The metabolomic quest for a biomarker in chronic kidney disease

Davies, R.

Original citation:
https://dx.doi.org/10.1093/ckj/sfy037

DOI: 10.1093/ckj/sfy037
ISSN: 2048-8505
ESSN: 2048-8513

Publisher: Oxford University Press (OUP)

© The Author(s) 2018. Published by Oxford University Press on behalf of ERA-EDTA.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Copyright © and Moral Rights are retained by the author(s) and/or other copyright owners. A copy can be downloaded for personal non-commercial research or study, without prior permission or charge. This item cannot be reproduced or quoted extensively from without first obtaining permission in writing from the copyright holder(s). The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the copyright holders.
The metabolomic quest for a biomarker in chronic kidney disease

Robert Davies

School of Biomedical and Healthcare Sciences, University of Plymouth School of Biological Sciences, Plymouth, UK

Correspondence and offprint requests to: Robert Davies; E-mail: robert.davies-8@students.plymouth.ac.uk

ABSTRACT

Chronic kidney disease (CKD) is a growing burden on people and on healthcare for which the diagnostics are neither disease-specific nor indicative of progression. Biomarkers are sought to enable clinicians to offer more appropriate patient-centred treatments, which could come to fruition by using a metabolomics approach. This mini-review highlights the current literature of metabolomics and CKD, and suggests additional factors that need to be considered in this quest for a biomarker, namely the diet and the gut microbiome, for more meaningful advances to be made.

Keywords: biomarkers, chronic kidney disease, diet, metabolomics

THE PROBLEM OF CHRONIC KIDNEY DISEASE

Globally, chronic kidney disease (CKD) has increased by 36.9% between 1990 and 2013 with increases in CKD due to diabetes by 106.5%, hypertension by 29.4% and other causes by 58.8% [1]. Global CKD prevalence is increasing with different rates between countries, ethnicities and sexes, reflecting health inequalities, and even within these categories there are differences with respect to CKD aetiology [1–3].

Although estimated glomerular filtration rate (eGFR), albuminuria and serum creatinine form part of the assessment along with clinical context and data [4, 5], there are limitations with the current diagnostic criteria. Estimation of GFR and creatinine is based on the Chronic Kidney Disease Epidemiology Collaboration creatinine equation, which requires a correction factor for sex and those of African–Caribbean or African background [5] and is dependent on muscle mass; therefore, those who embody extremes of muscle mass such as bodybuilders, amputees and those with sarcopenia or other muscle-wasting disorders may have exaggerated and erroneous results. Kidney biopsies are also used as diagnostic tools but are invasive, and require skilled professionals and resources to undertake [6].

Therefore, it would be beneficial to investigate other diagnostic measures to aid in further understanding CKD inception, progression and prognosis, to offer more suitable treatment options to patients and to advance and improve therapeutics [7]. Indeed, in 2016, the International Society of Nephrology identified key strategic points to enhance kidney-related research, of which diagnostic methods and CKD progression were highlighted [6].

As CKD is a condition of various aetiologies with complex networks of inter- and intra-molecular signalling, studies on CKD could utilize the ‘-omics’ approaches (Figure 1): genomics, transcriptomics, proteomics and metabolomics, which should enable the clinician and researcher to have a better understanding of the interconnecting genetic and molecular networks in CKD by how the disease affects different body systems and responses to stimuli such as diet, medication and the microbiome [8]. This mini-review will focus on contemporary human studies of CKD utilizing a metabolomics approach published between 2016 and 2017. The research strategy for this involved reviewing relevant
For metabolomics to be fruitful, metabolites need to be quantified. Biofluids and tissue samples can be used for metabolic analysis with technologies such as nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) coupled with a preparatory chromatographic separation step of the sample such as capillary electrophoresis MS (CE-MS), liquid chromatography MS (LC-MS) or gas-chromatography (GC-MS) (Table 2). NMR is an analytical technique that uses magnetic fields to yield molecular information. MS is a method that measures the mass-to-charge ratio of an intact ion and tandem MS (MS/MS) can be used to measure selected isolated ions that are then fragmented, and the mass-to-charge ratio of each fragment is measured and used for analysis [28]. Both methods require bioinformatic analysis for the data to be interpretable and meaningful. These methods provide information on identification and quantification of metabolites present in the sample. Additional factors such as sample preparation, sample matrix, and carryover effects should be considered when analysing and interpreting the data [24, 29]. For a more comprehensive review and analysis of metabolic techniques and methodology consult references [24–29].

This progress in technology has enabled the identification of endogenous and exogenous metabolites as potential disease biomarkers, which could place personalized and precision patient-centred medicine within reach [30]. Metabolomics can be used to identify metabolites from a range of samples and for CKD the most pertinent are blood and urine [31], with the dialysate fluid also offering potential benefits.

Blood

As the current diagnostics for CKD are not indicative of disease progression, Rhee et al. [9] investigated progression of CKD within a CKD cohort stratifying by stable and rapid decline based on eGFR slope. This study incorporated a mix of ethnic backgrounds and CKD aetiologies reflecting the phenotype of CKD, and suggested lower levels of threonine, methionine and arginine as potential biomarkers of renal dysfunction by analysing plasma samples processed by LC-MS. In a study by Kimura et al. [10] the authors aimed to identify prognostic biomarkers for CKD progression and mortality in participants with CKD Stages 3–5 over a 4-year period. Plasma samples were processed using CE and LC-MS from which 16 metabolites were identified with MasterHands software. These 16 metabolites were identified as intrinsic to variable metabolic pathways including nucleotides, glycolysis and amino acids, with others unidentified. Medical history was noted including CKD aetiology and presence of comorbidities, and some medications were listed but glycaemic agents were not. Furthermore, no changes in nutritional status, dietary intake or weight were reported; therefore, it is unknown whether the identified metabolites could arise from CKD or be derived from the diet, or gut microbiome as variation has been shown to occur both intra- and inter-individually in the blood metabolome largely due to dietary influences [32–34].

Lee et al. [11] attempted to identify prognostic biomarkers from blood serum comparing CKD patients with and without diabetes, versus a healthy control group using NMR spectroscopy analysis. Participants were placed in groups based on eGFR and diabetes diagnosis before enrolment, but the authors did not test participants in this study, which is a limitation as participants may have developed diabetes but have not yet been diagnosed. This study highlighted differences between healthy controls and the CKD groups with increases in...
Table 1. Summary of metabolomic studies included in this mini-review

<table>
<thead>
<tr>
<th>Proposed metabolite biomarkers</th>
<th>Study population group</th>
<th>Metabolomic platform</th>
<th>Biological matrix for metabolomic analysis</th>
<th>Study outcome</th>
<th>Bibliographic reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid, glucuronate, 4-hydroxymandelate, 3-methyladipate/pimelate, cytosine and homogentisate were higher in cases than in controls; threonine, methionine, phenylalanine and arginine were lower in cases than in controls</td>
<td>200 rapidly declining eGFR, and 200 stable eGFR</td>
<td>LC-MS</td>
<td>Plasma</td>
<td>CKD progression</td>
<td>Rhee et al. [9]</td>
</tr>
<tr>
<td>Isethionate, saccharate, TMAO, 4-oxopentanoate, cytidine, glucuronate, guanidinosuccinate, 2-hydroxyisobutyrate, uridine, 5-oxoproline, pimelate, N-acetylnereaminic, 3-methylhistidine, citramalate, phthalate</td>
<td>112 participants with CKD Stages 3–5 not on dialysis at start of study</td>
<td>CE-MS</td>
<td>Plasma</td>
<td>Composite: predictive value for CKD progression to ESRF, requiring RRT, all-cause death</td>
<td>Kimura et al. [10]</td>
</tr>
<tr>
<td>TMAO, creatinine, urea, glucose, higher in CKD than healthy controls; arginine, leucine, valine, glutamine, tyrosine, pyruvate, citrate, acetate and formate decreased in CKD compared with healthy group</td>
<td>291 pre-dialysis CKD patients with/without type 2 diabetes and 56 healthy controls</td>
<td>NMR</td>
<td>Serum</td>
<td>Progression of CKD</td>
<td>Lee et al. [11]</td>
</tr>
<tr>
<td>C-Glycosyltryptophan, pseudouridine, O-sulfotyrosine, N-acetyltreonine, N-acetylsersine, N6-carbamoyltheoynlyadenosine, N6-acetyllysine</td>
<td>158 patients with type 1 diabetes, proteinuria and CKD Stage 3</td>
<td>GC-MS and LC-MS</td>
<td>Serum</td>
<td>eGFR decline and progression to ESRF</td>
<td>Niewczas et al. [12]</td>
</tr>
<tr>
<td>4-Hydroxyphenylacetate, phenylacetylglutamine, hippurate and prolylhydroxyproline</td>
<td>Discovery cohort of 141 CKD patients on dialysis and an independent replication cohort of 180 CKD patients on dialysis</td>
<td>GC/LC-MS/MS</td>
<td>Plasma</td>
<td>Urinemic metabolites and impaired executive function</td>
<td>Tamura et al. [13]</td>
</tr>
<tr>
<td>Kynurenine and its metabolites (quinolinic acid, kynurenic acid, xanthurenic acid) and indoxyl sulphate</td>
<td>27 CKD patients</td>
<td>LC-MS/MS</td>
<td>Serum</td>
<td>Kidney function, tryptophan metabolism, markers for inflammation and oxidative stress, psychological/cognitive function</td>
<td>Karu et al. [14]</td>
</tr>
<tr>
<td>Citrulline, dimethylamine, proline, acetacetate, alphaketoisovaleric acid, valine, isobutyrate, D-Palmitly carnitine, histidine and N-methyl nicotinamide</td>
<td>15 patients with biopsy-proven FSG</td>
<td>NMR</td>
<td>Urine</td>
<td>Pathogenic pathways and molecular changes in FSG disease progression</td>
<td>Kalantari et al. [15]</td>
</tr>
<tr>
<td>Urinary excretion rate of 27 metabolites and plasma</td>
<td>First cohort: 22 non-diabetic CKD Stages</td>
<td>GC-MS</td>
<td>Plasma and urine</td>
<td>Metabolic pathway analysis of CKD</td>
<td>Hallan et al. [16]</td>
</tr>
</tbody>
</table>

(continued)
trimethylamine-N-oxide (TMAO), creatinine, urea and glucose that correlated with CKD progression. It was also shown that levels of arginine, leucine, valine, glutamine, tyrosine, pyruvate, citrate, acetate and formate decreased in CKD patients compared with the healthy group. However, these metabolites are not specific to kidney disease and may be influenced by a myriad of other factors such as age, diet, nutritional status, medication, nutritional supplements and other diseases not accounted for in this study. Indeed, this study did not exclude those with other CKD-associated conditions, namely hypertension and immune-related, subsequently, the results should be interpreted with caution. In another study on diabetes, Niewczas et al. [12] monitored patients with CKD Stage 3 and type 1 diabetes for a median of 11 years. Serum samples were analysed by Metabolon Inc. using GC-MS and LC-MS, and seven metabolites were identified that correlated with CKD progression. This study obtained repeat blood serum for metabolomic analysis, and urine samples for protein and renal function markers, which is a strength when investigating the progression of CKD. However, this study did not report on medication use and may obfuscate the study results because an improvement in medication treatment regimens and

Table 1. Continued

<table>
<thead>
<tr>
<th>Proposed metabolite biomarkers</th>
<th>Study population group</th>
<th>Metabolomic platform</th>
<th>Biological matrix for metabolomic analysis</th>
<th>Study outcome</th>
<th>Bibliographic reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>concentration of 33 metabolites differed significantly in CKD patients versus controls. Citric acid cycle was the most significantly affected</td>
<td>3–4 and 10 healthy adults. Second cohort: 45 non-diabetic CKD patients and 15 controls. Additional 155 patients from the European Renal cDNA Bank cohort and 31 kidney biopsies from healthy kidney transplant donors</td>
<td>LC-MS</td>
<td>Plasma and dialysate</td>
<td>Metabolic profile of ESRF patients on dialysis</td>
<td>Buijs et al. [8]</td>
</tr>
<tr>
<td>Significant differences in concentration of 214 metabolites between healthy control and ESRF patients’ pre-dialysis plasma (126 increased and 88 reduced in ESRF group). Pre-dialysis versus post-dialysis showed significant changes in 362 metabolites—including as yet unidentified metabolites</td>
<td>80 ESRF haemodialysis patients and 80 healthy controls</td>
<td>LC-MS/MS</td>
<td>Plasma</td>
<td>TMAO, inflammation and mortality in CKD patients</td>
<td>Missailidis et al. [18]</td>
</tr>
</tbody>
</table>

Table 2. Platforms for metabolomic analysis with possible advantages and disadvantages [24–27]

<table>
<thead>
<tr>
<th>Platform</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE-MS</td>
<td>Small sample volume, High separation efficiency, High resolution</td>
<td>Migration time variability, Poor concentration sensitivity, Low sample loading capacity</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Detects a large pool of metabolites, High sensitivity, High resolution</td>
<td>Destructive of sample, Time-consuming, Sample preparation required</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Wide dynamic range, High resolution, High sensitivity</td>
<td>Requires thermal stability, Destructive of sample, Sample preparation required</td>
</tr>
<tr>
<td>NMR</td>
<td>Minimal sample preparation, Non-destructive of the sample, High reproducibility</td>
<td>Low resolution, Low sensitivity, Expensive</td>
</tr>
</tbody>
</table>
patient adherence to glycaemic, hypertensive and dyslipidaemic agents may slow the decline in kidney function, and hence delay CKD progression [35].

Tamura et al. [13] sought to elucidate the impact of uraemic metabolites on executive function in a cohort of dialysis patients by using GC/LC-MS/MS in pre-dialysis plasma samples and analysis performed by Metabolon Inc. Four metabolites were associated with impaired executive function in those with CKD: 4-hydroxyphenylacetate, phenylacetylglutamine, hippurate and prolyl-hydroxyproline. However, these metabolites can be derived from the diet and gut microbial metabolism, which this study did not investigate [36–39]. Furthermore, cognitive impairment could result from other confounding factors in this study such as age, frailty, hypertension, incidence of neurological disorders and cardiovascular factors [40–46] rather than the metabolites identified. In a similar study into cognitive decline, Karu et al. [14] identified kynurenine and its metabolites (quinolnic acid, kynurenic acid, xanthurenic acid), and indoxyl sulphate as being greatly elevated in CKD patients, especially in those with cognitive impairment, compared with healthy controls. The proposed mechanism for this is that tryptophan is involved in the synthesis of the indoxyl sulphate (uraemic toxin) via colonic microbes [47, 48] and can affect brain activity through the kynurenine pathway. However, the same confounding factors are attributable to this study as were for Tamura et al. [13], and hypertension was documented in 78% of CKD participants in Karu et al. [14]; therefore, the cognitive decline may be as a result of hypertension or exacerbated by the co-presence of accumulating uraemic toxins and hypertension.

**Urine**

Metabolomic urinary analysis in CKD could be useful as it is non-invasive, easily obtained and provides a global state of physiological function. However, caution should be employed as there is evidence to suggest that urinary metabolites fluctuate throughout the day suggesting vigilance should be taken when interpreting results from such studies [49, 50]. Kalantari et al. [15] collected urine samples over 24 h from 15 patients with focal segmental glomerulosclerosis to identify 10 metabolites using NMR spectroscopy and ProMetab software, that were deemed to be prognostic when compared with kidney biopsy results. This study implemented a diet on its participants for 24 h prior to collecting urine as a mitigating measure to control for dietary influences on the urinary metabolome. However, urine samples were collected in 2011 but no information is given on how the samples were stored nor when the samples were processed, which could limit the reliability of these results [50, 51]. Hallan et al. [16] used GC-MS and MetaboAnalyst 3.0 software on urinary samples in non-diabetic CKD patients showing decreased excretion of citric acid cycle metabolites corroborated with analysis of kidney biopsies, showing a reduction in gene expression for citric acid cycle enzymes. These findings, however, could be accounted for by considering the nutritional status and dietary intake of these participants, which this study did not do.

**Dialysate**

The only currently identified study that applied metabolomics to the effect of haemodialysis on the metabolome and dialysate effluent was by Zhang et al. [17]. In this study, end-stage renal failure (ESRF) patients receiving dialysis were compared with matched healthy controls with samples collected from blood plasma and dialysate effluent at regular timings during the dialysis process but on a single occasion. The metabolome in the plasma samples was compared with both groups using ultra-performance LC-MS and MetaboAnalyst 3.0 software, which showed that the haemodialysis process not only removed, as expected, uraemic products (TMAO, indoxyl sulphate, p-cresol sulphate, p-cresol glucuronide, uric acid and hippuric acid), fluid and excessive electrolytes, but a plethora of metabolites—mainly amino acids (arginine, glutamine, alanine and phenylalanine) and lipids, which the authors concluded may be the cause of increased mortality within the CKD population. These changes in metabolites were also identified in the dialysate effluent when measured at the corresponding time intervals. However, the authors did not include information on the medical comorbidities or medications, CKD aetiology of the disease group, or whether those in this group had any residual kidney function. Although for the control group hypertension, cardiovascular disease and diabetes were exclusion criteria, no indication is given of diabetes prevalence within the ESRF group, which could have implications for interpreting the study’s results.

**DIET AND THE MICROBIOME—THE MISSING LINKS?**

**Diet and nutritional status**

Changes in amino acid metabolism are widely seen in those with CKD and on dialysis [9, 10, 17, 52, 53] but whether this is due to CKD progression, other diseases or concomitant with poor nutrition status and dietary protein intake remains elusive, compounded by the fact that very few studies that include an assessment of nutritional status or dietary intake. Diet is an important factor that should be assessed as those who display malnutrition and protein-energy malnutrition have worse outcomes and early mortality in CKD and on dialysis [54–56]. Utilizing a subjective global assessment (SGA) tool will enable the clinician and researcher to understand and appreciate whether the metabolites identified result from nutritional status, dietary intake or from disease [57–59].

Consideration should also be made of dietary regimes as these can have influence over the metabolome and microbiome composition [60–62], such as differences between vegetarians, vegans, pescatarians and carnivores. In Wu et al. [63], healthy vegans consumed more carbohydrates, but less protein and fat, than healthy omnivores, resulting in a 25% difference between the identified metabolites of omnivores and vegetarians of which lipid and amino acid metabolites were significantly elevated in omnivores and the metabolites often associated with CKD hippurate, catechol sulphate and 3-hydroxyhippurate were increased in vegetarians compared with omnivores. It is, therefore, necessary to account for differences in dietary intake when assessing the metabolites identified in CKD patients as what could have been considered to be a potential biomarker may be derived from or greatly influenced by factors other than kidney disease. Furthermore, it would also be informative to collect multiple samples across time-points for metabolomic analysis of dietary intake to understand how the metabolome changes especially for amino acids [32, 64, 65] in CKD patients. Indeed, current suggested dietary protein requirements for CKD patients are contentious and vary globally from 0.55 g/kg to 1 g/kg [66–71] with greater requirements for those on dialysis, 1.1–1.4 g/kg [58, 72], which may impact on the levels of amino acids and uraemic toxins seen in these metabolomic CKD studies.
Microbiome

Combining the study of diet and the microbiome in CKD studies with metabolomics would enable elucidation of these complex and interwoven relationships, especially for TMAO and uremic toxins [73, 74]. Phenylacetylglutamine is associated with levels of p-cresyl sulphate and indoxyl sulphate in CKD patients not yet on dialysis and is considered to be a risk factor for cardiovascular disease and mortality [39], but whether it is as a result of gut microbiome dysbiosis or due to impaired renal function is yet to be elucidated. TMAO is derived from the gut microbiota and l-carnitine and choline precursors derived from dietary intake of meat and eggs, and p-cresyl sulphate and sulphate are uremic toxins derived from the metabolism of amino acids by commensal gut microbiota, consequently greatly influenced by dietary intake [62, 75], and TMAO is implicated in greater mortality amongst those with CKD concomitant with progressing impaired renal function [76] and increased cardiovascular events [77]. Missailidis et al. [18] assessed plasma samples from those with CKD Stages 3–5 from various aetiologies, comorbidities and nutritional status using SGA, but lacked an assessment of dietary intake. TMAO increased as CKD stage progressed and was associated with greater mortality in a 5-year follow-up. However, the nutritional status score also increased with progressive CKD, which may have a more negative impact on mortality than the presence of TMAO. CKD patients had higher TMAO levels than controls, which continued to increase as renal function declined. When a study participant received a kidney transplant, TMAO levels decreased and nutritional status improved. It was demonstrated that CKD patients with the highest TMAO levels had a significantly lower survival rate, which the authors deemed to suggest that high levels of TMAO predicted reduced 5-year survival. Although this study did consider nutritional status by utilizing the SGA tool examining weight loss, anorexia and vomiting, muscle wasting, oedema and loss of fat mass, it did not consider dietary intake or the microbiome, which can both have an impact on TMAO levels [62, 73–75]. Furthermore, it could have collected faecal samples to assess the gut microbiome and its influence on TMAO levels [31]. Stubbs et al. [78] also assessed the impact of TMAO on CKD and demonstrated the beneficial effect of transplant on decreased levels of TMAO compared with pre-transplant, therefore suggesting that increased levels of TMAO are a consequence of decreased renal function and urinary excretion.

It would have been advantageous to assess levels of TMAO in conjunction with an assessment of dietary intake for a more comprehensive investigation of the relationship between TMAO, CKD, diet and the microbiome [79]. Stubbs et al. [78] did not comment on the effect of diet and Missailidis et al. [18] concluded that dietary changes could not explain the normalized levels of TMAO after kidney transplant; however, it is not documented whether the participants receiving the transplant were urinating [80, 81] as this would allow TMAO levels to decrease due to it being excreted in the urine [82]. It remains unclear if TMAO can be used as a biomarker in CKD and cardiovascular dysfunction as it may just be a marker of poor renal clearance or poor nutritional status; therefore, TMAO should be monitored in those with CKD along with an assessment of dietary intake and gut microbiome to further elucidate this mechanism and potential biomarker.

Very few studies have been identified that incorporate the study of the metabolome with dietary and microbiome considerations. Pallister et al. [38] identified that metabolites are influenced by microbiome diversity, particularly hippurate, which was associated with intakes of coffee, fruit and wholegrains; and p-cresol sulphate and phenylacetylglutamine from the putrefaction of undigested dietary proteins by colonic bacteria. Furthermore, Lees et al. [36] suggested that hippurate excretion is associated with co-excretion of metabolic intermediates, especially citrate, succinate and 2-oxoglutarate, and has been associated with a range of conditions besides kidney disease including liver disease, hypertension, diabetes, atherosclerosis and psychiatric disorders, but is also dependent on intestinal microbiota diversity. Diversity and abundance of human microbiome varies widely even among healthy subjects and important factors such as diet need to be considered due to its effect on microbiome composition and metabolism, and wider effects on health status and disease [20, 83–85]. Furthermore, dietary advice given to those with CKD and on dialysis may negatively impact the microbiome of the kidney–gut axis due to reducing the ability to produce beneficial short-chain fatty acids [86, 87] as fruit and vegetable consumption is rationed to prevent electrolyte derangement [57, 86, 88]. Short-chain fatty acids are thought to be implicated in CKD through their deleterious depletion and consequential effect on increasing oxidative stress, fibrosis and the immune response [86, 89]. Utilizing faecal samples to assess the microbiome may offer further insights in the pathological progression of CKD [31] and provide potential dysbiotic targets for treatment of CKD [73].

CONSIDERATIONS FOR FURTHER CKD METABOLICOMICS

Single biomarker

As studies incorporate metabolomics into their methodology to identify potential biomarkers, it should also be embedded how to evaluate the clinical usefulness of these biomarkers, to elucidate to what degree they can be used in clinical care, drug development and therapy, and, ultimately, point-of-care testing devices [30, 90]. A single biomarker may be elusive, but a panel of biomarkers based on ratios of identified altered metabolites may offer potential benefits [47] such as glutamate-glutamine, which may indicate nervous system disorders and energy dysmetabolism in uraemic patients, and tryptophan-kynurenine, which may indicate immune responses and increased atherosclerosis risk in uraemic patients [53].

Samples

Results from metabolomic studies are not always reproducible due to differences in patient demographics, samples used, methodology and computational analysis [20, 23, 91–94]. Studies should report on when samples are taken and what presampling checks have been done to limit variability, as well as on the time between sample acquisition and sample processing, because this may increase the possibility of metabolite degradation and yielding false-positive results [50, 93, 95–99]. Each patient is an individual, and each has their own individual metabolic phenotype that is subject to dynamic daily changes due to diet and diet–microbiome interactions [32–34, 49]. It is problematic when studies rely on a single sample from which to extrapolate prognostic markers with hindsight, and studies seeking to investigate prognostic questions should have at least two measurements over the study time period in order to monitor dynamic changes and allow for more meaningful interpretation of the data [90].
The other ‘-omics’

Current limitations with metabolomics in CKD studies stem from the inability to identify all metabolites in the metabolome and concomitant lack of overlap of metabolite coverage in comparable studies and validation [100]. Corroborating identified metabolites with other physiological functions would make the results more robust and may offer great benefits in ascertaining phenotypical data for an individual patient [16, 101], which will probably become even more useful with advances in technology and the ability to have wider coverage. Genetics coupled with metabolomics could provide valuable information on an individual’s metabolic profile, coined as metabolotype, which has been demonstrated in genome-wide association studies (GWAS) through the identification of genetic variation and its effect on metabolic functionality [19, 102–105]. GWAS data sets can be utilized by researchers to enrich the study of metabolic dysregulation and be applied to metabolomic studies of CKD, but such studies are currently lacking [106].

Advancement of metabolomics, and the wider ‘-omics’ family, requires a collaborative effort to share and store metabolite data such as the CKDdb database [100]. Once the technology is available, it could conceivably progress to readily available point-of-care devices such as lateral flow devices, dipsticks, breath testing and wearable technology utilizing biosensors and chemometric-based analyses [8] to monitor for disease inception, progression and prognosis, with additional benefits arising from measuring dietary and microbiome influences [107].

CONCLUSIONS

This mini-review has highlighted the current need for better diagnostic and prognostic markers for CKD. Further studies on CKD should utilize the metabolomic approach, but also examine the diets and microbiome of the individual participants with CKD. Multiple samples should be taken over a pre-determined time period and assessed for changes in the metabolome and cross-referenced with the CKD phenotype. Studies should also stratify patients based on their ethnicity, sex and CKD aetiology, and perform further analysis based on nutritional status, dietary intake and on the microbiome particularly to elucidate the interconnectedness of amino acid metabolism, uraemic toxins, dietary factors and the gut microbiome. This approach would strengthen the research output on CKD fostering greater understanding of how metabolites change, and through what influences, so that biomarkers for CKD inception, prognosis and prognostics may be identified.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


54. Zha Y, Qian Q. Protein nutrition and malnutrition in CKD and ESRD. Nutrients 2017; 9: 208


haemodialysis or peritoneal dialysis. J Hum Nutr Diet 2013; 26: 315–328
global assessment and malnutrition inflammation score for monitoring the nutritional status of older adults on hemodialysis. Clin Nutr 2018; 37: 604–611
60. Poesen R, Mutsaers HAM, Windey K et al. The influence of dietary protein intake on mammalian tryptophan and phe-
61. Schmidt JA, Rinaldi S, Scalbert A et al. Plasma concentra-
tions and intakes of amino acids in male meat-eaters, fish-
63. Wu GD, Compher C, Chen EZ et al. Comparative metabolomics in vegans and omnivores reveal constraints on diet-
64. Andersen M-BS, Rinnan Å, Manach C et al. Untargeted metabolomics as a screening tool for estimating compli-
65. Scalbert A, Brennan L, Manach C et al. The food metabo-
66. Fouque D, Laville M. Low protein diets for chronic kidney
67. Kovesdy CP, Kopple JD, Kalantar-Zadeh K. Management of
68. Piccoli GB, Vigotti FN, Leone F et al. Low-protein diets in
CKD: how can we achieve them? A narrative, pragmatic re-
73. Zhang LS, Davies SS. Microbial metabolism of dietary com-
genomemedicine.biomedcentral.com/articles/10.1186/s13073-016-0296-x (28 November 2017, date last accessed)
74. Fernandez-Prado RE, Perez-Gomez M, Gracia-Iguacel C et al. Nutrients turned into toxins: microbiota modulation of nu-
75. Schmedes M, Aadland KE, Sundekilde UK et al. Lean-sea-
food intake decreases urinary markers of mitochondrial lipid and energy metabolism in healthy subjects: Metabolomics results from a randomized crossover inter-
vention study. Mol Nutr Food Res 2016; 60: 1661–1672
77. Kim RB, Morse BL, Djurdjev O et al. Advanced chronic kid-
ney disease populations have elevated trimethylamine
-N-oxide levels associated with increased cardiovascular events. Kidney Int 2016; 89: 1144–1152
78. Stubbs JR, House JA, Ocque AJ et al. Serum trimethylamine-
-N-oxide is elevated in ckd and correlates with coronary ath-
81. Lai Q, Pretagostini R, Poli L et al. Early urine output predicts
82. Moraes C, Fouque D, Amaral ACF et al. Trimethylamine
84. Hall AB, Tolonen AC, Xavier RJ. Human genetic variation and
85. Mendes-Soares H, Chia N. Community metabolic modelling
86. Felizardo RJF, Castoldi A, Andrade-Oliveira V et al. The
88. Mitch WE, Remuzzi G. Diets for patients with chronic kid-
89. Li L, Ma L, Fu P. Gut microbiota-derived short-chain fatty
90. Ravani P, Parfrey PS, Dicks E et al. Clinical research of
91. Everett JR. A new paradigm for known metabolite identifi-
cation in metabonomics/metabolomics: metabolite identi-
93. Emwas A-H, Roy R, McKay RT et al. Recommendations and
standardization of biomarker quantification using nmr-
based metabolomics with particular focus on urinary
94. Hajduk J, Matysiak J, Kokot ZJ. Challenges in biomarker