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Novel associations between blood metabolites and kidney function among Bogalusa Heart Study and Multi-Ethnic Study of Atherosclerosis participants

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Author Contributions

JLN, TNK, JH, SL, LB, HH, AHA, and WC designed the study; JLN, CL, XG, MS, ACR, and XM analyzed the data; JMK contributed to the logistics and optimization of the untargeted metabolomics; CMR, JC, ASL, LAI, and MS performed replication of the study results; JLN made the figures; JLN and TNK drafted and revised the manuscript; all authors read and approved the final article.

Data Availability

The metabolomic data sets generated and analyzed during this study are available as supplementary data.

Conflict of interest

JLN, TNK, JH, SL, LB, HH, AHA, WC, CL, XG, MS, ACR, XM, CMR, JC, ASL, LAI, and MS declare that they have no conflict of interest.

JMK is employed by Metabolon, Inc. He contributed to the logistics, optimization, and interpretation of the untargeted metabolomics. Metabolon, Inc. was not involved in the study design, statistical analysis, or interpretation of the results.

Research involving human participants

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional review boards at participating institutions and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Abstract

Introduction: Chronic kidney disease (CKD) is a major public health challenge given its high global prevalence and associated risks of cardiovascular disease and progression to end stage renal disease. Although it is known that numerous metabolic changes occur in CKD patients, identifying novel metabolite associations with kidney function may enhance our understanding of the physiologic pathways relating to CKD.

Objectives: The objective of this study was to elucidate novel metabolite associations with kidney function among participants of two community-based cohorts with carefully ascertained metabolomics, kidney function, and covariate data.

Methods: Untargeted ultrahigh-performance liquid chromatography-tandem mass spectrometry was used to detect and quantify blood metabolites. We used multivariate adjusted linear regression to examine associations between single metabolites and creatinine-based estimated glomerular filtration rate (eGFRcr) among 1,243 Bogalusa Heart Study (BHS) participants (median eGFRcr: 94.4, 5^{th}–95^{th} percentile: 66.0–119.6 mL/min/1.73 m^2). Replication, determined by statistical significance and consistent effect direction, was tested using gold standard measured glomerular filtration rate (mGFR) among 260 Multi-Ethnic Study of Atherosclerosis (MESA) participants (median mGFR: 72.0, 5^{th}–95^{th} percentile: 43.5–105.0 mL/min/1.73 m^2). All analyses used Bonferroni-corrected alpha thresholds.

Results: Fifty-one novel metabolite associations with kidney function were identified, including 12 from previously unrelated sub-pathways: N6-carboxymethyllysine, gulonate, quinolinate, gamma-CEHC-glucuronide, retinol, methylmalonate, 3-hydroxy-3-methylglutarate, 3-aminoisobutyrate, N-methylpipecolate, hydroquinone sulfate, and glycine conjugates of $C_{10}H_{12}O_2$ and $C_{10}H_{14}O_2$(1). Significant metabolites were generally inversely associated with kidney function and smaller in mass-to-charge ratio than non-significant metabolites.

Conclusion: The 51 novel metabolites identified may serve as early, clinically relevant, kidney function biomarkers.

Keywords

metabolome; metabolomics; kidney; kidney disease; glomerular filtration rate

1. Introduction

Chronic kidney disease (CKD) is a heterogeneous disorder defined by reduced kidney function or the presence of kidney damage (Levey and Coresh 2012). CKD is an important public health challenge due to its high prevalence (Mills et al. 2015) and associated risks of end stage renal disease and cardiovascular disease (CVD) (Jha et al. 2013; Sarnak et al.

2003). However, clinical measurement of kidney function remains imprecise (Levey et al. 2014). The study of the human metabolome, which reflects endogenous and exogenous processes and their interactions, provides a unique opportunity to directly identify small molecule markers of reduced kidney function (Nicholson and Lindon 2008). These proximal metabolites could serve as clinically relevant biomarkers for earlier and more accurate detection of CKD.

Previous studies have identified metabolites associated with kidney function in healthy populations (Goek et al. 2013; E. P. Rhee et al. 2013; Sekula et al. 2016; Yu et al. 2014) and in patients with CKD (Eugene P. Rhee et al. 2016; Shah et al. 2013; Toyohara et al. 2010) and diabetes (Niewczas et al. 2014, 2017; Solini et al. 2016). Although these findings are promising, limitations of past works include the use of estimated glomerular filtration rate (eGFR) without validation using gold-standard measured glomerular filtration rate (mGFR) (Goek et al. 2013; Niewczas et al. 2017; E. P. Rhee et al. 2013; Eugene P. Rhee et al. 2016; Sekula et al. 2016; Shah et al. 2013; Solini et al. 2016; Toyohara et al. 2010; Yu et al. 2014), sole use of targeted metabolomics approaches which are restricted to pathways with presumed biological relevance (Goek et al. 2013; E. P. Rhee et al. 2013; Eugene P. Rhee et al. 2016; Toyohara et al. 2010), small numbers of metabolites tested (110 to 258 metabolites) (Goek et al. 2013; Niewczas et al. 2017; E. P. Rhee et al. 2013; Eugene P. Rhee et al. 2016; Shah et al. 2013; Yu et al. 2014), and small sample sizes (30 to 286 participants) (Niewczas et al. 2014, 2017; Shah et al. 2013; Toyohara et al. 2010). This study was designed to examine the relationship between serum metabolites and kidney function leveraging agnostic metabolomic profiling, and utilizing replication with gold-standard mGFR.

2. Materials and Methods

2.1 Study Design

The current study utilized untargeted metabolomic profiling to identify novel blood metabolites crosssectionally associated with glomerular filtration rate (GFR). Two complementary cohorts were chosen to ensure reproducibility and assess generalizability of the study findings: the Bogalusa Heart Study (BHS) and the Multi-Ethnic Study of Atherosclerosis Kidney (MESA-Kidney).

2.2 Populations

The BHS is a community-based long-term study investigating the natural history of CVD among an ethnically diverse sample (35% African-American and 65% Caucasian) of residents from Bogalusa, Louisiana (Berenson et al. 1995). From 1973 to 2016, 7 surveys were conducted in children and adolescents aged 4 to 17 years, and 10 surveys were conducted among adults aged 18 to 51 years who had been examined previously as children. The BHS has been described in detail elsewhere (Berenson et al. 1995). The current BHS study population includes 1,298 participants born between 1959 and 1979 who were screened at least two times during childhood and two times during adulthood. Data and specimens collected in the recent 2013 to 2016 follow-up visit were leveraged in this cross-sectional study. Those missing metabolomics (n=37), creatinine based eGFR (eGFRcr)

(n=8), or covariable (n=10) data were excluded. A total of 1,243 participants remained for the analyses.

MESA is a community-based cohort designed to study subclinical CVD in 4 race and ethnic groups in the United States who were free of clinical CVD at the baseline visit (2000–2002) (Bild et al. 2002). MESA-Kidney is an ancillary study of 307 African American and Caucasian MESA participants who completed visit 5 (2010 to 2011), as well as those that completed visit 3 or 4 but not visit 5, at the Johns Hopkins University MESA field center in Baltimore, MD (Inker et al. 2016). The present study included 260 MESA-Kidney participants with mGFR, metabolomics, and covariable data.

2.3 Exposures

Untargeted, ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) of BHS serum samples was conducted by Metabolon Inc. (Durham, NC) (Evans et al. 2009) using samples that were stored at −80°C since the 2013 to 2016 visit. All methods used Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution, further details on the chromatography and mass spectrometry are provided in Online Resource 1. Rigorous quality assurance was conducted during metabolomic profiling which included the use of blanks, blind duplicates (5% of samples), and standard biochemical compounds which were integrated into every run. Batch effects were assessed using principal components analysis, which revealed no evidence of clustering of metabolite data by run-days.

Untargeted metabolomic profiling resulted in the detection and relative quantification of 1,466 metabolites, including: 1,055 known biochemical compounds in pathways related to amino acids (n=201), carbohydrates (n=25), cofactors and vitamins (n=35), energy (n=9), lipids (n=435), nucleotides (n=42), peptides (n=52), and xenobiotics (n=256); 18 known biochemical compounds whose pathways have yet to be determined (referred to as ‘partially characterized molecules’); and 393 unnamed compounds currently lacking chemical standards. Our metabolite identification procedure included matching data to a library using three variables, mass-to-charge ratio (m/z), retention index (RI) and ms^n scan, followed by review by a human curator, yielding high confidence the metabolite identification calls. Pathway and sub-pathway information was derived from the literature or from internal expertise at Metabolon Inc. (Durham, NC). Metabolite identification levels, presented in the Supplementary Data as numbers 1 through 4, were determined according to the metabolomics standards initiative (Sumner et al. 2007). The unnamed compounds may be identified upon the eventual acquisition of a matching purified standard (or via classical structural analysis). Additional information on metabolite identification and relative quantification is provided in Online Resource 1.

Metabolite values were scaled to set the median of detected values for each metabolite equal to 1. Similar to previous analyses (Zheng et al. 2013), the 1,035 metabolites above the detection limit in greater than 50% of samples were analyzed as continuous variables, where the minimum observed value was imputed for metabolites with below-the-detection-limit

values. The 167 metabolites below the detection threshold in 50% to 80% of the samples were analyzed as ordinal variables after categorization into one of three mutually exclusive groups: 1) below-the-detection-limit; 2) below the median of measured values; or 3) greater than or equal to the median. Data filtering excluded 213 metabolites that were missing or below the detection threshold in more than 80% of samples and 51 metabolites with a reliability coefficient <0.3 based on blind duplicate analysis. The 1,202 metabolites passing quality control were retained for analysis.

The metabolomics methods used in MESA-Kidney and have been described previously (Coresh et al. 2018). In brief, MESA-Kidney plasma samples were stored at −70°C until untargeted metabolomics profiling was conducted by Metabolon Inc. using the same methods as the BHS. MESA-Kidney also used the same scaling, imputation, and categorization procedures as the BHS. Metabolite A total of 1,447 metabolites were detected among MESA-Kidney participants.

2.4 Primary Outcome and Covariables

Among BHS participants, phenotype and covariable data were collected following stringent protocols (Foster and Berenson 1987). Questionnaires were administered to obtain information on demographic characteristics, lifestyle risk factors, and personal medical history. Anthropometric measures were obtained by trained staff with participants in light clothing without shoes. During each visit, body weight and height were measured twice to the nearest 0.1 kg and 0.1 cm, respectively. The mean values of height and weight were used to estimate body mass index (BMI), which was calculated as weight in kilograms divided by height in meters squared.

BHS participants were instructed to fast for 12 hours prior to blood sample collection. Serum creatinine level was measured by Laboratory Corporation of America (LabCorp, Burlington, NC) using the kinetic Jaffe method. eGFRcr was calculated using the 2009 CKD-EPI equation (Levey et al. 2009).

Among MESA-Kidney participants, covariable and phenotype data were also collected using stringent protocols (Inker et al. 2016). mGFR was determined through gold-standard plasma clearance of iohexol, using previously described methods (Inker et al. 2016).

2.5 Statistical Methods

Characteristics of BHS and MESA-Kidney participants were presented as means and standard deviations (SDs) for continuous variables and as percentages for categorical variables.

Multivariable linear regression models were used to analyze associations between each metabolite (exposure) and untransformed eGFRcr (outcome) among BHS participants, after adjustment for age, gender, BMI, education, cigarette smoking, and alcohol drinking. Analyses were performed according to race, and in a combined analysis after additional adjustment for race. A Bonferroni corrected alpha threshold ($0.05/1202=4.2\times10^{-5}$) was used to provide the best control of Type 1 error in multiple testing. To ensure that findings were generalizable across populations, metabolite findings were considered robustly significant if

they were significant at the Bonferroni corrected level in the combined analysis and in either race with a consistent effect direction and nominal significance ($p<0.05$) in the other race. Sensitivity analyses were conducted with additional adjustment for diabetes status and after exclusion of participants with CKD (defined as eGFRcr<60 mL/min/1.73 m^2). An identified association was considered novel if the metabolite had not previously been reported to associate with kidney function phenotypes (eGFR, eGFR decline, incident CKD, prevalent CKD, CKD subtypes compared to healthy controls, diabetic nephropathy, albuminuria, ESRD, or renal failure) in prior human or animal metabolomics studies (conducted using serum, plasma, or urine). The correlations between metabolite m/z and eGFRcr-metabolite association statistics (p value and beta coefficient) were assessed using Spearman's method.

Novel metabolite associations that were robustly identified in BHS were tested for replication among MESA-Kidney participants, after adjustment for race, age, gender, BMI, education, cigarette smoking, and alcohol drinking. The same criteria and cut-points were used for categorical variables among BHS and MESA-Kidney participants. A Bonferroni corrected alpha threshold for 131 metabolites tested ($0.05/131=3.8\times10^{-4}$) was used to control for multiple testing in the replication sample.

All statistical analyses were performed using SAS (version 9.4; SAS Institute, Cary, NC) and R (version 3.4.3).

3. Results

BHS and MESA-Kidney participant characteristics are presented in Table 1. The cohorts were similar in racial composition, but different in age, GFR range, and method of determining GFR. A flow chart depicting the numbers of participants and metabolites at each stage of the analysis is presented in Figure 1.

In total, 334 metabolites were robustly associated with eGFRcr among BHS participants, representing 28% of the metabolites tested; 330 were negatively associated with eGFRcr and 4 were positively associated with eGFRcr (Figure 2). The pathways and sub-pathways of metabolites robustly identified in BHS are presented in Online Resource 2. Of these metabolites, 242 had novel associations with kidney function, including 131 with known chemical identities (Online Resource 3) and 111 with unknown chemical identities. The remaining 92 metabolites were previously reported to associate with kidney function phenotypes and included 12 known uremic solutes (from the European Uremic Toxins Working Group) (Online Resource 4). Results were similar in sensitivity analyses with additional adjustment for diabetes status (data not shown), which was expected, as eGFR did not differ according to diabetes status among BHS participants ($p=0.77$). Results were also similar in sensitivity analyses conducted after excluding participants with CKD (Online Resource 5).

The 131 metabolites with novel kidney function associations and known chemical identities were carried forward for replication among MESA-Kidney participants. Among them, 105 had been measured in MESA-Kidney participants; 51 and 65 of these showed evidence of replication using mGFR and eGFRcr respectively (Online Resource 6). In addition to having

novel kidney function associations, 12 of these metabolites were in sub-pathways also not previously associated with kidney function (Table 2). These 12 metabolites all had above-the-detection values in the majority of BHS participants (78% to 100%) and all had been tested as continuous variables in both BHS and MESA-Kidney. All 12 of these metabolites replicated using both mGFR and eGFRcr.

The m/z of metabolites examined in this study ranged from 74.07 Da/z to 972.73 Da/z. Metabolites with smaller m/z, when compared to those with larger m/z, were more likely to have smaller p-values ($r=-0.27$; $p=2.2\times10^{-21}$) (Online Resource 7) and larger negative effect estimates ($r=0.21$; $p=1.3\times10^{-13}$) (Online Resource 8). Concordance with this trend varied across pathways (Online Resource 9; Online Resource 10).

4. Discussion

The current study identified 12 metabolites with novel kidney function associations, in sub-pathways that were also not previously associated with kidney function. Novel sub-pathways included carbohydrate (advanced glycation end-product (AGE)), cofactor and vitamin (ascorbate & aldarate, nicotinate & nicotinamide, tocopherol, and vitamin A), lipid (mevalonate and fatty acid & branched-chain amino acids (BCAA)), nucleotide (pyrimidine, thymine containing), and xenobiotic (drug and bacterial/fungal) sub-pathways, along with two which have not been fully characterized. This study also identified 39 novel metabolites in sub-pathways previously associated with kidney function. Similar to classic glomerular filtration studies (Brenner et al. 1978; Haraldsson et al. 2008), metabolites with lower m/z tended to be more statistically significant. Similar to prior kidney function metabolomics studies (E. P. Rhee et al. 2013; Sekula et al. 2016; Toyohara et al. 2010; Yu et al. 2014), the vast majority of significant metabolites from all pathways studied tended to negatively associate with GFR, including known uremic toxins such as creatinine, dimethylarginine, and pseudouridine. These data suggest that many of the identified metabolites are likely a consequence and not a cause of reduced kidney function. However, because the discovery stage of this study was carried out in a healthy population with low CKD prevalence, these findings also suggest that the identified metabolites may serve as early and clinically relevant indicators of declining kidney function. Additionally, this study found significant relationships between 12 uremic solutes, substances known to be retained in acute kidney injury and CKD (Lisowska-Myjak 2014), and eGFRcr in the discovery cohort, and replicated many of the previously published findings from MESA-Kidney in BHS. These metabolites may begin accumulating well before clinical onset of CKD.

The N6-carboxymethyllysine (CML) metabolite from the carbohydrates pathway was identified in the current study. This analyte has been widely employed as a marker for AGEs in food analysis. High levels of AGEs are present in the Western diet, especially in foods processed at high temperatures (Semba, Nicklett, et al. 2010). CML and other AGEs can also form in the body endogenously (Semba, Nicklett, et al. 2010). CML has been previously associated with eGFRcr and CKD in aging studies (Semba et al. 2009; Semba, Fink, et al. 2010), and diabetic kidney disease in American Indians (Saulnier et al. 2016). AGEs have been implicated in kidney disease progression and toxicity to multiple organ systems, and CVD mortality in CKD (Mallipattu and Uribarri 2014). Although this is the

first untargeted metabolomics study to implicate the CML metabolite in kidney function, the totality of evidence from disparate lines of research support future studies to examine the effects of lowering exogenous AGEs on kidney function (Mallipattu and Uribarri 2014).

Four metabolites from the cofactors and vitamins pathway were identified by this analysis. Gulonate, in the ascorbate and aldarate sub-pathway, has been previously implicated in kidney function, as the reduction of glucuronate to gulonate occurs in the kidney cortex during inositol catabolism (Barski et al. 2005). Quinolinate, in the nicotinate and nicotinamide sub-pathway, has been found in uremic human serum (Niwa et al. 1991) and plasma (Pawlak et al. 2009), and has been associated with CKD severity (Schefold et al. 2009). Gamma carboxyethyl hydroxychroman (CEHC) glucuronide*, in the tocopherol sub-pathway, was recently suggested as a novel uremic solute in a small study of 9 cases and 6 controls (Tanaka et al. 2015). Retinol (vitamin A), had the strongest measure of association among the metabolites in this study's main findings, and has previously been shown to have high levels in CKD patients (Hamamura et al. 2016; Handelman and Levin 2011). A recent mouse study demonstrated reductions in renal dysfunction when 5/6 nephrectomy mice were fed a retinol free diet (Hamamura et al. 2016). Future studies may be warranted to explore the longitudinal effects of dietary vitamin A on kidney function.

Two metabolites from the lipids pathway were also related to kidney function. Methylmalonate (MMA), in the fatty acid (also BCAA) sub-pathway, has been suggested as a novel uremic toxin in a small study of adult hemodialysis patients and controls (Eugene P Rhee et al. 2010), and induces kidney damage when administered to rats (Schuck et al. 2013). MMA is known to build up in methylmalonic acidemia/aciduria, a disease characterized by progressive neurodegeneration and kidney failure (Zsengellér et al. 2014), and has been suggested to affect the kidney through megamitochondria formation in the proximal tubules and electron transport chain dysfunction (Zsengellér et al. 2014). Further research on the potential for MMA lowering to serve as a target for kidney function therapies is warranted. Three-hydroxy-3-methylglutarate, in the mevalonate sub-pathway, is primarily metabolized in the kidney (Wiley et al. 1977), and mevalonate metabolism has been shown to have a direct relationship with kidney failure (Scoppola et al. 1997). Three-hydroxy-3-methylglutarate may also serve as a marker of early stage renal dysfunction.

Furthermore, this study identified 3-aminoisobutyrate, which is in the nucleotide pathway and the pyrimidine, thymine containing sub-pathway, and is also known as β-aminoisobutyric acid (BAIBA). It was identified in uremic serum in 1976 (Gejyo et al. 1976) and has also been discussed in cardiometabolic (Katakami et al. 2019; Rietman et al. 2016; Roberts et al. 2014) studies. N-methylpipecolate, in the xenobiotic pathway and the bacterial/fungal sub-pathway has also been examined in few relevant studies. Hydroquinone sulfate, in the xenobiotic pathway and drug sub-pathway, has environmental exposures and been linked to renal tumors and spontaneous chronic progressive nephropathy in rats (Hard et al. 1997). The two partially characterized molecules identified (glycine conjugates of $C_{10}H_{12}O_2$* and $C_{10}H_{14}O_2$ (1)*) have not been previously examined, and thus their clinical importance is less clear. Further examination of these compounds, with potential to serve as biomarkers of reduced kidney function, is warranted.

Molecular size has been previously associated with renal filtration (Brenner et al. 1978; Haraldsson et al. 2008). Small solutes, such as urea (60 Da, 1.8 Å), glucose (180 Da, 8.5 Å), and inulin (5,200 Da, 14 Å) pass freely across glomerular capillary endothelia, while large solutes, such as albumin (69,000 Da, 36 Å) are blocked in individuals with healthy kidney function. Metabolites associated with kidney function in the current analysis tended to be smaller than those not associated with GFR. As expected, metabolites associated with non-renal clearance, including clearance in bile (from the primary or secondary bile acid and hemoglobin & porphyrin sub-pathways) were not associated with eGFRcr. These findings suggest that many of the identified metabolites may be markers of kidney filtration and not etiologically relevant in kidney function decline.

This study had several important strengths. The BHS and MESA-Kidney designs provided a unique opportunity to study this relationship in well phenotyped racially diverse cohorts. Differences in mean kidney function and methods of GFR determination between the two studies allowed for identification of important metabolites that are likely to be generalizable across varying populations. Examination of both mGFR and eGFRcr in MESA-Kidney provided more rigorous replication than utilizing either method alone. This study tested a larger number of metabolites for association with kidney function than many other previous metabolomics studies. In addition, the identification of known uremic solutes adds credence to the robustness of the metabolites reported here.

This study also has several limitations. The cross-sectional study design does not allow examination of whether the identified metabolites precede or follow kidney function decline. We recognize that our decision to prioritize generalizability across ancestry groups may have led to missing ancestry specific signals. There were 26 novel metabolites identified in the discovery analysis in BHS that were not available for replication in MESA. Although not formally replicated, these metabolites had compelling evidence in different race groups and are thus worth following up in further studies. In addition, although metabolites identified in serum and plasma have been shown to be highly correlated (Breier et al. 2014), there is the possibility that some metabolites that were associated with kidney function may not have replicated due to differences in serum and plasma metabolite profiling; therefore it is possible that there are false negative associations due to not using the same biofluid (Kaluarachchi et al. 2018). There were also 111 novel metabolites identified by this study that may be relevant to kidney function but, as of yet, have unknown chemical identities.

5. Conclusion

In aggregate, the current study identified 51 metabolites with novel kidney function associations in both BHS and MESA-Kidney participants, including 12 metabolites in sub-pathways that have not been reported by previous kidney function metabolomics studies. In addition, the discovery stage analysis replicated findings for 92 metabolites from previous studies, and identified 111 metabolites with novel kidney function associations in unknown pathways. Significant metabolites tended to be inversely associated with kidney function and smaller in m/z than non-significant metabolites. While longitudinal research is warranted to discriminate upstream predictors from downstream consequences of kidney function,

metabolites identified here have potential to serve as early, clinically relevant biomarkers of reduced kidney function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AGE	Advanced glycation end-product
BAIBA	β-aminoisobutyric acid
BCAA	Branched-chain amino acids
BHS	Bogalusa Heart Study
BMI	Body mass index
CEHC	Carboxyethyl hydroxychroman
CKD	Chronic kidney disease
CML	N6-carboxymethyllysine
CVD	Cardiovascular disease
eGFR	Estimated glomerular filtration rate
eGFRcr	Creatinine based eGFR
GFR	Glomerular filtration rate
MESA	Multi-Ethnic Study of Atherosclerosis
mGFR	Measured glomerular filtration rate
MMA	Methylmalonate
SD	Standard deviation
UPLC-MS/MS	Ultrahigh performance liquid chromatography-tandem mass spectrometry

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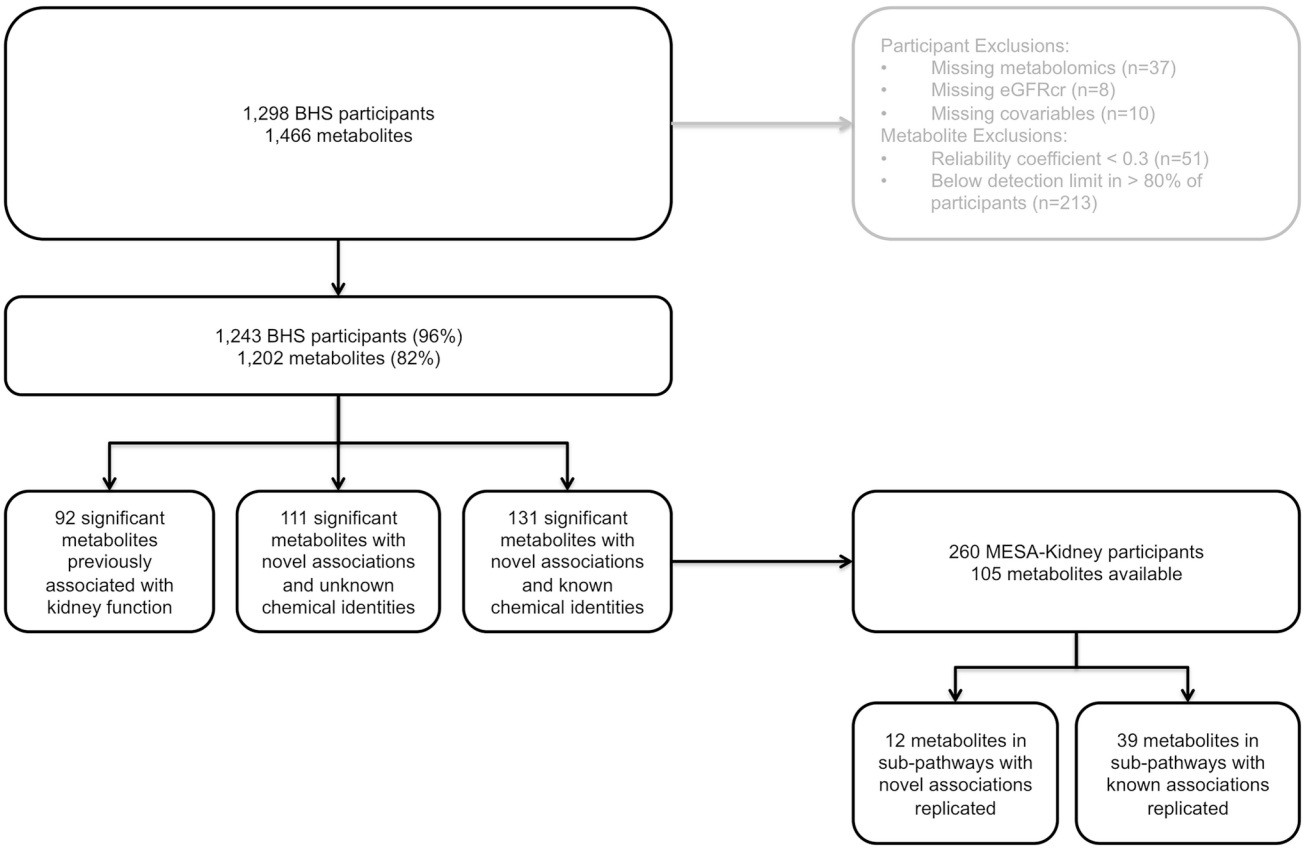

Fig. 1. Analysis flow chart

Summary of analytic workflow showing numbers of participants and metabolites at each stage of the analysis. BHS=Bogalusa Heart Study; eGFRcr=creatinine-based estimated glomerular filtration rate; MESA=Multi-Ethnic Study of Atherosclerosis.

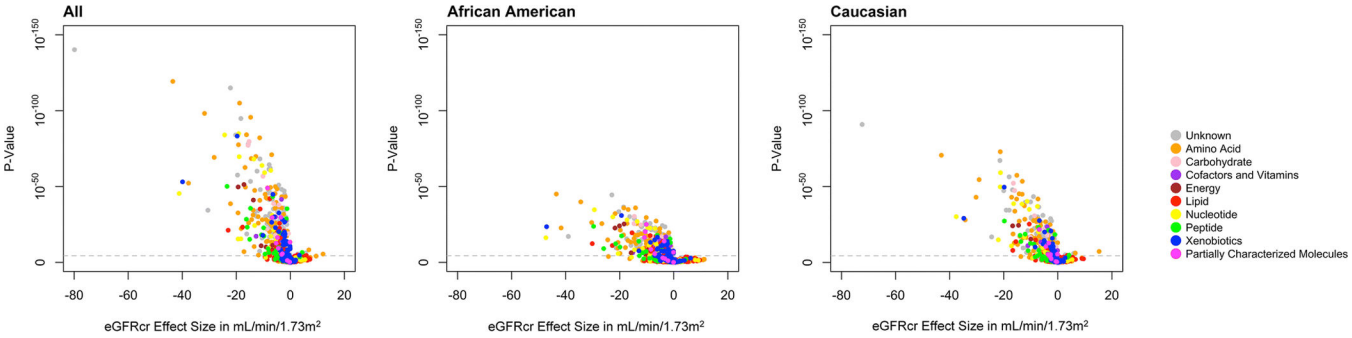

Fig. 2. P-Values against eGFRcr effect size among BHS participants

Grey=unknown; orange=amino acid; light pink=carbohydrate; purple=cofactor and vitamin; brown=energy; red=lipid; yellow=nucleotide; green=peptide; blue=xenobiotic; dark pink=partially characterized molecule. BHS=Bogalusa Heart Study; eGFRcr=creatinine-based estimated glomerular filtration rate.

Creatinine is not shown in this figure (All: $\beta=-60.3$ and $p=7.0\times10^{-271}$, African American: $\beta=-60.8$ and $p=5.8\times10^{-97}$, Caucasian: $\beta=-60.3$ and $p=9.0\times10^{-170}$).

The grey dashed line indicates the Bonferroni corrected α threshold ($0.05/1202=4.2\times10^{-5}$) that was used for this study.

Table 1.

Characteristics of BHS and MESA-Kidney Study Participants

	BHS (N=1243)	MESA-Kidney (N=260)
Age, years, mean (SD)	48 (5)	71 (9)
Male, n (%)	511 (41)	138 (53)
White, n (%)	816 (66)	140 (54)
Post-high school education, n (%)	610 (49)	196 (75)
Smoking, n (%)		
Never	634 (51)	123 (47)
Former	363 (29)	112 (43)
Current	246 (20)	25 (10)
Drinking, n (%)		
Never	154 (12)	27 (10)
Former	397 (32)	62 (24)
Current	692 (56)	171 (66)
BMI, kg/m^2, mean (SD)	31.4 (7.8)	29.8 (5.5)
SBP, mmHg, mean (SD)	123.3 (16.8)	125.5 (19.7)
Hypertension[*], n (%)	773 (62)	117 (45)
Glucose, mg/dL, mean (SD)	107.8 (38.5)	96.5 (26.2)
Diabetes[†], n (%)	208 (17)	31 (12)
GFR[‡], mL/min/1.73 m^2, mean (SD)	94 (17)	73 (19)
CKD (GFR[‡]<60 mL/min/1.73 m^2), n (%)	39 (3)	63 (24)

BHS=Bogalusa Heart Study, BMI=body mass index, CKD=chronic kidney disease, DBP=diastolic blood pressure, GFR=glomerular filtration rate, MESA=Multi-Ethnic Study of Atherosclerosis, SBP=systolic blood pressure, SD=standard deviation.

[*]Hypertension was defined as SBP≥130 mmHg, DBP≥80 mmHg, or use of antihypertensive medication.

[†]Diabetes was defined as fasting plasma glucose≥126 mg/dL or use of diabetes medication.

[‡]Estimated GFR in BHS, measured GFR in MESA-Kidney

Table 2.

Novel Metabolites in Novel Sub Pathways

Metabolite	Sub-Pathway	ID Level*	Detected (%)	BHS, eCFRcr						MESA, mGFR (N=260)	
				African American (n=427)		Caucasian (n=816)		All BHS (N=1,243)			
				Beta (SE)	P**	Beta (SE)	P**	Beta (SE)	P**	Beta (SE)	P***
Carbohydrate											
N6-carboxymethyllysine	Advanced Glycation End-product	1	89	−4.44 (0.49)	8.58×10^{-18}	−3.92 (0.43)	1.44×10^{-18}	−4.25 (0.32)	1.46×10^{-38}	−5.59 (1.11)	8.63×10^{-7}
Cofactors and Vitamins											
Gulonate	Ascorbate and Aldarate Metabolism	2	94	−2.92 (0.33)	1.35×10^{-17}	−3.95 (0.38)	2.84×10^{-24}	−3.27 (0.23)	2.59×10^{-42}	−8.57 (1.06)	2.38×10^{-14}
Quinolinate	Nicotinate and Nicotinamide Metabolism	1	100	−5.21 (0.63)	1.86×10^{-15}	−6.28 (0.81)	3.37×10^{-14}	−5.48 (0.46)	1.06×10^{-30}	−6.34 (0.94)	1.17×10^{-10}
Gamma-CEHC glucuronide	Tocopherol Metabolism	2	78	−1.29 (0.18)	8.60×10^{-12}	−3.12 (0.31)	4.85×10^{-22}	−1.61 (0.14)	2.22×10^{-28}	−3.43 (0.58)	9.04×10^{-9}
retinol (Vitamin A)	Vitamin A Metabolism	1	100	−15.15 (2.93)	3.64×10^{-7}	−11.06 (1.59)	8.29×10^{-12}	−12.68 (1.45)	5.85×10^{-18}	−16.40 (3.52)	5.17×10^{-6}
Lipid											
Methylmalonate (MMA)	Fatty Acid Metabolism (also BCAA Metabolism)	1	90	−11.16 (1.75)	4.92×10^{-10}	−1.62 (0.54)	2.67×10^{-3}	−2.95 (0.56)	1.43×10^{-7}	−7.41 (1.49)	1.25×10^{-6}
3-hydroxy-3-methylglutarate	Mevalonate Metabolism	1	100	−6.51 (0.72)	7.34×10^{-18}	−10.56 (0.97)	5.00×10^{-26}	−7.59 (0.53)	1.04×10^{-42}	−10.43 (1.63)	7.36×10^{-10}
Nucleotide											
3-aminoisobutyrate	Pyrimidine Metabolism, Thymine containing	1	100	−5.72 (1.01)	2.46×10^{-8}	−4.72 (0.89)	1.39×10^{-7}	−5.27 (0.64)	5.75×10^{-16}	−6.06 (1.53)	9.29×10^{-5}
Xenobiotic											
N-methylpipecolate	Bacterial/ Fungal	1	95	−4.75 (0.68)	1.16×10^{-11}	−2.54 (0.26)	2.14×10^{-21}	−2.98 (0.26)	5.77×10^{-29}	−2.25 (0.58)	1.34×10^{-4}
Hydroquinone sulfate	Drug	1	91	−2.25 (0.30)	4.58×10^{-13}	−1.96 (0.27)	3.82×10^{-13}	−2.14 (0.19)	2.56×10^{-27}	−2.70 (0.61)	1.54×10^{-5}
Partially Characterized Molecules											
Glycine conjugate of C10H1202		2	89	−10.14 (0.90)	9.58×10^{-26}	−6.88 (0.68)	1.81×10^{-22}	−8.43 (0.54)	8.96×10^{-50}	−7.40 (1.27)	1.60×10^{-8}
Glycine conjugate of C10H1402 (1)		2	97	−10.53 (1.06)	6.35×10^{-21}	−4.44 (0.66)	3.57×10^{-11}	−6.73 (0.57)	2.75×10^{-30}	−6.71 (1.43)	4.55×10^{-6}

BHS=Bogalusa Heart Study; eGFRcr=creatinine-based estimated glomerular filtration rate; mGFR=measured glomerular filtration rate; MESA=Multi-Ethnic Study of Atherosclerosis; SE=standard error.

*Identification level. Level 1: Identified metabolites; Level 2: Putatively annotated compounds without chemical reference standards.

** A stringent Bonferroni correction for testing 1,202 metabolites was employed, using an α-threshold of 4.16×10^{-5} (0.05/1202) to determine statistical significance.

*** A stringent Bonferroni correction for testing 131 metabolites was employed, using an α-threshold of 3.82×10^{-4} (0.05/131) to determine statistical significance.