
Modern metabolomic profiling technologies, including mass spectroscopy and nuclear magnetic resonance spectroscopy, can reliably quantify metabolites in tissues and body fluids in targeted and nontargeted manners.1 These platforms provide means for metabolite profiling that are high throughput, inexpensive, and scalable to a large number of plasma, urine, or tissue samples. The ability to generate metabolite profiles from genetic cohorts with available DNA provides an opportunity to quantify the extent of genetic contributions to metabolite homeostasis. Metabolite quantitative trait locus mapping and metabolite genome-wide association studies (mGWAS) are systems genetics techniques that use a conventional genome-wide association study approach with individual metabolite concentrations in lieu of clinical traits. Thus, mGWAS allows for unbiased genome-wide mapping of genetic loci for specific metabolite levels, providing another dimension to similar techniques routinely used for transcriptomic and proteomic data. Integrated multidimensional quantitative trait locus maps for transcripts, proteins, and metabolites improve the interpretability of human genetic variation by providing insights into specific molecular consequences of variants driving the disease process. Such molecular drivers represent excellent candidates for mechanistic disease biomarkers and can be targeted therapeutically in individuals at high genetic risk (Figure 1).

In 2008, the first mGWAS using sera of 284 healthy individuals was reported.2 Since then, numerous mGWAS have discovered >150 genetic loci that were significantly associated with the concentrations of >300 metabolites in healthy individuals.3 These loci include several common variants in genes previously associated with severe metabolic disorders due to rare mutations. For example, CPS1 encodes the rate-limiting liver enzyme that catalyzes the first step of the hepatic urea cycle by synthesizing carbamoyl phosphate from ammonia and bicarbonate. Rare loss-of-function mutations in CPS1 cause carbamoyl phosphate synthetase I deficiency, a severe recessive disorder characterized by hyperammonemia. A common variant in CPS1 recently identified through mGWAS is reproducibly associated with increased glycine levels, which are consistent with a milder carbamoyl phosphate synthetase dysfunction.4

Many of the mGWAS loci have been linked to clinically relevant phenotypic traits, including CKD. For example, the common CPS1 allele described above is also associated with increased risk of CKD, suggesting a potential role in the pathogenesis of renal disease.5 Several other established risk loci for CKD overlap with mGWAS findings, including variants in NAT8 (encoding a kidney-detoxifying enzyme responsible for the synthesis of mercapturic acid), SLC6A13 (encoding a major kidney transporter for γ-aminobutyric acid, taurine, and betaine), and SLC7A9 (encoding a transporter that reabsorbs positively charged amino acids from the urine, including cystine).3,6 However, given the cross-sectional nature of mGWAS and the fact that the genetic associations for CKD and metabolites were established in separate cohorts, the

See related article, “Intracellular Chloride and Scaffold Protein Mo25 Cooperatively Regulate Transepithelial Ion Transport through WNK Signaling in the Malpighian Tubule,” on pages 1449–1461.

Insights into CKD from Metabolite GWAS
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Under steady state, blood levels of human metabolites are determined by their baseline rates of production and clearance. These physiologic processes, in turn, are influenced by various environmental factors (e.g., diet and medication exposure), genetic variation (e.g., in genes encoding metabolic enzymes and transport mechanisms), and disease states (e.g., kidney or liver disease) as well as by more complex gene-environment and gene-disease interactions. Major alterations in the physiologic processes involved in metabolite generation or clearance can lead to a dysregulation of metabolite levels with serious health consequences. In nephrology, active metabolomic research efforts are motivated by the fact that the kidney plays a central role in metabolite homeostasis.

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precise cause-effect relationships between metabolites and the disease risk are difficult to disentangle. The key question thus becomes whether the observed metabolite associations at CKD risk loci represent true causal drivers of kidney disease, or if these are changes secondary to underlying renal dysfunction, or perhaps, they are completely independent of CKD.

In this issue of the *Journal of the American Society of Nephrology*, Li et al. report a comprehensive mGWAS in 1168 participants with CKD in the German CKD Study. The authors performed genome-wide single-nucleotide polymorphism genotyping with imputation and mass spectroscopy–based target metabolomics followed by genetic association analyses of 139 blood metabolites and 41 urine metabolites. Significant genetic associations were detected for 25 metabolites in blood and two in urine, and 259 blood and 14 urinary metabolite ratios. In eGFR-adjusted analyses, the study replicated numerous metabolite-associated genetic loci from previous population-based studies, including genome-wide significant associations of CKD risk variants in NAT8 with N-acetyl-ornithine and CPS1 with glycine.

Among novel findings were serum metabolite loci detected uniquely in patients with CKD that were not previously detected in population-based studies. Such new gene-metabolite associations may reflect genetic effects unmasked by renal dysfunction. This is exemplified by the AOCI locus (encoding an enzyme catalyzing the oxidative deamination of polyamines) that was significantly associated with blood levels of serum putrescine, a known uremic toxin. The effect of the index single-nucleotide polymorphism at this locus on serum putrescine levels was twice as large in individuals with severe CKD (eGFR < 30 ml/min per 1.73 m²) compared with those with mild CKD (eGFR ≥ 60 ml/min per 1.73 m²), suggesting a potential gene-disease interaction.

Another new finding included the association of a carrier status for rare deleterious missense variants in ACADM (encoding a medium-chain acyl-CoA dehydrogenase) with increased serum ratios of medium-chain acylcarnitines. Reccessive inheritance of deleterious variants in ACADM causes Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, a metabolite disorder detectable by newborn blood screening for acylcarnitine. Interestingly, carrier status for these variants has a large effect on metabolite levels and is relatively common in adult European populations, with carrier frequency estimated at approximately 2%. This association was likely missed in prior mGWAS that did not systematically test rare variants against metabolite ratios.

Lastly, the study provided new insights into metabolite associations of the SLC7A9 variant previously associated with the risk of CKD. The authors replicated the previously reported association of this locus with low urinary lysine concentration. In addition, they showed that the associations of this variant with ratios of lysine to neutral amino acids were much stronger than the association with lysine concentration alone. These findings are consistent with SLC7A9 functioning as an
apical exchanger of urinary cationic amino acids against neutral amino acids in the proximal tubule.

Importantly, rare mutations in SLC7A9 are known to cause recessive cystinuria characterized by CKD and recurrent nephrolithiasis.9 Similarly, Slc7a9 knockout mice have reduced GFR and excrete high amounts of lysine, cystine, arginine, and ornithine in urine.10 Therefore, the association of the CKD risk allele with lower urinary lysine (and presumably, cystine) levels is perplexing and remains unexplained by this study. Notably, cystine, which had been hypothesized to be a mediator of the CKD association, was not measured in this or prior genetic studies.8 We are thus left guessing if the true mechanism of the disease risk due to the SLC7A9 locus has anything to do with its effects on urinary cystine or other metabolites. As an alternative explanation, lower urinary lysine may simply represent a consequence of CKD, perhaps as a result of compensatory upregulation of proximal tubule transporters, with the true disease mechanism remaining independent of urine metabolite levels. More work is needed to pinpoint a specific causal mechanism operating at this locus.

Other limitations of this study relate to the use of single-spot urine measurements of metabolites, which are known to have a large degree of circadian variability, reducing the statistical power. Furthermore, this work involves relatively low metabolite coverage and may be missing several important metabolites that accumulate in CKD, such as hippuric acid, myoinositol, or other uremic toxins. The power to detect rare variant associations as well as gene-disease interactions is also limited in this cohort, and systematic exploration of such effects will have to await larger and more comprehensive studies. Nevertheless, this work represents an important step in deciphering the genetic regulation of the human metabolome in the setting of disease perturbation. For future studies in this area, the critical task will be to systematically tackle the cause-effect relationship between metabolite levels and kidney disease through formal mediation analyses anchored in the known CKD loci, longitudinal metabolite analyses in prospective cohorts, and/or functional studies in animal models.

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DISCLOSURES

None.

REFERENCES


See related article, “Genome-Wide Association Studies of Metabolites in Patients with CKD Identify Multiple Loci and Illuminate Tubular Transport Mechanisms,” on pages 1513–1524.

Using Large Datasets to Understand CKD

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