Metabolomics, the systematic analysis of small molecules present in a biologic specimen, is an increasingly useful tool for research in CKD.1 Ongoing methodologic improvements have expanded the capabilities of metabolomics platforms. Untargeted (or nontargeted) methods now simultaneously detect hundreds of known compounds from multiple pathways, including those involved in sugar, amino acid, organic acid, nucleotide, acylcarnitine, and lipid metabolism, as well as unknown compounds that are reproducibly found but yet to be identified.2 Initial metabolomic studies in CKD showed associations between several blood metabolites, eGFR, and future development of CKD.3,4 Continued technologic improvements, collaborative work across metabolomic platforms, and metabolite profiling in large cohort studies should yield new biomarkers, insights on CKD pathophysiology, and therapeutic opportunities.

Statistical methods in metabolomics parallel those used in genetics, a field that has benefitted from widespread improvement in DNA sequencing methodologies over the past two decades. Figure 1A shows a traditional “Manhattan plot,” depicting P values from the associations of 953 serum metabolites with mortality risk among individuals with CKD and showing dozens of highly significant associations.5 This layout underscores the multiplicity of statistical comparisons and the need to adjust thresholds for statistical significance accordingly. Although there is no consensus approach, our view is that Bonferroni adjustment (i.e., adjusting for every metabolite) can be overly conservative, because many metabolites are highly intercorrelated, particularly within a given metabolite class or biochemical pathway. Alternative approaches include controlling for a false discovery rate, adjusting for the number of meaningful metabolite clusters determined by principal component analysis, or adopting a more lenient statistical threshold for discovery but requiring external validation.

Although there are similarities between metabolomic and genetic analyses, important differences also exist. First, genetic data are relatively constant within an individual over time, whereas metabolites can and do vary according to a wide range of intrinsic and environmental factors, including diet, medications, and the microbiome (all of which contribute metabolites that can be detected in blood and other biologic specimens). This individual variability can reduce power to detect true associations. Some metabolomic studies have required a degree of medium-term stability, evaluating only those metabolites with low individual variation.6 Second, genetic studies can evaluate associations between genetic variants and phenotypes without regard to temporality given near certainty that the genetic variant was the antecedent event. In contrast, metabolite studies must consider longitudinal relationships, with careful accounting for baseline confounders of the relationship between metabolite and clinical outcomes.

One of the most important confounders in metabolomics studies of CKD is kidney function. Typically assessed as GFR, kidney function is related to approximately one third of detected metabolites in both general and CKD populations.7,8 Figure 1B shows the association between the same blood metabolites and risk of subsequent death depicted in Figure 1A after adjusting for baseline GFR, revealing a dramatic attenuation in the number of statistically significant metabolite associations. Thus, careful measurement and consideration of baseline GFR are critical. However, even perfect adjustment for GFR may not fully address the effect of kidney function on metabolite levels. Metabolites might vary because of differences in tubular secretion or resorption and disruptions in kidney catabolism or anabolism. Furthermore, there may be indirect effects of CKD, such as modifications to diet, the microbiome, and medications that may co-occur with CKD progression.

At present, even the best metabolomics platforms provide incomplete coverage of the human metabolome, detecting less than one quarter of the currently known endogenous and exogenous metabolites in a given biologic specimen. Different platforms provide different coverage of the metabolome, such that a metabolite detected on one platform may not be identified on another, complicating comparisons across studies. Within a platform, not all detected metabolites are measured equally well.9 Thus, it may be prudent to corroborate the assignments of promising metabolites with experiments that analyze samples spiked with the authentic standard.10 Development of dedicated targeted assays may also be necessary.
to improve precision and enable absolute quantitation, because untargeted assays typically report only relative results.

Figure 1. The number of statistically significant associations between metabolites and subsequent mortality is greatly reduced after adjustment for GFR. Figure depicts P-values of the demographic-adjusted associations between metabolites identified in an untargeted screen and future risk of mortality among 963 participants with CKD in the African American Study of Kidney Disease and Hypertension (A) before and (B) after adjustment for GFR measured by urinary clearance of iothalmate.

Taken together, metabolomics is emerging as a powerful tool for CKD research. However, it must be coupled with thoughtful study design, careful consideration of potential confounders, and rigorous quality control to yield meaningful results. Meta-analysis across large datasets significantly enhanced the
Nephrolithiasis is an illness of worldwide significance. Over the course of a lifetime, about 9% of the United States population will develop kidney stones, and the incidence is rising. About 60%–80% of stones are composed of calcium oxalate, and urinary metabolic abnormalities, including hyperoxaluria, hypercalciuria, and hypocitraturia, increase kidney stone risk. Although nephrolithiasis can be associated with specific genetic and metabolic disorders, most stones are idiopathic; why kidney stones are becoming more common is not well understood.

In this issue of the Journal of the American Society of Nephrology, Tasian et al. focused on antibiotic exposures as one explanation for an increasing stone prevalence. In the United States, >250 million courses of antibiotics are prescribed annually to outpatients, with >40 million of these prescribed to children under 18 years of age. A conservative estimate is that 30% of the antibiotics prescribed in the outpatient setting in the United States are unnecessary, with comparable estimates in other developed countries.

Although many antibiotic courses are absolutely necessary, antibiotics are also overused for marginal indications in part on the basis of the belief that their long-term side effects are minimal. The prevailing belief for three generations has been that, after a brief period of instability accompanying an antibiotic course, “everything will bounce back to normal.” However, this assumption may not be correct, and ultimately, reassessment may force changes in the practice of medicine.

We now know that the “everything” that we are counting to “bounce back to normal” refers largely to the human microbiome, the collection of microorganisms that live in and on us. The microbiome, the collection of microorganisms that we are counting to our body, serves as a sort of “dynamic unmet need in the field. We view metabolomics studies as an important starting point for discovery and hypothesis generation, with subsequent efforts required for independent replication, individualized assay development, and mechanistic investigation.

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Does the Receipt of Antibiotics for Common Infectious Diseases Predispose to Kidney Stones? A Cautionary Note for All Health Care Practitioners

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