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Metabolomics and Renal Disease

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Abstract

Purpose of review—This review summarizes recent metabolomics studies of renal disease, outlining some of the limitations of the literature to date.

Recent findings—The application of metabolomics in nephrology research has expanded from initial analyses of uremia to include both cross-sectional and longitudinal studies of earlier stages of kidney disease. Although these studies have nominated several potential markers of incident CKD and CKD progression, lack of overlap in metabolite coverage has limited the ability to synthesize results across groups. Further, direct examination of renal metabolite handling has underscored the substantial impact kidney function has on these potential markers (and many other circulating metabolites). In experimental studies, metabolomics has been used to identify a signature of decreased mitochondrial function in diabetic nephropathy and a preference for aerobic glucose metabolism in PKD; in each case, these studies have outlined novel therapeutic opportunities. Finally, as a complement to the longstanding interest in renal metabolite clearance, the microbiome has been increasingly recognized as the source of many plasma metabolites, including some with potential functional relevance to CKD and its complications.

Summary—The high-throughput, high-resolution phenotyping enabled by metabolomics technologies has begun to provide insight on renal disease in clinical, physiologic, and experimental contexts.

Keywords

Metabolomics; metabolite profiling; renal metabolism

INTRODUCTION

Metabolomics, or metabolite profiling, refers to the systematic analysis of metabolites (i.e., sugars, amino acids, organic acids, nucleotides, bile acids, acylcarnitines, lipids, etc.) in a biologic specimen [1, 2]. Metabolomic approaches are particularly promising in nephrology research because of the broad impact kidney function has on circulating metabolite levels and because metabolites may themselves play functional roles in CKD pathogenesis and its complications. These concepts are not new – indeed, decades of research utilizing traditional

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tools in biochemistry have outlined numerous plasma metabolite alterations in uremia [3, 4]. Instead, technological advances, for example in chromatography and mass spectrometry, have simply enabled more metabolites to be measured simultaneously, more rapidly, and in smaller sample volumes. These increases in breadth and throughput have had at least two salutary effects. First, and consistent with the rationale of all “-omic” approaches, they have enabled unbiased examinations of blood and urine from individuals with kidney disease, in some cases highlighting novel metabolite perturbations. Second, they have catalyzed examination of large cohorts, enhancing statistical power for biomarker studies of cross-sectional phenotypes and longitudinal outcomes. This article provides an update on publications within the last ~18 months that illustrate these points, reviewing both clinical and experimental renal metabolomics studies as well as select non-metabolomics studies that are of interest to the field. With the goal of providing a coherent synthesis primarily anchored around adult CKD, metabolomics studies of renal transplantation [5, 6], renal cell carcinoma [7], and pediatric nephrology [8] are not discussed.

METABOLOMICS OVERVIEW

Although a comprehensive review of available technologies is beyond the scope of this article, Fig. 1 outlines notable strengths and limitations of select metabolomic approaches. For a detailed overview of the technical aspects of metabolomics, the reader is directed to other recent manuscripts [9-11]. Nuclear magnetic resonance spectroscopy (NMR) uses the magnetic properties of select atomic nuclei to determine the structure and abundance of metabolites in a specimen. It requires relatively little sample preparation and does not require up-front chromatography. However, because of limited sensitivity and high data complexity, unambiguous identification is typically limited to <100 metabolites. Mass spectrometry (MS)-based approaches, generally coupled to an array of separation techniques including liquid chromatography (LC) and gas chromatography (GC), have higher sensitivity and rely on a combination of chromatographic separation and mass-to-charge ratio (m/z) resolution for metabolite identification. By permitting passage of a select precursor ion, inducing precursor ion fragmentation (into product ions), and then monitoring for a defined product ion across its three quadrupoles, triple quadrupole instruments can be particularly sensitive and specific. However, because each metabolite's precursor and resultant product ions must be known a priori, these triple quadrupole based ‘tandem’ MS methods are restricted to *targeted* analyses of ~100s of metabolites of established identity. By contrast, *nontargeted* methods that measure ~1000s of metabolite peaks (only a subset of which have assigned identities) generally utilize time-of-flight and ion trap mass spectrometers. Instead of monitoring pre-specified precursor ion-product ion pairs, these instruments leverage their superior mass accuracy relative to triple quadrupole instruments to facilitate metabolite identification, with current instruments providing m/z resolution to the fourth decimal place. Whereas the majority of renal metabolomics studies to date have applied NMR or *targeted* MS-based methods, increasing interest is being directed towards *nontargeted* MS-based approaches, in parallel with efforts to assign unambiguous identities to many of the resulting unknown analyte peaks.

CLINICAL STUDIES

Given long-standing interest in small molecules as uremic toxins, initial applications of metabolomics in nephrology research examined plasma or dialysate from individuals with ESRD [12-15]. Although these studies generated a broad view of the metabolite alterations that accompany ESRD, they were unable to identify the alterations of greatest biologic or clinical significance. First, because of the advanced and widespread physiologic derangements in ESRD, these studies could not disentangle the relative contributions of decreased urinary clearance, hemodialysis, underlying comorbidities, impaired nutrition, changes in the microbiome, etc. on the metabolome. Second, the cross-sectional nature of these studies did not permit association of select metabolite alterations with longitudinal outcomes of interest. Recent studies have begun to address some of these limitations.

Cross-sectional studies

Metabolomic surveys of earlier stages of CKD have provided insight on how metabolite alterations vary across levels of kidney function [16-19]. Duranton *et al.* used a commercial LC-MS metabolomics vendor to measure amino acids and amino acid derivatives in plasma and urine from 52 individuals across different stages of CKD and plasma only from 25 individuals on dialysis [20]. By examining paired plasma and urine, they were able to determine that uremic elevations in plasma ADMA are attributable to decreased urinary clearance, whereas elevations in plasma citrulline are due to overproduction. Posada-Ayala *et al.* used NMR based discovery and LC-MS based validation to demonstrate that a panel of seven urinary metabolites could distinguish 31 individuals with CKD from 30 individuals without CKD [21]. Although plasma samples were not examined in this study, the finding of elevated urinary levels of trimethylamine-N-oxide (TMAO), guanidoacetate, and phenylacetylglutamine in CKD subjects suggests that these established uremic retention solutes are overproduced in CKD.

Longitudinal studies

Because early markers may provide more clinical and biologic insight than changes that occur in later stages of disease, recent studies have examined whether baseline metabolite profiles are associated with future CKD or CKD progression. Yu *et al.* used a commercial LC-MS/GC-MS based platform to measure 204 metabolites in plasma from 1921 African-American participants of the Atherosclerosis Risk in Communities study [22]. The authors found that lower levels of 5-oxoproline and 1,5-anhydroglucitol were associated with new onset CKD, as defined by an eGFR < 60 mL/min per 1.73 m² and < 75% of baseline, or a CKD-related hospitalization or death. The authors speculate that higher levels of 5-oxoproline may report on increased glutathione bioavailability. The association between lower 1,5-anhydroglucitol levels and incident CKD is interesting because this metabolite is primarily derived from diet and may be a marker of short-term glycemic control [23]. Notably, this study did not replicate findings from prior studies of incident CKD performed in the Framingham Heart Study (FHS) [24] and the KORA Study [25], both of which are comprised primarily of Caucasian study participants. The strongest signal shared in these cohorts was an association between higher levels of tryptophan metabolites of the kynurenine pathway and incident CKD. To what extent the differences across these studies

reflects differences in platform coverage, sample or data quality, or racial composition is uncertain [26].

In a study of early diabetic nephropathy, Pena *et al.* applied a commercial LC-MS metabolomics platform to plasma and urine from 90 individuals [27]. Whereas no metabolites were associated with the transition from normo- to microalbuminuria, several plasma (histidine and butenoylcarnitine) and urine (hexose, glutamine, and tyrosine) metabolites were associated with the transition from micro- to macroalbuminuria. Interestingly, urine metabolites increased the AUC of the receiver operating curve and the integrated discrimination index more than plasma metabolites over a reference prediction model that included eGFR and albuminuria only. Larger studies will be required to clarify the relative predictive value of plasma versus urine metabolites, and to what extent this depends on whether outcomes are defined in relation to blood or urine based end-points.

Whereas studies of incident or early CKD compare individuals who do or do not cross an eGFR or albuminuria threshold, identifying markers of a hard endpoint like progression to ESRD is arguably of greater interest. Using a nested case-control study, Niewczas *et al.* [28] used a commercial LC-MS/GC-MS based platform to measure 262 plasma metabolites in 40 individuals with diabetic nephropathy who progressed to ESRD and 40 individuals with diabetic nephropathy who did not progress over 8-12 years of follow-up. They identified two major classes of metabolite associations with case status. First, *increased* levels of several previously identified uremic retention solutes, including p-cresol sulfate, several polyols, and nucleotide derivatives were associated with an increased risk of progression to ESRD, even after adjusting for baseline differences in HgbA1c, proteinuria, and eGFR. Conversely, *depletion* of several essential amino acids and their derivatives were associated with increased risk following multivariable adjustment. As discussed below, these findings may still reflect differences in renal health (e.g. secretory capacity) between cases and controls not captured by serum creatinine, rather than implicate these molecules as causal factors. The investigators also examined the stability of metabolite signals within individuals over time. More specifically, they profiled a second plasma sample from a subset of 10 individuals obtained ~2 years after baseline and found that uremic solute levels but not most essential amino acid levels were strongly correlated at both time points. In the renal literature to date, information on repeated metabolite measures in the same individuals over time is generally lacking.

Physiologic studies

Two recent studies of samples obtained via invasive catheterization have directly examined the impact of human kidney function on the plasma metabolome. First, LC-MS based profiling of plasma obtained from the aorta and renal vein of nine individuals cataloged the mean venous to arterial ratio of 225 metabolites [24]. Select markers of future CKD in the FHS were found to decrease substantially more than creatinine from aorta to vein, either because they undergo tubular secretion or metabolism within the organ. These findings suggest that integrating markers of different renal functions, including glomerular filtration, tubular secretion, and metabolism provides a more complete picture of renal health and prognosis (Fig. 2). Further, these findings illustrate why metabolites associated with disease

outcomes may still be reporting on baseline differences in kidney function, even if their statistical association with disease persists after adjusting for GFR. For example, for any given plasma creatinine elevation, uremic solutes that undergo significant tubular secretion can be elevated several fold higher [29]. Notably, although the majority of polar analytes were found to decrease from aorta to renal vein, some metabolite levels were actually higher in the renal vein than aorta, signifying net renal release. Whether loss of renal anabolic capacity contributes to select metabolite depletion in CKD or ESRD requires further study.

In a second study designed to ascertain the human kidney's impact on circulating metabolites, LC-MS based metabolite profiling was performed on venous effluent obtained from both kidneys of 16 individuals with unilateral renal artery stenosis [30]. This study design permitted each individual to be used as his or her own control, circumventing the confounding that arises in comparisons across individuals. Surprisingly, no metabolite differences were identified in venous plasma from stenotic versus contralateral kidneys, despite a measurable loss of kidney volume and blood flow on the affected side. These findings suggest that the kidneys are able to adjust to changes in blood flow over time to maintain a range of metabolic functions.

EXPERIMENTAL STUDIES

Although clinical metabolomics studies have begun to identify potential biomarkers of CKD and CKD progression, they have largely been unable to implicate specific metabolic pathways in disease pathogenesis. Sharma *et al.* have pushed this boundary by utilizing a combination of clinical and experimental metabolomic approaches to study diabetic nephropathy. Using GC-MS, these investigators quantified 94 metabolites in urine obtained from a total of 158 individuals, including subjects with diabetes and CKD, diabetes without CKD, and healthy controls [31]. A decrease in the urine levels of 13 metabolites, many potentially related to mitochondrial function, was found to be associated with diabetic CKD. Using these cross-sectional metabolite findings as a springboard, the authors then used a variety of approaches including cytochrome C oxidase immunostaining and PGC1 α mRNA profiling in tissue and exosome mtDNA quantitation in urine to demonstrate decreased mitochondrial activity in human diabetic kidney disease. In a parallel study, the authors further showed that mitochondrial biogenesis and activity of AMPK, a major energy-sensing enzyme, are reduced in kidneys from mice with streptozotocin-induced diabetes [32]. In turn, a small molecule AMPK activator was able to rescue mitochondrial biogenesis and reduce albuminuria in streptozotocin treated mice. Urine metabolite profiles and their response to AMPK activation in this model, however, were not assessed. Using NMR, Stec *et al.* found decreased levels of several urine metabolites in models of type 1 (*eNOS*^{-/-} mice treated with streptozotocin) and type 2 diabetes (*eNOS*^{-/-} *db/db* mice) compared to controls [33], although without significant overlap (aside from aconitate) with the human findings described above. Whether these differences reflect the different platforms used, or whether mouse models can faithfully recapitulate the metabolite signatures of human kidney disease is an area of ongoing study [34].

Whereas metabolic alterations are expected in diabetes, one recent study has raised significant interest in altered glucose metabolism in PKD. Using NMR, Rowe *et al.* found

lower glucose and higher lactate concentrations in cultured media from mouse embryonic fibroblasts isolated from *Pkd1*^{-/-} embryos compared to cells isolated from *Pkd1*^{+/+} littermates [35]. These findings were associated with increased transcription of genes encoding glycolytic enzymes in the *Pkd1*^{-/-} cells, consistent with the metabolic switch to aerobic glycolysis exhibited by many tumor cells (Warburg effect). Further, the authors confirmed that this glucose dependence in cells was *Pkd1* dependent, and ¹³C isotope labeling studies demonstrated increased glucose uptake and conversion to lactate in cystic kidneys in a mouse model of PKD. Finally, the authors showed that inhibiting glycolysis with 2-deoxyglucose reduced cyst growth in two distinct mouse models of PKD. These elegant studies reveal a fundamental metabolic link between PKD cysts and neoplasia, i.e. a shift towards aerobic glycolysis, and motivate clinical studies of glycolysis inhibition in ADPKD [36].

Finally, in contrast to the relative paucity of metabolomics studies of human AKI [37], there have been several recent metabolomics studies of AKI in rodents, for example following ischemia reperfusion injury (IRI) and nephrotoxic drug exposure [38-40]. One study of note by Wei *et al.* applied a commercial LC-MS/GC-MS based platform to plasma, kidney cortex, and kidney medulla samples obtained from mice 2 hours, 48 hours, and 1 week after bilateral IRI [41]. In addition to outlining numerous metabolic perturbations that follow IRI, this study demonstrates how plasma provides an incomplete picture of organ-specific metabolism and illustrates how repeated measures over time provide insights not captured in a single snapshot.

MICROBIOME AND GUT-DERIVED METABOLITES

As studies continue to expand the impact of gut microbes on host health and disease, increasing interest has been directed towards the microbiome in nephrology research [42]. Gut microbiota are clearly an important contributor to the plasma metabolome, particularly in the context of uremia [43, 44]. This review highlights recent updates on select gut-derived metabolites relevant to kidney disease. Perhaps most interesting has been the emergence of short chain fatty acids (SCFAs), generated from the gut microbial fermentation of complex carbohydrates, as agonists for specific G-protein coupled receptors. For example, select SCFAs can modulate systemic inflammation through receptors (Gpr41 and Gpr43) expressed on immune cells [45] or by inhibiting histone deacetylases in macrophages [46]. Pluznick *et al.* have spearheaded studies demonstrating the expression of select olfactory receptors in mouse kidneys [47] and shown that that SCFAs act through both Olfr78 and Gpr41 to modulate renin release and blood pressure in mice [48]. Most recently, Andrade-Oliveira *et al.* have shown that intraperitoneal injection of SCFAs ameliorates IRI-induced AKI in mice [49], although the precise mechanism remains to be determined.

Studies highlighting plasma TMAO levels as a biomarker and potential causal factor in atherogenesis among individuals with normal kidney function has received considerable attention [50, 51]. It is unknown if these findings extend to ESRD, where TMAO levels are elevated ~3-fold above normal [52]. In an LC-MS based case-control study of 500 incident hemodialysis patients, baseline TMAO levels were not associated with 1-year cardiovascular mortality [53]. Plasma levels of indoxyl sulfate, another established uremic retention solute,

also had no association with 1-year cardiovascular mortality in this study. By contrast, plasma indoxyl sulfate levels were associated with a first heart failure event in a study of 258 prevalent hemodialysis patients [54]. In the longitudinal metabolomics studies cited above, plasma levels of indoxyl sulfate had no association with incident CKD [24] or with CKD progression (although p-cresol sulfate, another gut-derived uremic solute, was associated with disease progression) [28]. One important caveat to these studies is that most LC-MS based methods use organic solvents to precipitate out plasma proteins prior to sample analysis. This permits measurement of *total* plasma levels of indoxyl sulfate and other highly protein bound hydrophobic metabolites, whereas *free* metabolite levels may be of more biologic significance [55]. Nevertheless, a large randomized control trial of AST-120, a carbon adsorbent that lowers the absorption of indole and other gut-derived molecules, did not show a benefit for slowing CKD progression [56]. Whether alternative approaches such as using drugs to change gut flora [57] or increasing fiber intake [58] may offer clinical benefit via modulation of the plasma metabolome remains uncertain.

CONCLUSION AND FUTURE DIRECTIONS

Metabolomics studies have begun to outline metabolite alterations in blood and urine at different stages of CKD, nominate markers of disease progression, expand our view of renal metabolite handling, provide insight on cellular metabolism in diabetic nephropathy and PKD, and highlight a role for the gut microbiome in kidney disease. However, several challenges limit interpretation of the current literature (Table 1), particularly in regards to clinical biomarker studies. Perhaps most important, incomplete coverage of the metabolome and the lack of overlap in metabolite coverage by different platforms preclude the ability to fully synthesize findings across groups. These limitations will likely be addressed with ongoing improvements in MS sensitivity and mass accuracy [59], along with efforts to annotate currently unknown m/z peak identities and standardize reagents and nomenclature. These advances will yield data that is more amenable to meta-analysis across studies, thus permitting replication of signals across cohorts, pooling of relatively rare disease phenotypes (e.g. a specific etiology of CKD), and a better accounting of how factors like comorbidities, race, and geography impact the metabolome. Further, combined data sets will provide increased statistical power for the integration of metabolomics data with genomics and other functional genomics outputs [60] – in turn, these efforts will provide insight on the genetic determinants of select metabolite alterations and whether metabolite markers of kidney disease belong to causal pathways previously highlighted by GWAS or linkage studies. Finally, these efforts at an epidemiologic scale will need to be interpreted in combination with physiologic and experimental studies that provide more direct insight on the organ-specificity and potential mechanistic implications of select metabolite alterations. Ultimately, these various applications of metabolomics will seek to determine if select metabolites are clinically useful biomarkers of renal endpoints, play causal roles in human kidney disease, and/or reveal novel insights on the metabolic underpinnings of renal disease.

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KEY POINTS

- To date, no consistent metabolite signature of incident CKD or CKD progression has emerged.
- Current platforms generally provide incomplete and only partially overlapping coverage of the metabolome.
- Interpretation of metabolomics studies of disease prediction should consider the broad impact kidney function has on circulating metabolites and recognize that differences in kidney function are not fully captured by GFR.
- Recent studies in diabetic nephropathy and PKD demonstrate how metabolite signatures can provide insight into disease pathogenesis and therapeutic opportunities.
- SCFAs and other gut microbiota-derived metabolites have potential functional relevance in kidney disease.

Figure 1. Overview of Metabolomics Technologies

NMR is robust, requiring relatively little sample preparation and no chromatographic separation. However, because of limited sensitivity and high data complexity, unambiguous identification is typically limited to <100 metabolites. MS-based approaches have higher sensitivity and rely on a combination of chromatographic separation and mass-to-charge ratio (m/z) resolution for metabolite identification. In MS-based platforms, triple quadrupole instruments are generally used for *targeted* analyses, where ~100s of metabolites of known identity are measured, whereas time-of-flight and ion trap instruments are often used for *nontargeted* analyses of ~1000s of metabolite peaks (only a subset of which have assigned identities). Relative advantages (+) and disadvantages (–) of the different approaches are detailed in the figure.

Figure 2. Different Axes of Renal Health and Prognosis

Although kidney function is defined clinically by the excretion of select nitrogenous waste products, profiling of plasma from the aorta and renal vein has demonstrated the breadth and heterogeneity of renal metabolite handling [24]. Select markers of tubular secretion (kynurenic acid) and renal metabolism (citrulline and choline), which decreased more than creatinine across the renal circulation, were found to predict incident CKD in a community-based cohort, even after adjusting for eGFR, proteinuria, and other CKD risk factors. Metabolite structures were downloaded from www.hmdb.ca.

Table 1

Challenges in Metabolomics Study Interpretation

	Current limitations	Solutions or Future Directions
Metabolome coverage	<ul style="list-style-type: none"> given the wide range of metabolite size and polarity, different analytical approaches (Fig. 1) are required for different subsets of the metabolome different methods are biased towards different metabolite classes most studies of renal disease to date have employed <i>targeted</i> methods measuring ~100s of metabolites 	<ul style="list-style-type: none"> improvements in MS sensitivity and mass accuracy will increasingly facilitate <i>nontargeted</i> profiling of ~1000s of metabolites; however, unambiguous identification of individual MS peaks remains a challenge
Platform overlap	<ul style="list-style-type: none"> different analytical approaches result in non-overlapping metabolite coverage across studies 	<ul style="list-style-type: none"> even if individual metabolites are not shared, concordant signals along metabolic pathways may be evident across studies <i>nontargeted</i> platforms approaches should increase overlap in coverage
Quantitation	<ul style="list-style-type: none"> unlike NMR, MS-based methods are semi-quantitative; i.e. data are reported in arbitrary units that cannot be compared across studies 	<ul style="list-style-type: none"> stable isotope dilution permits absolute quantitation in MS; this increases cost and requires prior knowledge of metabolites to be quantitated
Static snapshots	<ul style="list-style-type: none"> metabolite levels at a single time point do not report on metabolic flux; e.g., a metabolite may be elevated because of increased flux upstream or decreased flux downstream in a given pathway 	<ul style="list-style-type: none"> interpretation of patterns of metabolite elevation and depletion, e.g. at key enzymatic nodes, can provide hints about flux through pathways tracking stable isotopes over time can quantify flux, although this is limited to cellular and (rarely) animal experiments
Biofluids	<ul style="list-style-type: none"> ease of access makes blood and urine attractive for biomarker studies, but these biofluids may not report on metabolism at the organ or cellular level most methods measure <i>total</i> metabolite levels in biofluids, whereas <i>free</i> metabolite levels may be of greater biologic interest 	<ul style="list-style-type: none"> a combination of human physiologic, animal-based, and cell-based studies is required to characterize renal metabolism for hydrophobic, highly protein bound metabolites, results should be interpreted carefully in relation to serum or urine albumin levels