Metabolomics Approaches Confer a Deeper Biologic Understanding of Kidney-Related Diseases

By Alexander M. Buko

A Q&A

Human Metabolome Technologies (HMT) Vice President Alex Buko, PhD, addresses the use of metabolomics to further understanding of kidney diseases. At HMT, Dr. Buko provides scientific and statistical support for preclinical and clinical studies in metabolomics.

1 Metabolomics is fast becoming a significant technology applied to kidney disease research. What is metabolomics?

Metabolomics is the measurement and analysis of small organic molecules in biologic samples: cells, tissues, organs, and biofluids. It provides a snapshot of the biologic system as a whole, taking into account internal and external factors such as genetics, microbiome, lifestyle, and disease. The organic compounds analyzed cover a wide range of chemical species including sugars, amino acids, organic acids, nucleic acids, acylcarnitines, small-to-very-long-chain fatty acids, bile acids, and a whole cast of steroids and lipids. Owing to the wide range of chemical species, different analytic methods need to be used to capture the kidney, urine, or plasma metabolome. Concerning kidney function, metabolites of research interest include creatinine, urea, uric acid, glucose, triglycerides, kynurenines, amino acids, and bile acids. Current clinical approaches in nephrology include measuring metabolites like urea, creatinine, and uric acid, which complement other tests, such as cystatin C, parathyroid hormone, and albumin.

The number of endogenous metabolites present in the human body, according to the Human Metabolome Database (HMDB) (1), is >90,000. Altogether, metabolites in the HMDB are linked to >660 diseases. Small molecule metabolites are linked to >27,000 single nucleotide polymorphisms, 2000 enzymes, and hundreds of pathways. They build a network of signaling and information flow representing the biochemical profile of an individual. In patients with chronic kidney disease (CKD), many metabolomics studies have revealed associations among blood metabolites, estimated glomerular filtration rate (eGFR), and clinical phenotypes representing disease status and progression.

The central dogma of molecular biology states that DNA makes RNA, and RNA makes proteins. These proteins turn over metabolites, with metabolites representing an endpoint of protein expression. Whereas an individual’s DNA is static, the metabolome is dynamic and a functional system of cellular programming. In addition, the metabolome differs throughout the body, so the analysis of organs such as the kidneys will be different from that of other organs or biofluids such as blood and urine. Even within tissues, there can be heterogeneity across an organ’s cross-section. Hence, metabolic profiles of patients with kidney diseases can represent their clinical status at the molecular level.

2 Many metabolites can then be measured. Why would kidney disease be an emerging area for applying metabolomics?

Changes in circulating metabolites may be of interest for different reasons, based on the specific cohort or clinical study in a host of different diseases. To this point, kidney disease is not unique; however, there are some special reasons that may enable metabolomics to be particularly adaptive and successful in this area. The kidney has a broad and complicated impact on circulating metabolites because of its unique biologic function to filter blood and remove circulating toxins. Hence, an enhanced level of a metabolite may be reflective of kidney failure and could provide early detection of chronic disease, measure disease prognosis, be a marker for therapeutic efficacy, shadow organ health, or provide researchers with a better understanding of the complex kidney biochemistry. Some of these metabolites may in fact act as ligands for specific receptors elsewhere in the body and can facilitate interaction with other organs such as the liver and brain.

3 Where can metabolomics enable CKD research?

One of the most common measurements for kidney disease is the GFR. However, because human biology is so complex, GFR does not fully reflect individual kidney functions or discriminate between disease cause and progression. The effects of diet, lifestyle, microbiome, medication, and comorbidities require a finer understanding of the disease phenotype than the general measurements of cholesterol, uric acid, glucose, creatinine, and eGFR typically provide to the clinician. Many metabolites have been observed to change in blood with kidney dysfunction (2–4), among them the amino acids citrulline, glutamine, and several others. The impact of kidney function on peripheral metabolites in plasma and serum and on urine metabolism is complex, with both direct and indirect effects. Intra-organ communication and feedback add further complexity, which is not completely understood. Despite the diversity among patients, metabolomics offers new insights and new directions to understanding CKD and drug development (5–7).

At HMT, we have a lot of experience with the metabolome of the gut microbiome and its interactions with other organs and the brain. Whereas many metabolomics studies continue to identify the best mix of biomarkers for CKD diagnosis, an emerging subject is the effect of the microbiome on CKD development (8). Indoxyl sulfate and p-cresyl sulfate, which are colon-derived metabolites of bacterial origin, are found at higher levels in end-stage kidney disease (ESKD) than in healthy individuals and are not removed by dialysis. Phenyl sulfates, another gut metabolite, has also been observed in diabetic kidney disease. Phenyl sulfate was observed to correlate with albuminuria. In addition, another gut metabolite, trimethylamine-N-oxide (TMAO), has been associated with cardiovascular disease (CVD) and cholesterol transport (9). TMAO is primarily excreted by the kidney and is associated with ESKD (10). These biomarkers and others are under investigation for larger validation studies and other related diseases (11).

4 You mentioned many different types of metabolites, from TMAO to lipids. How can we decide which technology would best be used to discover biomarkers for disease progression or patient stratification?

Part of the problem understanding the complex biochemistry of kidney function is how to apply the measure the large metabolome space covered by polar and nonpolar metabolites. The presence of polar metabolites, such as asymmetric dimethylarginine (ADMA), TMAO, and phosphate sugars, and nonpolar long-chain fatty acids, bile acids, steroids, and lipid complexes presents an analytic challenge. Many different technologies are successfully used to measure different segments of this metabolic space, including nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), various forms of liquid chromatography (LC)-MS, imaging MS, and capillary electrophoresis (CE)-MS. Each method has its advantages and limitations. The differences among these technologies include unique coverage of metabolic space, sensitivity for certain classes of metabolites, ability to identify novel metabolites, sample throughput, and instrument dynamic range. NMR has the advantage of being nondestructive, and samples do not need preparation or extraction and can be reused. However, NMR lacks the sensitivity, resolution, and specificity of the MS-based techniques.

Many successful NMR applications have been published for kidney disease studies. MS-based techniques, however, are the most widespread. Owing to the large variations and complexities of the metabolome, no single MS-based technique is capable of systematic broad detection and measurements. Various reverse-phase LC methods coupled with high-performance MS are mainstream methods to sample a broad range of polar to nonpolar spaces. Very polar metabolites require a different approach with hydrophilic interaction LC (HLIC) CE methods. The complexity and number of isobaric metabolites in the lipid space place an even higher burden on specialized chromatography and MS. In the search for the right method to use in a study, prior knowledge of a metabolite of interest, focused metabolic pathway, or specific chemical class allows for more unique solutions. Unbiased methods—called untargeted metabolomics—generally provide nonquantitative metabolite data but cover a large range of metabolic space. If a particular metabolite or family is known to be of interest, a targeted approach providing quantitation may be the method of choice. It may be advisable to consider several different methods before choosing one or more methods for metabolomic studies.

Most laboratories today use extensive metabolite libraries for the annotation of known and validated metabolite identification and high-resolution mass spectrometers that can provide elemental formulas for the identification of novel or unknown metabolites. As the size of the metabolome continues to grow, metabolite libraries continue to expand.
Metabolomics is a growing, powerful, and enabling tool for CKD research. Coupled with a strategic cohort study, metabolomics research in chronic kidney disease is the incredible mentorship I have received over the years.

The issues today, concerning not only biomarkers but also clinical measurements, are found in the translation from discovery and preclinical to clinical use. In detail, these issues include acceptance of common practices, variations in size and composition of study groups, reliance on the discovery of single analytes, the lack of appropriate validation studies, and the challenges of combining different datasets from orthogonal methods (combining RNA with proteomics with metabolomics, for example). Hence, aside from the accepted clinical measurements, there is a growing literature of promising new metabolites and panels that can be used for research. However, owing to different methodologies, pathologic conditions, and cohort selections, investigators today must conduct their own targeted or untargeted research to feel confident about any metabolite panel chosen for disease progression or clinical study. The good news is that organizations such as ASN are bringing together data and studies, facilitating data sharing, and providing the driver to move these discoveries to a common consensus that pushes commercialization of critical biomarkers and also focuses on the needed biochemistry to enable drug discovery.

Valiant work is needed for a deeper understanding of kidney function and dysfunction. We at HMT view metabolites as an important starting point for biomarker discovery and hypothesis generation. Metabolomics has the capability to measure polar and lipid metabolites in kidney tissue, circulating plasma, and urine, linking disease status to molecular processes. The technologies to measure metabolites in patient samples already exist, so the technology and assay development from research to validation to commercialization are already in place. In the future, we expect to see metabolomics playing a leading role in CKD study, diagnosis, and drug development.

References


Cultivating Interest in Nephrology by Engaging in Opportunities and Seeking out Mentors

By Tanim A Arora

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The crux of my interest and passion for nephrology is the incredible mentorship I have received over the years.

A starry-eyed international medical student looking for opportunities to gain clinical experience in the United States, I was beyond thrilled to receive an offer from Yale School of Medicine to complete a month-long clerkship in pediatric nephrology. Did I want to pursue a career in nephrology back then as a final-year medical student? Honestly, I wasn’t sure. Kidney physiology, as fascinating as it is, was also extremely daunting to me. As a medical student, I would have a subclinical panic attack anytime I was asked to, for instance, “calculate eGFR in CKD” or explain “renal tubular acidosis” in detail. I was nervous, to say the least, but I did decide to keep an open mind and take this remarkable opportunity to learn! Absolutely, yes! And so my journey in the world of nephrology began, surrounded by exceptional mentors, interesting patients, fascinating research, and most important, a sense of belonging. In addition to the complexity of kidney pathophysiology and satisfaction of caring for and improving the quality of life of critically ill patients, what struck me the most was the passion and commitment of the workforce toward creating an innovative and fun learning environment for students. Thus, invigorated and inspired by this intense, albeit short, exposure to nephrology, I decided to dedicate myself toward kidney research and gain more experience in this wonderful field.

My postdoctoral training (a full-time research trainee position) at Yale allowed my academic and professional growth to flourish, as I gained independence in conducting and managing research projects, presenting posters, publishing papers, contributing at conferences, and building connections, both in person and via social media. My budding interest in nephrology was recognized by numerous members of the kidney world, and I was lucky enough to partake in wonderful opportunities to participate in unique educational activities such as ASN Kidney STARS, ASN Kidney TREKS, and NephSIM Nephrons. The crux of my interest and passion for nephrology is the incredible mentorship I have received over the years. Not only was I blessed with extraordinary mentors at Yale, but through participating in various educational activities, I also connected with some of the brightest minds in nephrology and gained more mentors. Each provided support, motivation, and guidance and created a nurturing, stimulating environment for me to consistently face challenges, grasp new opportunities, and grow, both professionally and personally.

That sense of belonging that I first felt during my nephrology clerkship has only grown stronger, and I feel confident that as I embark on this next phase of my career, the kidney world will take care of me and continue to cultivate my passion for nephrology.

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