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Original Article

ORIGINAL ARTICLE

Consistency of metabolite associations with measured glomerular filtration rate in children and adults

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ABSTRACT

Background. There is interest in identifying novel filtration markers that lead to more accurate GFR estimates than current markers (creatinine and cystatin C) and are more consistent across demographic groups. We hypothesize that large-scale metabolomics can identify serum metabolites that are strongly influenced by glomerular filtration rate (GFR) and are more consistent across demographic variables than creatinine, which would be promising filtration markers for future investigation.

Methods. We evaluated the consistency of associations between measured GFR (mGFR) and 887 common, known metabolites quantified by an untargeted chromatography- and spectroscopy-based metabolomics platform (Metabolon) performed on frozen blood samples from 580 participants in Chronic Kidney Disease in Children (CKiD), 674 participants in Modification of Diet in Renal Disease (MDRD) Study and 962 participants in African American Study of Kidney Disease and Hypertension (AASK). We evaluated metabolite–mGFR correlation association with metabolite class, molecular weight, assay platform and measurement coefficient of variation (CV). Among metabolites with strong negative

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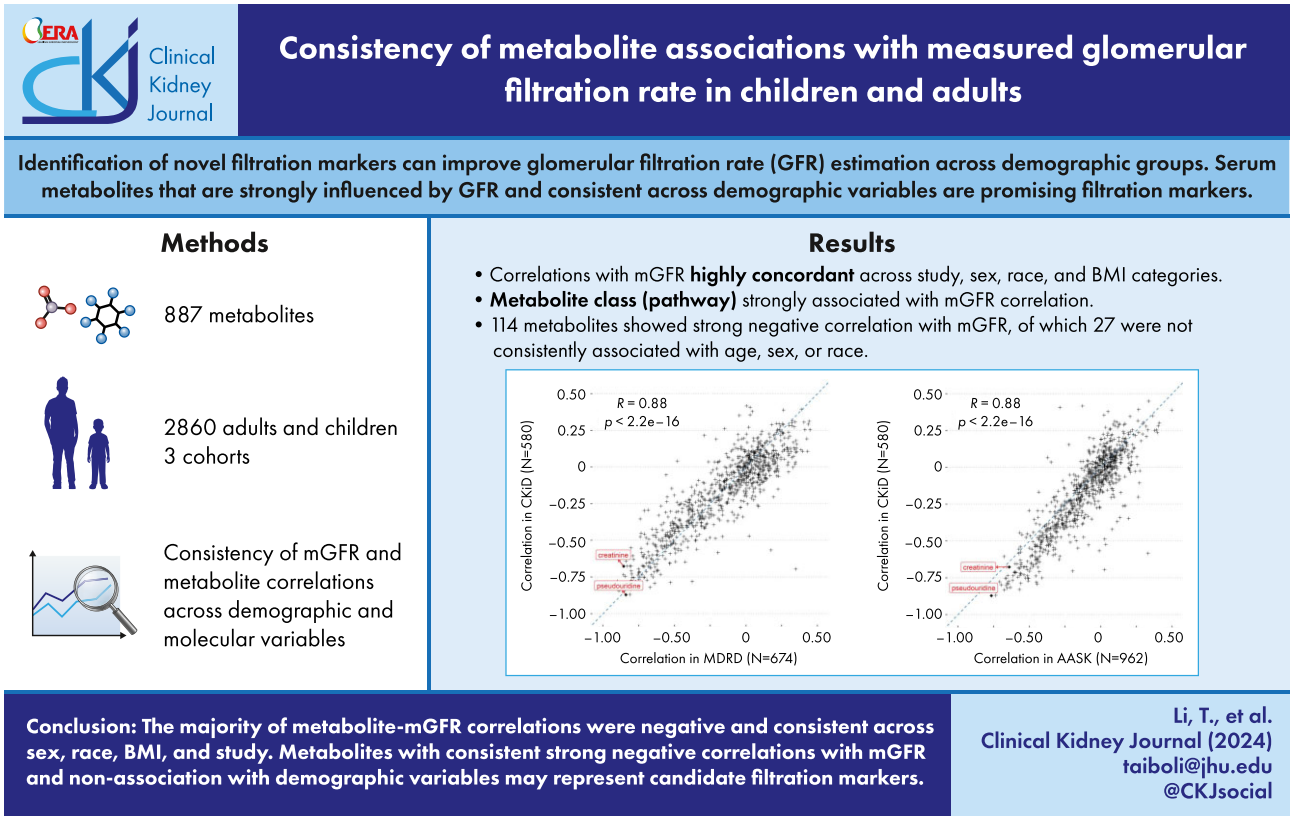
correlations with mGFR ($r < -0.5$), we assessed additional variation by age (height in children), sex, race and body mass index (BMI).
Results. A total of 561 metabolites (63%) were negatively correlated with mGFR. Correlations with mGFR were highly consistent across study, sex, race and BMI categories (correlation of metabolite–mGFR correlations between 0.88 and 0.95). Amino acids, carbohydrates and nucleotides were more often negatively correlated with mGFR compared with lipids, but there was no association with metabolite molecular weight, liquid chromatography/mass spectrometry platform and measurement CV. Among 114 metabolites with strong negative associations with mGFR ($r < -0.5$), 27 were consistently not associated with age (height in children), sex or race.
Conclusions. The majority of metabolite–mGFR correlations were negative and consistent across sex, race, BMI and study. Metabolites with consistent strong negative correlations with mGFR and non-association with demographic variables may represent candidate markers to improve estimation of GFR.

GRAPHICAL ABSTRACT



Keywords: filtration markers, GFR, kidney function, metabolites, metabolomics

KEY LEARNING POINTS

What was known:

- More accurate estimation of glomerular filtration rate (GFR) is needed in adults and children. Errors in estimated GFR reflect the contribution of non-GFR factors to serum levels of endogenous filtration markers, such as creatinine, which differ systematically across demographic characteristics.
- A key step in improving GFR estimation is identifying novel filtration markers that are consistently associated with measured GFR (mGFR) across different patient characteristics, and less strongly related to patient demographics than creatinine.
- Large-scale metabolomics studies now systematically quantify the levels of hundreds of metabolites in addition to creatinine, enabling the identification of novel markers of GFR.

This study adds:

- We evaluated the consistency of associations between mGFR and 887 common, known metabolites in frozen blood samples from 2860 adults and children with chronic kidney disease from three studies.

- A total of 561 metabolites (63%) were negatively correlated with mGFR. Correlations with mGFR were highly consistent across study, sex, race and body mass index categories (correlation of metabolite–mGFR correlations between 0.88 and 0.95).
- Among 114 metabolites with strong negative associations with mGFR ($r < -0.5$), 27 were consistently not associated with age (height in children), sex or race.

Potential impact:

- Metabolites with consistent strong negative correlations with mGFR and non-association with demographic variables may represent candidate markers to improve estimation of GFR.
- Inclusion of these novel markers in panel of filtration markers in an estimating equation may allow a unified GFR estimating equation, which enables consistent GFR estimates from pediatric to adult practices and across different ethnicities.
- For next steps, it will be necessary to develop targeted assays of the metabolites most highly correlated with mGFR, and test the panel in a larger number of cohorts in diverse population settings.

INTRODUCTION

Improved estimation of glomerular filtration rate (GFR) is needed to minimize errors in the definition, classification and management of chronic kidney disease (CKD) in adults and children [1]. The gold standard for GFR assessment, measured GFR (mGFR), is laborious [2], hence estimated GFR (eGFR) is generally used in clinical decision-making and research studies [3, 4]. Estimating equations for GFR incorporate blood levels of filtration markers (metabolites and low molecular weight serum proteins), the most common of which is creatinine, an amino acid metabolite (113 g/mol) (eGFRcr). Creatinine is easily and reliably measured, generated at a relatively constant rate by muscle and cleared primarily by glomerular filtration, making it an attractive option for estimating GFR. However, creatinine levels are also affected by a variety of non-GFR factors including differences in amino acid metabolism in liver or muscle, diet, kidney tubular secretion and gastrointestinal elimination [5–7]. In both adults and children, there are differences in the relationship between creatinine and mGFR across age, race and sex [8–11]. As a result, eGFRcr equations use demographic factors as a surrogate for muscle mass to increase accuracy, but still have errors >30% of their target measured GFR in over 10% of patients.

There is much interest in identifying filtration markers that improve the accuracy of GFR estimation without reliance on patient demographics, since demographic factors may be surrogates for non-GFR determinants of metabolites other than creatinine [12]. Large-scale metabolomics studies now systematically quantify the levels of hundreds of small molecules in patient samples, enabling the identification of novel markers of GFR [13–18]. A key step in improving GFR estimation is identifying candidate metabolites—metabolites that are consistently associated with mGFR across different patient characteristics, and less strongly related to patient demographics than creatinine. In addition, it is also useful to understand how different metabolite classes relate to mGFR correlations.

We hypothesize that large-scale metabolomics can identify serum metabolites that are strongly influenced by GFR and are more consistent across demographic variables than creatinine which would be promising filtration markers for future investigation. The aim of the current study was to quantify the associations between metabolites and mGFR in three cohorts including adults and children with CKD in the USA. We tested the central hypothesis that correlations of metabolites with mGFR would be highly similar by age, race, sex and research study, and describe how they vary by metabolite class and other metabolite characteristics. For those metabolites with strong negative correlations with mGFR, we sought to determine whether there were markers that were not associated with demographic variables.

MATERIALS AND METHODS

Study population

The Chronic Kidney Disease in Children (CKiD) study is an ongoing multicenter observational cohort study of children and adolescents aged between 6 months and 16 years with eGFR between 30 and 90 mL/min/1.73 m^2 from 59 centers in North America [19, 20]. mGFR was determined by plasma clearance of iohexol administered by intravenous bolus [19]. Global metabolomics and mGFR were performed at the 6-month and 12-month visits, respectively. For this analysis, we excluded participants with missing metabolites or phenotype information, resulting in a total of 580 individuals.

The Modification of Diet in Renal Disease (MDRD) study was a multicenter randomized clinical trial that enrolled patients with GFR between 25 and 55 mL/min/1.73 m^2 to examine the effects of protein restriction and blood pressure control on CKD progression [21]. mGFR was determined by the urinary clearance of ^{125}I-iothalamate administered by subcutaneous injection. Global metabolomics and mGFR were performed at the 12-month visit. For this analysis, we excluded participants with missing metabolites or phenotype information, resulting in a total of 674 individuals.

The African American Study of Kidney Disease and Hypertension Cohort Study (AASK) was a multicenter observational study consisted of 1094 individuals recruited from 21 centers with CKD attributed to hypertension, urine protein–creatinine ratio ≤2500 mg/g, mGFR between 20 and 65 mL/min/1.73 m^2, and without a diagnosis of diabetes mellitus [22, 23]. mGFR was determined by urinary^{125}I-iothalamate clearance, similar to the MDRD study [24]. Global metabolomics and mGFR were performed at the baseline visit. For this analysis, we excluded participants with missing metabolites or phenotype information, resulting in a total of 962 individuals.

Metabolomic profiling

Serum metabolite profiling was conducted using untargeted mass spectrometry (MS) by Metabolon, Inc. [25] (Morrisville, NC, USA). Details of sample handling, global metabolomics assay methods and processing of metabolomics data have been described previously [26–29]. Briefly, metabolites were standardized to a median value of 1.0, log transformed to reduce skewness and then correlated to log-transformed mGFR. Despite

Table 1: Clinical characteristics of participants in the current study.

	CKiD (N = 580) N (% of total)	MDRD (N = 674) N (% of total)	AASK (N = 962) N (% of total)
Race (white)	501 (86.4)	578 (85.8)	0 (0.0)
Sex (female)	228 (39.3)	254 (37.7)	374 (38.9)
Diabetes	0 (0.0)	33 (4.9)	0 (0.0)
	Mean (SD)	Mean (SD)	Mean (SD)
Age	12.1 (4.2)	52.1 (12.0)	54.5 (10.6)
BMI (kg/m^2)	20.6 (5.8)	26.8 (4.1)	30.6 (6.6)
Z_BMI	0.37 (1.2)	NA[c]	NA
mGFR (mL/min/1.73 m^2)[a]	52.7 (24.4)	29 (13.2)	46 (13.0)
Urine PCR (mg/g)[b]	324 (123, 1000)	185 (53, 865)	80 (28, 359)
Serum creatinine (mg/dL)	1.38 (0.89)	2.73 (1.47)	1.87 (0.68)
Serum cystatin C (mg/L)	1.57 (0.73)	2.50 (0.91)	1.59 (0.51)
BUN (mg/dL)	28 (15)	34 (16)	24 (10)

[a]In CKiD, GFR is measured by iohexol rather than iothalamate as in AASK and MDRD.
[b]Median and interquartile range are shown.
[c]Z-scores for BMI were not calculated for adults in MDRD or AASK.
BUN, blood urea nitrogen.

using the same methods, the exact metabolites which crossed the limit of detection in each study varied. We focused on the 887 metabolites that were profiled in all three cohorts, which were involved in a total of 97 pathways across 8 classes (amino acid, carbohydrate, cofactors and vitamins, energy, lipid, nucleotide, peptide and xenobiotics). Molecular weight and the optimal liquid chromatography/MS (LC/MS) platform (late, polar, positive early, positive late) to characterize each metabolite was provided by Metabolon. Coefficient of variation (CV) across masked replicates were obtained from an external study [30] which examined replicability of metabolomics measurements using samples from 49 individuals with CKD during two study visits.

Statistical analysis

Baseline characteristics were summarized as frequency, mean and percentiles. To compare metabolite–mGFR correlations across cohorts, the Pearson correlation was calculated for each cohort and metabolite, and compared using scatter plots for pairs of cohorts. Subgroup analyses by sex, race (Black, white and other), and body mass index (BMI) category (<25, 25–30 and >30 kg/m^2) were conducted by calculating correlations with mGFR within subgroups in each study and then comparing them across subgroups. To minimize differences driven by the different range of mGFR across studies, a sensitivity analysis was conducted limiting the data to mGFR between 30 and 60 mL/min/1.73 m^2.

To investigate whether physiological and technical factors relate to metabolite–mGFR correlation, we examined in univariate and then multiple regression analysis the association of metabolite–mGFR correlation with biological pathways (class of metabolites), molecular weight groups (≥500 g/mol and <500 g/mol), CV and LC/MS platforms. A mixed effects linear regression model was constructed using the aggregate data from all three cohorts (CKiD, MDRD and AASK), where metabolite–mGFR correlation was regressed on cohort, pathway (metabolite class), molecular weight, CV and LC/MS platform, using metabolite as a random effect. In this model, the 369 metabolites negatively associated with mGFR ($r < 0$) with complete data on molecular weight, CV, pathway (metabolite class) and LC/MS platform were used. For subgroup analysis with sex and race, metabolite–mGFR correlations for the metabolites were calculated separately for each sex and race, and an individual linear mixed model was constructed using cohort, age or sex, molecular weight, CV, pathway (metabolite class) and LC/MS platform as fixed effects, and metabolite as random effects. Analyses were performed with raw correlation values and after applying a Fisher-transformation for normalization. Since results were similar between raw correlation and Fisher-transformed correlations, results are presented on the original correlation scale for ease of interpretation.

For metabolites strongly negatively associated with mGFR ($r < -0.5$), we tested for independence from demographic variables. In each study, we regressed log-transformed metabolite levels on age (height for children), sex and race, while adjusting for mGFR and urine protein–creatinine ratio. Bonferroni correction was then performed in each study to identify metabolites with significant associations with demographic variables (2.5×10^{-4} for CKiD, 6.6×10^{-4} for MDRD and 2.7×10^{-4} for AASK). We considered non-significant associations with all demographic variables in two or three of the three studies as consistently not associated. We also tested for interactions of demographic variables with mGFR.

A threshold of $\alpha = 0.05$ was used to determine significance of each covariate. All statistical tests and plots were generated using R software version 4.0.3, with ggpubr package version 0.4.0.

RESULTS

Study population

Study populations differed in level of GFR, age, race, clinical characteristics and cause of kidney disease. Mean [standard deviation (SD)] mGFR was 53 (24) in CKiD, 29 (13) in MDRD and 46 (13) mL/min/1.73 m^2 in AASK. The distribution of demographic variables and clinical characteristics for the three studies is detailed in Table 1. In CKiD, 6% of participants had hemolytic uremic syndrome, 25% had non-HUS glomerular disease, 62% had hereditary causes of CKD and 7% had other causes of kidney disease. In MDRD, 24% of participants had polycystic kidney disease, 28% had glomerular disease and 47% had other causes of CKD. In AASK, all participants had hypertension as the primary assigned

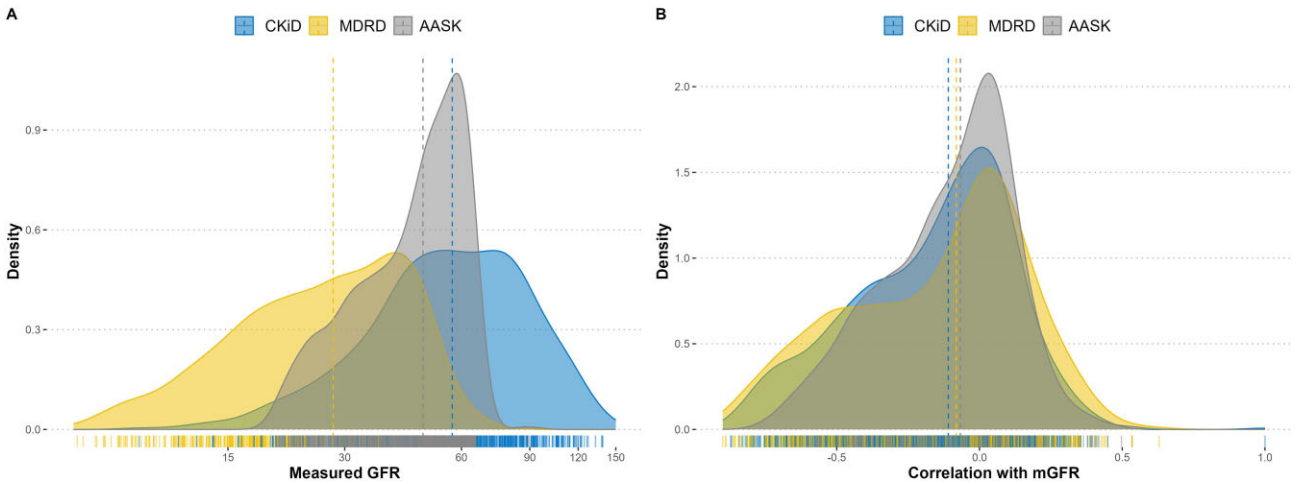


Figure 1: Distribution of directly mGFR and correlation with metabolites. (**A**) Kernel density plot of mGFR of 580 participants in CKiD, 674 participants in MDRD and 962 participants in AASK. The x-axis is plotted in log scale. (**B**) Kernel density plot of metabolite–mGFR correlation in CKiD, MDRD and AASK (887 metabolites). Dotted lines represent the median value for each group.

cause of CKD. The distribution of mGFR is shown in Fig. 1A. mGFR values in CKiD and MDRD spanned a >5-fold range of variation compared with a <3-fold range in AASK (5th–95th percentiles: 20–97, 10–50 and 24–64 mL/min/1.73 m^2, respectively).

Comparison of metabolite–mGFR correlations across studies

The distribution of 887 metabolite–mGFR correlations was wide and similar in CKiD, MDRD and AASK [median (5th to 95th) percentiles of −0.11 (−0.68 to 0.22), −0.08 (−0.71 to 0.29) and −0.07 (−0.54 to 0.20), respectively]. A total of 561 of the correlations were negative (63%), with more left skew observed for MDRD and CKiD compared with AASK (Fig. 1B, Supplementary data, Table S1). The metabolite–mGFR correlation distributions were even more similar across cohorts in a sensitivity analysis restricted to only those individuals with mGFR between 30 and 60 mL/min/1.73 m^2 (Supplementary data, Fig. S2).

The correlations between metabolites and mGFR were highly consistent among studies. The correlation of correlations for CKiD vs MDRD was 0.88, for CKiD vs AASK was 0.88 and for MDRD vs AASK was 0.91 (Fig. 2A–C). The most negatively correlated individual metabolites (class) were: pseudouridine (pyrimidine metabolism); N6-carbamoylthreonyladenosine (purine metabolism); C-glycosyltryptophan (tryptophan metabolism); 1-methylguanidine (guanidino and acetamido metabolism), N-acetylneuraminate and erythronate (aminosugar metabolism); O-sulfo-L-tyrosine (xenobiotics); and 4-acetamidobutanoate (polyamine metabolism) (Supplementary data, Table S1). For pseudouridine, the correlation with mGFR was −0.87 in CKiD, −0.84 in MDRD and −0.77 in AASK, vs −0.68, −0.86 and −0.64, respectively, for serum creatinine.

Comparison of metabolite–mGFR correlations across sex, race and BMI

We observed a similar consistency of metabolite–mGFR correlations within each study across all categories of sex, race and BMI (correlation of correlations for sex, $r > 0.93$; race, $r > 0.89$; BMI, $r > 0.89$ in adults and $r > 0.76$ in children, Fig. 2D–H, Supplementary data, Fig. S3).

Other metabolite factors related to correlation with mGFR

Several biological and technical factors were associated with the strength of metabolite–mGFR correlations. The strongest factor associated with metabolite–mGFR correlation was metabolite class (Fig. 3). Lipids tended to have weaker correlations with mGFR than the other classes. More negative correlations were seen among amino acid, nucleotides and carbohydrates compared with lipids; this was consistent across all three studies (Supplementary data, Fig. S4).

Univariate analyses of metabolites with a negative correlation with mGFR showed that larger metabolites (molecular weight ≥500 g/mol) were significantly less correlated with mGFR compared with smaller metabolites ($P < .001$ for all three cohorts, Supplementary data, Fig. S5). Similarly, metabolites best characterized on the LC/MS positive early and polar platforms tended to have more negative metabolite–mGFR correlation than those characterized on the LC/MS negative and positive late platforms (Supplementary data, Fig. S6). Metabolite–mGFR correlation did not vary by CV across technical replicate samples (Supplementary data, Fig. S7). Of note, biological pathway was related to both molecular weight and LC/MS platform (Supplementary data, Figs S8–S10).

Linear mixed model to simultaneously assess multiple determinants of metabolite–mGFR correlations

In adjusted models of metabolite–mGFR correlations (Table 2), we found that metabolite class remained a strong predictor of negative correlations, with statistically significant differences (more negative correlations) for molecules belonging to amino acid, carbohydrate, cofactors and vitamins, and nucleotides compared with lipids (all P-values <.01). Molecular weight, CV and LC/MS platforms were not associated with metabolite–mGFR correlations in adjusted analysis. Study cohort was associated with metabolite–mGFR correlation, with weaker correlations in AASK than the other cohorts likely due to different ranges of mGFR across these studies. In fact, AASK had slightly stronger correlations than the other cohorts even when restricting the range of GFR to similar values in all studies (30–60 mL/min/1.73 m^2). We repeated the regression analy-

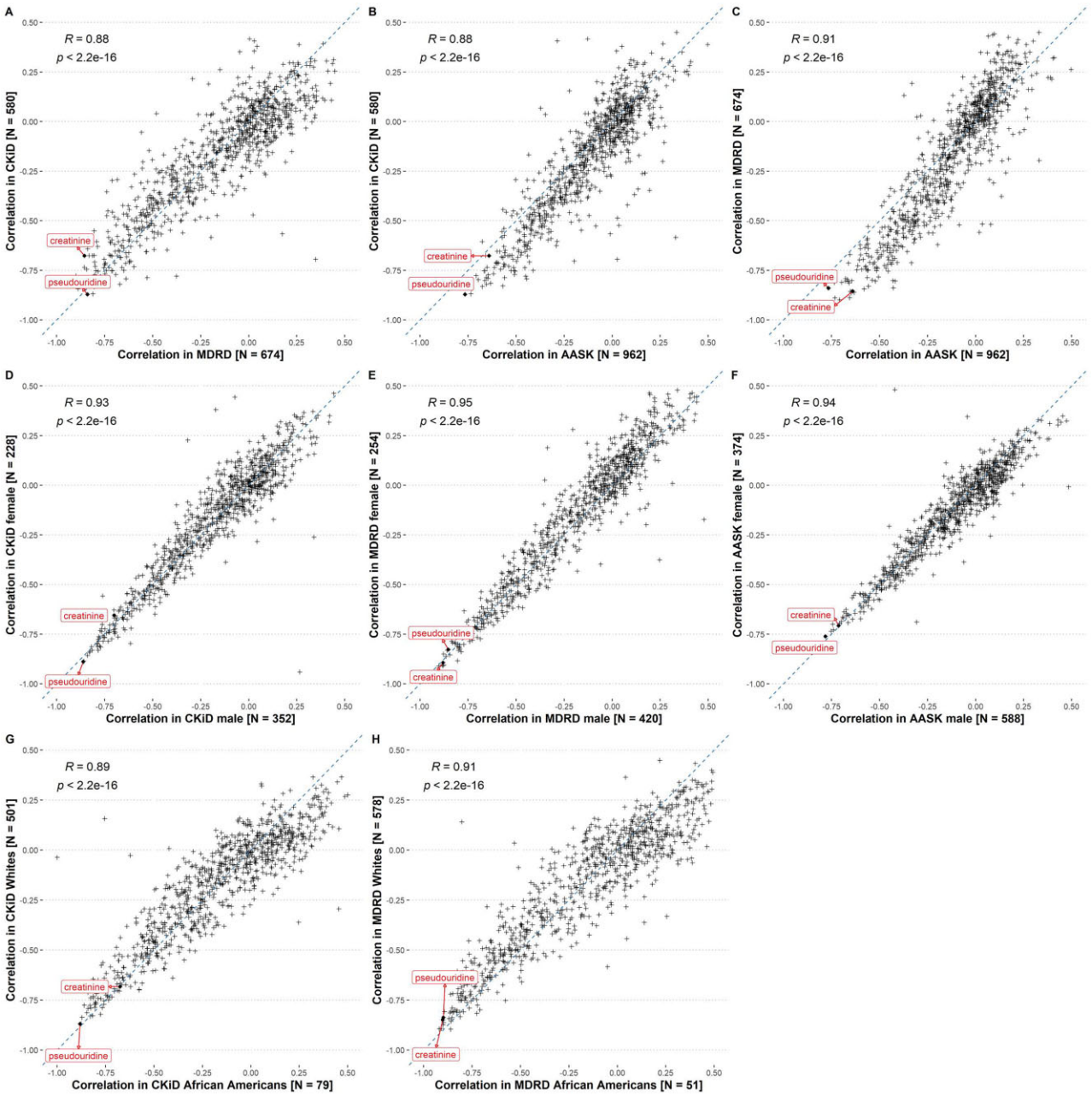


Figure 2: Concordance of metabolite–mGFR correlations across studies (**A–C**), sex (**D–F**) and race (**G**, **H**). Scatter plot of metabolite–mGFR correlation values for each of the 887 metabolites in three cohorts as pairwise comparisons. Creatinine and pseudouridine are labeled. Dotted lines represent the line of identity. Pearson correlation coefficient (R) and its associated *P*-value are shown. The number of participants in each comparison is shown in the axis labels.

sis after stratification by sex and race to allow comparisons across these factors. Race and sex were significant covariates for metabolite–mGFR correlation but with small effects (<0.1), while metabolite class remained highly significant in each of the subgroup regression analyses.

Regression of metabolite levels on demographic variables

For the 114 metabolites with strong negative correlations with mGFR ($r < -0.5$) in any of the three studies, we regressed log metabolite level on demographics adjusted for mGFR and urine protein–creatinine ratio to directly estimate these associations at the individual level in each study. In CKiD, 43 of the 83 (51.8%) metabolites were significantly associated with height ($P < 6 \times 10^{-4}$ after Bonferroni correction; height was used in place of age due to its known association with mGFR), 13 (15.7%) were significantly associated with sex and 26 (31.3%) were significantly associated with African American race. Creatinine was associated with height ($P < .001$) while not significantly associated with sex or African American race after adjustment ($P = .03$ for sex and $P = .21$ for race) in CKiD. In MDRD, 14 of the 101 (13.9%) metabolites were significantly associated with age ($P < .001$), 30 (29.7%) were significantly associated with sex and 4 (4.0%) were significantly associated with Black race. Creatinine was significantly associated with each of the demographic characteris-

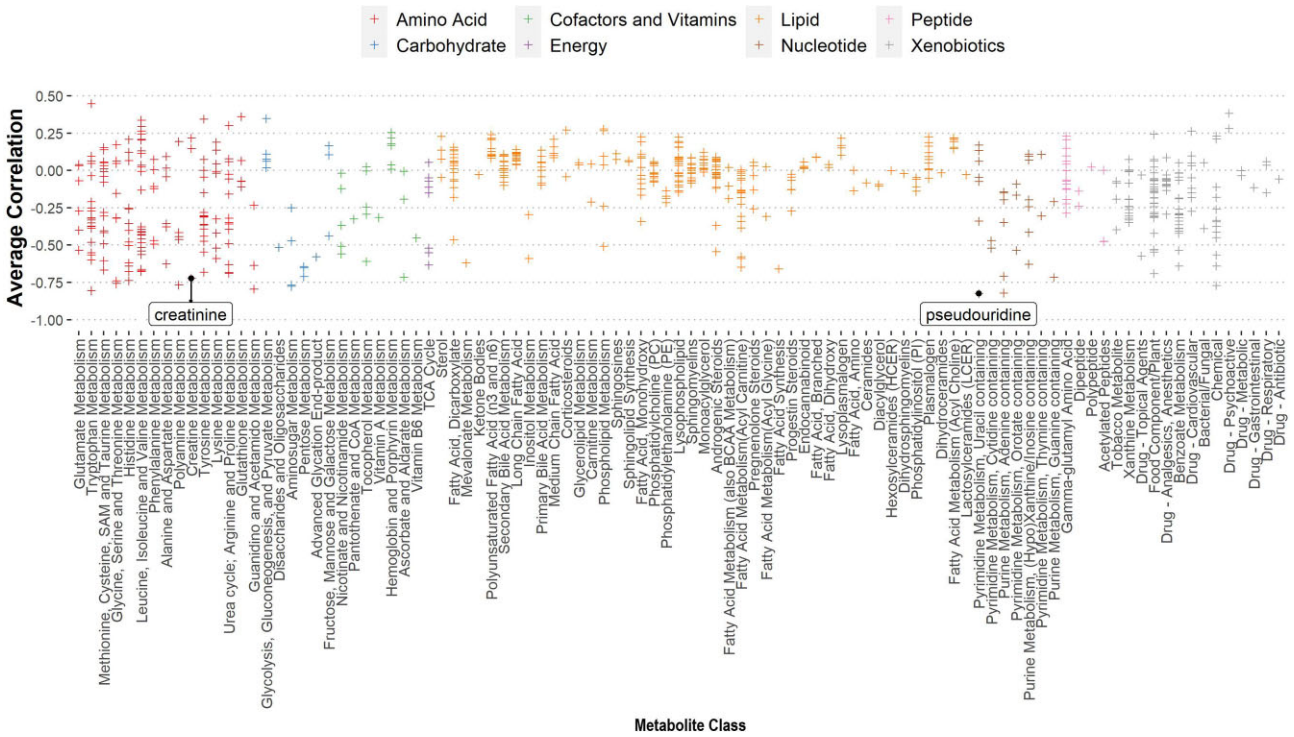


Figure 3: The relationship between biological pathways and metabolite–mGFR correlations. Metabolite–mGFR correlation as a function of pathways, which are colored by category. Correlation is calculated as average across all three cohorts (CKiD, MDRD and AASK). Creatinine and pseudouridine are labeled.

tics ($P < .001$ for each). In AASK, 6 of the 47 (12.8%) metabolites were significantly associated with age ($P < .001$) and 24 (51.1%) were significantly associated with sex. Creatinine was significantly associated with age, sex and race ($P < .001$ for CKiD and MDRD). Overall, among the metabolites with strong negative correlations with mGFR, there were 23 metabolites in CKiD, 64 in MDRD and 22 in AASK not associated with any of the three demographic variables after adjustment for GFR and urine protein–creatinine ratio (Table 3). One of these metabolites was shared in all studies (erythritol) and 27 (24%) were shared in at least two studies (Supplementary data, Table S2). We found no interactions between metabolite levels and demographic variables.

DISCUSSION

In three US cohorts with diverse representation in terms of age, sex, race and CKD etiology, we evaluated the correlations of 887 metabolites with mGFR. We found that the majority of correlations (63%) were negative, and that they were highly consistent across sex, race, BMI and study. Metabolite class was associated withinfluenced by non-GFR factors [31, 32]. The most widely used GFR estimation equation relies on serum creatinine levels and demographic factors. While demographic factors are surrogates for some of the non-GFR factors and improve the accuracy of GFR estimation, significant debate has emerged about their use for this purpose, in particular the use of race [33–35]. The use of novel filtration markers or a panel of filtration markers is likely needed to eliminate or decrease reliance on demographic factors and increase accuracy of GFR estimates [36]. Equations using panels of metabolites or low molecular weight proteins have been demonstrated to show improved accuracy without use of race [16, 36–39]. Our study suggests that many more metabolites with strong negative correlations with mGFR and weaker associations with age, sex, race and height than creatinine potentially could be incorporated into multi-marker panels to improve estimation of GFR.

Metabolite class was a strong factor in metabolite association with mGFR. Lipid metabolites had the lowest correlation with mGFR, presumably as a result of binding to serum proteins or incorporation into lipoproteins which prevent them from being freely filtered. The most negative correlations were seen among amino acids followed by nucleotides, and carbohydrates compared with lipids. However, there remained residual variation of metabolite–mGFR correlation within each class. Interestingly, we found that within the same class, some end products of metabolic pathways had highly negative correlations with mGFR, as compared with intermediate members of the pathway. For example, creatinine is an end product of the creatine metabolism pathway with a correlation of $r = -0.72$ (averaged across the three studies), whereas the intermediate products such as creatine and guanidinoacetate had correlations of $r = 0.15$ and $r = 0.22$, respectively [40–42]. Similarly, in the urea cycle, we found that end products such as urea (correlation $r = -0.63$) and homocitrulline (correlation $r = -0.63$) were highly negatively associated with mGFR, compared with the intermediates such as arginine (correlation $r = 0.06$) and ornithine (correlation $r = 0.08$). In inosine metabolism, inosine showed little correlation with mGFR (average correlation $r = 0.07$) compared with its derivative N1-methylinosine (average $r = -0.63$). It may be that among metabolites classes not bound to plasma proteins, end products within a pathway are unregulated, thereby accumulating as GFR declines, whereas intermediates may be highly regulated, limiting their correlation with GFR. These findings suggest that an optimal GFR estimating equation might be developed by including molecules from a diverse classes and pathways to avoid being susceptible to non-

Table 2: Association of study and participant characteristics with metabolite correlations with mGFR.

	Full model		Full model for mGFR 30–60		Sex specific correlations		Race specific correlations	
	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value
Study: MDRD	Reference		Reference		Reference		Reference	
Study: AASK	0.09	<.001	−0.02	.002	0.05	<.001	0.03	<.001
Study: CKiD	0.00	.93	0.01	.07	−0.06	<.001	−0.04	<.001
Sex: female						Reference		
Sex: male					−0.01	.03		
Race: white							Reference	
Race: African American							−0.03	<.001
Race: other							−0.01	.24
Molecular weight Q4	Reference		Reference		Reference		Reference	
Molecular weight Q1	0.03	.50	0.02	.34	0.02	.62	0.09	.12
Molecular weight Q2	−0.05	.21	0.00	.86	−0.04	.38	−0.01	.89
Molecular weight Q3	−0.05	.22	0.01	.63	−0.07	.17	−0.01	.79
CV	0.00	.87	0.00	.51	0.00	.95	0.00	.86
Class: lipid	Reference		Reference		Reference		Reference	
Pathway: amino acid	−0.21	<.001	−0.11	<.001	−0.25	<.001	−0.26	<.001
Pathway: carbohydrate	−0.36	<.001	−0.22	<.001	−0.44	<.001	−0.45	<.001
Class: cofactors and vitamins	−0.15	.005	−0.06	.06	−0.14	.02	−0.16	.02
Pathway: energy	−0.12	.11	−0.06	.18	−0.17	.06	−0.21	.04
Pathway: nucleotide	−0.20	<.001	−0.11	<.001	−0.26	<.001	−0.27	<.001
Pathway: peptide	0.01	.81	0.04	.27	−0.00	.96	−0.00	.97
Pathway: xenobiotics	−0.08	.03	−0.02	.38	−0.06	.12	−0.09	.04
Platform: LC/MS late	Reference		Reference		Reference		Reference	
Platform: LC/MS polar	−0.08	.03	−0.05	.03	−0.08	.06	−0.07	.12
Platform: LC/MS pos early	−0.02	.43	−0.01	.42	−0.03	.37	−0.07	.03
Platform: LC/MS pos late	0.07	.06	0.03	.21	0.08	.12	0.14	.01

Coefficients are the difference in correlation with mGFR (dependent variable) estimated from a linear mixed model with a random effect for each metabolite. Full model includes all the variables in the table. mGFR (30–60 mL/min/1.73 m^2) denotes limiting the metabolite–mGFR correlations to individuals in this GFR range to provide a more similar range of mGFR across all three cohorts. Sex- and race-specific correlations were calculated within these subgroups to allow for comparison across these factors of the metabolite correlation to mGFR.
pos, positive.

Table 3: Number (%) of candidate filtration marker metabolites[a] associated with demographic variables in CKiD, MDRD and AASK.

		CKiD ($N = 83$)	MDRD ($N = 101$)	AASK ($N = 47$)
Age/height	Associated	43 (52)	14 (14)	6 (13)
Sex	Associated	13 (16)	30 (30)	24 (51)
Race	Associated	26 (31)	4 (4)	NA[b]
Any of above	Associated	60 (72)	37 (37)	25 (53)
	Not associated	23 (28)	64 (73)	22 (47)
	Not associated in any study		1 (Erythritol)	

[a]Association at Bonferroni level ($P < .05$/# candidate metabolites) in each cohort.
[b]Race analysis was not performed in AASK because all participants were African American.
Candidate metabolites defined as having a correlation with mGFR less than –0.5.
Age was replaced with height in CKiD.

GFR influences affecting in any one metabolite class or pathway, setting or disease [43].

Our findings have two immediate implications. First, because metabolite–mGFR correlations were highly concordant across different parameters, we can nominate a large number of metabolites as potentially useful filtration markers across a broad range of age and mGFR. Second, we show that multiple metabolites are less affected by age, sex and race than creatinine. Inclusion of these novel markers in a panel of filtration markers in an estimating equation may therefore allow a unified GFR estimating equation, which enables consistent GFR estimates from pediatric to adult practices and across different ethnicities [33, 44]. Overall, our findings were encouraging for the search for generalizable filtration markers and robust algorithms for GFR estimation.

Previous studies identified a handful of metabolites reliably associated with estimated GFR [45], but to our knowledge, the present study is the first to rigorously assess metabolite correlations with mGFR across a range of patient and marker characteristics in studies that include children and adults. A potential limitation of the study is that the current metabolomic methods provide relative rather than absolute concentrations of each metabolite. For this reason, we focus on correlations which show relative agreement rather than directly applying

measured metabolite levels to estimate GFR or fit a single regression or meta-analysis model across all cohorts. Thus, in the current study, we examined the correlations with mGFR rather than focusing on developing an equation to estimate GFR. Another limitation is the different ranges of mGFR and different mGFR measurement methods in the three studies, and the 6-month interval between mGFR measurement and samples for global metabolomics in CKiD. AASK had a narrower range of log mGFR values compared with CKiD and MDRD, reducing the magnitude of correlations with mGFR. When restricting to mGFR in the same range in all studies (30–60 mL/min/1.73 m^2), the significance of study differences in the linear mixed model became much weaker and reversed direction. We conclude that even though metabolites measured in AASK had a weaker correlation with mGFR compared with CKiD and MDRD, the striking similarities among the three cohorts and across a wide range of demographic variables remain valid. CKiD measured GFR using plasma clearance of iohexol rather than urinary clearance of iothalamate, but our analysis focused on correlations makes the results robust to systematic differences between GFR measurement methods. Possibly, correlations in CKiD may have been stronger if samples for global metabolomics had been obtained simultaneously with mGFR measurements, as in MDRD and AASK. Another limitation is that we did not include low molecular weight proteins, such as cystatin C, in our analysis, which are assayed using different platforms. Use of low molecular proteins, such as cystatin C, in addition to metabolites, as filtration markers in a panel (of metabolites) for estimating GFR may be useful, but would require an additional assay. For this reason, we confined this analysis to metabolites. Of note, recent studies using proteomic assays have demonstrated that a significant fraction of the plasma proteome is associated with GFR and may therefore include novel filtration markers, with cystatin C demonstrating the strongest correlation with mGFR in a Swedish cohort [46]. Possibly, a combination of metabolites and low molecular weight proteins may further improve GFR estimation.

For the next steps, it will be necessary to develop targeted assays of the metabolites most highly correlated with mGFR in order to use absolute metabolite concentrations for multi-marker equation development, and to test the panel in a larger number of cohorts in diverse population settings, take into account differences in mGFR methods, and compare performance with existing equations using creatinine and cystatin C and panels with low molecular weight proteins.

In conclusion, the correlations of metabolites with mGFR were consistent across a wide range of demographic variables including age, sex, race and BMI categories. These findings inform identification of novel kidney filtration markers from which improved GFR estimating equations applicable to diverse populations may be developed.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest relevant to the data presented in this manuscript.

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