ, Haines S, et al. Genetic dissection of a blood pressure quantitative trait locus on rat chromosome 1 and gene expression analysis identifies SPON1 as a novel candidate hypertension gene. *Circ Res*. 2007;100(7):992-999. doi:10.1161/01.RES.0000261961.41889.9c
31. Lee Y, Choe J, Park OH, Kim YK. Molecular mechanisms driving mRNA degradation by m6A modification. *Trends Genet*. 2020;36(3):177-188. doi:10.1016/j.tig.2019.12.007
32. Wang JN, Wang F, Ke J, et al. Inhibition of METTL3 attenuates renal injury and inflammation by alleviating TAB3 m6A modifications via IGF2BP2-dependent mechanisms. *Sci Transl Med*. 2022;14(640):eabk2709. doi:10.1126/scitranslmed.abk2709
33. Liu P, Zhang B, Chen Z, et al. m6A-induced lncRNA MALAT1 aggravates renal fibrogenesis in obstructive nephropathy through the miR-145/FAK pathway. *Aging (Albany New York)*. 2020;12(6):5280-5299. doi:10.18632/aging.102950
34. Ramalingam H, Kashyap S, Cobo-Stark P, et al. A methionine-Mettl3-N6-methyladenosine axis promotes polycystic kidney disease. *Cell Metab*. 2021;33(6):1234-1247.e7. doi:10.1016/j.cmet.2021.03.024
35. Chen J, Wei X, Yi X, Jiang DS. RNA Modification by m6A methylation in cardiovascular disease. *Oxid Med Cell Longev*. 2021;2021:8813909. doi:10.1155/2021/8813909
36. Cao Y, Li R, Shen M, et al. DDRGK1, a crucial player of ufmylation system, is indispensable for autophagic degradation by regulating lysosomal function. *Cell Death Dis*. 2021;12(5):416. doi:10.1038/s41419-021-03694-9
37. Neziri D, Pajenda S, Amuge R, et al. DDRGK1 in urine indicative of tubular cell injury in intensive care patients with serious infections. *J Nephropathol*. 2016;5(2):65-71. doi:10.15171/jnp.2016.13