Serum metabolites are associated with all-cause mortality in chronic kidney disease

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Chronic kidney disease (CKD) involves significant metabolic abnormalities and has a high mortality rate. Because the levels of serum metabolites in patients with CKD might provide insight into subclinical disease states and risk for future mortality, we determined which serum metabolites reproducibly associate with mortality in CKD using a discovery and replication design. Metabolite levels were quantified via untargeted liquid chromatography and mass spectroscopy from serum samples of 299 patients with CKD in the Modification of Diet in Renal Disease (MDRD) study as a discovery cohort. Six among 622 metabolites were significantly associated with mortality over a median follow-up of 17 years after adjustment for demographic and clinical covariates, including urine protein and measured glomerular filtration rate. We then replicated associations with mortality in 963 patients with CKD from the African American Study of Kidney Disease and Hypertension (AASK) cohort over a median follow-up of ten years. Three of the six metabolites identified in the MDRD cohort replicated in the AASK cohort: fumarate, allantoin, and ribonate, belonging to energy, nucleotide, and carbohydrate pathways, respectively. Point estimates were similar in both studies and in meta-analysis (adjusted hazard ratios 1.63, 1.59, and 1.61, respectively, per doubling of the metabolite). Thus, selected serum metabolites were reproducibly associated with long-term mortality in CKD beyond markers of kidney function in two well characterized cohorts, providing targets for investigation.

Kidney International (2018) ■, ■–■; https://doi.org/10.1016/j.kint.2018.03.008

KEYWORDS: chronic kidney disease; metabolomics; mortality; uremic toxins

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Received 24 July 2017; revised 31 January 2018; accepted 2 March 2018

Chronic kidney disease (CKD) is a public health problem with a global prevalence of 13.4% and mortality rate of 1.2 million per year worldwide.[1–3] The kidney is an important regulator of homeostasis by filtration, reabsorption, secretion, synthesis, and degradation of metabolites in numerous pathways.[4] As such, kidney disease directly leads to a wide range of circulating metabolic abnormalities.[5]

Untargeted serum metabolite profiling, which is a nondirected, high-throughput approach to identify and quantify metabolites, may provide insight into subclinical disease states and risk for future adverse events beyond the prognostic information available from reduced glomerular filtration rate (GFR). The metabolome refers to small molecules between 50 and 1500 Da in size that are the intermediates and products of metabolism, including amino acids, carbohydrates, cofactors, vitamins, lipids, nucleotides, peptides, and xenobiotics. The metabolome has multiple endogenous and exogenous determinants, including the genome, transcriptome, and proteome, and diet and the microbiome. Metabolites that associate with subsequent risk of adverse events may shed light on underlying pathophysiology or represent novel treatment targets.

To date, an untargeted metabolomics approach to identify metabolites associated with mortality has been used only in the general population.[6,7] Because of the interrelatedness of kidney function and metabolite levels, associations between metabolites and mortality may be even more informative in CKD. Thus, we examined the associations between more than 600 serum metabolites identified in untargeted profiling and the risk of all-cause mortality, using a discovery cohort of participants in the Modification of Diet in Renal Disease (MDRD) Study, followed by replication in the African American Study of Kidney Disease and Hypertension (AASK). In addition, we specifically evaluated associations between known uremic toxins and mortality with and without adjustment for clinical covariates.[8]

RESULTS

Baseline characteristics of discovery study and replication study participants

Study participants in the discovery cohort (MDRD; $N = 299$) had a mean age of 55 years and mean body mass index of 27 kg/m^2; 42% were women, 8% were African American, 10% had diabetes mellitus, 18% had coronary heart disease, and

9% were current smokers (Table 1). The mean measured GFR was 30 ml/min per 1.73 m^2. The median follow-up period was 16.5 years, ranging from 1.1 to 18.6 years, during which 151 participants (51%) died.

Study participants in the replication cohort (AASK; $N = 963$) had a mean age of 55 years and a mean body mass index of 31 kg/m^2; 39% were women, 100% were African American, 0% had diabetes, 52% had coronary heart disease, and 58% were current smokers. The mean measured GFR was 47 ml/min per 1.73 m^2. The median follow-up period was 9.7 years, ranging from 1 day to 12 years, during which 220 participants (23%) died.

Metabolites associated with mortality in the discovery study

We investigated 622 nondrug, named metabolites (Supplementary Table S1). The mean molecular weight of the investigated compounds was 298 Da, ranging from 74 to 811 Da. In global analysis adjusted for multiple clinical covariates including ^{125}I-iothalamate clearance and log-transformed 24-hour urine protein, there were 6 metabolites that had statistically significant associations with mortality in the MDRD study (discovery threshold $P < 0.001$). These included glutamine (hazard ratio [HR] per doubling of metabolite = 0.3, $P = 0.0002$), alpha-ketoglutarate (HR = 1.5, $P = 0.0004$), ribonate (HR = 1.6, $P = 0.0006$), fumarate (HR = 1.7, $P = 0.0007$), allantoin (HR = 1.6, $P = 0.0007$), and gamma-glutamylglutamine (HR = 0.6, $P = 0.00095$) (Figure 1). These metabolites are part of the amino acid, energy, carbohydrate, energy, nucleotide, and peptide pathways, respectively (Supplementary Figure S1).

Metabolites associated with mortality in the replication study

Three of the 6 discovered metabolites in MDRD were significant in AASK in the Bonferroni-corrected replication study (replication threshold $P < 0.0083$) adjusted for clinical covariates including ^{125}I-iothalamate clearance and log-transformed 24-hour urine protein: ribonate (HR = 1.7, $P = 0.0005$), fumarate (HR = 1.6, $P = 0.001$), and allantoin (HR = 1.6, $P = 0.001$) (Figure 2). All associations were in the same direction with similar magnitude across the 2 cohorts, with higher levels of metabolites associated with higher mortality. Kaplan-Meier survival curves stratified at the median levels of ribonate, fumarate, and allantoin showed separation over time (all log rank $P < 0.05$ except for allantoin in MDRD; Figure 3).

Table 1 | Baseline characteristics of participants from the Modification of Diet in Renal Disease study (MDRD) and African American Study of Kidney Disease and Hypertension (AASK) included in the metabolomics study

Characteristic	MDRD	AASK
n	299	963
Age, mean (SD)	55 (12)	55 (11)
Female, *n* (%)	126 (42)	375 (39)
African American, *n* (%)	23 (8)	963 (100)
Diabetes, *n* (%)	31 (10)	0 (0)
Coronary heart disease, *n* (%)	53 (18)	498 (52)
Smoking, *n* (%)	27 (9)	561 (58)
Body mass index, kg/m^2, mean (SD)	27 (4)	31 (7)
Systolic blood pressure, mm Hg, mean (SD)	130 (18)	150 (24)
Urine protein, g/d, mean (SD)	0.69 (1.04)	0.53 (0.95)
Glomerular filtration rate, ml/min per 1.73 m^2, mean (SD)	30 (13)	47 (14)
High-density lipoprotein, mg/dl, mean (SD)	41 (15)	48 (16)
Total cholesterol, mg/dl, mean (SD)	203 (42)	212 (46)

Metabolites associated with mortality in a meta-analysis

In a meta-analysis of the 2 cohorts, all 3 replicated metabolites (ribonate, fumarate, allantoin) were significantly associated with mortality after Bonferroni correction ($P < 0.00008$) (Figure 4). Each of the replicated metabolites showed a 1.5- to 1.6-fold higher risk of mortality per metabolite doubling. If all 622 metabolites were considered, 5 additional metabolites were also statistically significant in the meta-analysis: N-acetylcarnosine (HR = 0.67, $P = 8.3E-8$), 1-palmitoyl-2-linoleoyl-GPE (HR = 1.43, $P = 8.2E-6$), malate (HR = 1.63, $P = 3.2E-5$), 1-linoleoyl-GPE (HR = 1.47, $P = 6.6E-5$), and hydroxycotinine (HR = 1.08, $P = 7.2E-5$). Each of these metabolites was positively associated with mortality except for N-acetylcarnosine, which had a protective effect.

Sensitivity analysis

In sensitivity analyses adjusting for baseline medication classes in the MDRD study, the effect estimates for the 3 metabolites significantly associated with mortality remained largely the same (Supplementary Table S2). In sensitivity analysis adjusting for baseline liver function tests in the AASK study, the effect sizes for the 3 replicating metabolites were also largely unchanged (Supplementary Table S3).

Correlations between mortality-related metabolites and other metabolites in their respective pathways

Allantoin, ribonate, and fumarate were all positively correlated with each other. Allantoin was negatively correlated with GFR and positively correlated with urea (Supplementary Figures S2 and S3). With respect to other metabolites in the (hypo)xanthine/inosine-containing purine pathway, allantoin was generally only weakly correlated (Supplementary Table S4). Only one other purine pathway metabolite, N1-methylinosine, was associated with mortality, and this was only in AASK, but not MDRD.

Fumarate was negatively correlated with GFR, but not significantly so. With respect to other metabolites in the tricyclic acid cycle pathway, fumarate had the most consistently strong correlation with malate, which was also associated with mortality in both AASK and MDRD (Supplementary Table S5). Alpha-ketoglutarate was associated with mortality in MDRD, but not AASK.

Ribonate was negatively correlated with GFR and positively correlated with urea. With respect to other metabolites in the pentose metabolism pathway, ribonate had strong correlations with arabitol/xylitol and arabonate/xylonate (Supplementary Table S6). Arabitol/xylitol was associated with mortality in both MDRD and AASK, and arabonate/xylonate was associated with mortality in MDRD only.