Interpreting Metabolomics and Proteomics in Kidney Disease: A Practical Guide

Insa M. Schmidt and Eugene P. Rhee

Metabolomics and proteomics, the systematic analysis of small molecules and proteins, respectively, have been increasingly used in kidney disease research to identify potential biomarkers and to provide biologic insights.[1] Both methodologies generate large datasets that require rigorous biostatistical and bioinformatic analysis, and both share key challenges, including variability in data quality and the potential for residual confounding, which occurs in observational studies when unmeasured or inadequately controlled variables distort observed associations.[2] In this Brief Guide, we summarize key principles and provide practical insights into the interpretation of metabolomics and proteomics studies, with a focus on blood-based analyses in human cohorts (Fig 1).

Overview of Metabolomics and Proteomics Methodologies

Different technologies are available to analyze the metabolome and proteome (Table 1).[1,3-5] Mass spectrometry (MS) has been a cornerstone for both metabolomics and proteomics, differentiating metabolites (amino acids, sugars, lipids, xenobiotics, etc) or peptides resulting from protein digestion based on their molecular weight. MS-based techniques often require up-front sample preparation (especially for proteomics) and separation steps such as liquid or gas chromatography to reduce sample complexity; however, these steps also reduce throughput and can influence the breadth of analytes detected. Nuclear magnetic resonance spectroscopy has also been used for metabolomics, leveraging the magnetic properties of atomic nuclei to determine the structure and concentration of metabolites within a sample without the need for up-front analyte separation, but the number of metabolites assayed by nuclear magnetic resonance is less than by MS.[6] In part because they do not require the up-front sample preparation required for MS-based approaches, multiplexed "affinity-based" approaches that use nucleotide-labeled antibodies or aptamers to assay proteins have gained considerable popularity.[7-9] The throughput of these affinity-based approaches has enabled blood proteomic profiling of population-based cohorts composed of thousands of individuals.

Although increasingly powerful, there is no entirely comprehensive metabolomics or proteomics methodology. Further, there are often considerable areas of non-overlap across platforms, even when the technology used is similar, as seen across different liquid chromatography–MS-based metabolomics platforms or across leading affinity-based proteomic platforms such as Olink and SomaLogic.[1,10] Many metabolomics and proteomics methods provide relative metabolite and protein quantitation in arbitrary units, rather than absolute concentrations. For any given method, there is also heterogeneity in how well individual analytes are measured, with differences in sensitivity and reproducibility.

A Brief Guide to Interpreting Metabolomics and Proteomics Studies

What is the purpose of the study?

Although the distinction is not always clear-cut, it is helpful to start with the question of whether a study seeks to identify new disease biomarkers or to discover new biology. For example, biomarker discovery studies that assess associations between metabolite or protein levels and clinical outcomes typically benefit from external validation in independent cohorts to ensure reproducibility and generalizability. Studies focused on biological discovery often pursue functional validation through experimental models. Metabolomics and proteomics can be powerful tools for both pursuits, but the study design, data analysis, and validation approaches often differ considerably depending on the goal.

Is the data high quality?

Strong track records of publication, including citations that provide methodologic details and information on assay specificity and precision, enhance confidence in data quality. Preanalytical factors such as sample temperature fluctuations, storage duration, and freeze-thaw cycles can impact both metabolite and protein stability.[1] For blood-based samples, it is important to clearly state the type of sample analyzed, as different sample types (eg, serum, EDTA plasma, or citrate plasma) can influence metabolite and protein measurements owing to differences in coagulation, proteolytic activity, and matrix composition.[11,12] These factors are sometimes, but not consistently, described in manuscript methods. Analytic platform differences (eg, mass spectrometry vs antibody- or aptamer-based assays) can also affect the comparability of measured values for the same metabolite or protein, depending on differences in sensitivity, specificity, and what molecular form is being captured.[13,14] Additional analytic factors, including outliers and batch effects, also require attention. Study methods sections should clearly delineate how quality control measures were implemented, including use of internal standards, spike-in controls, and batch effect corrections.[15,16]

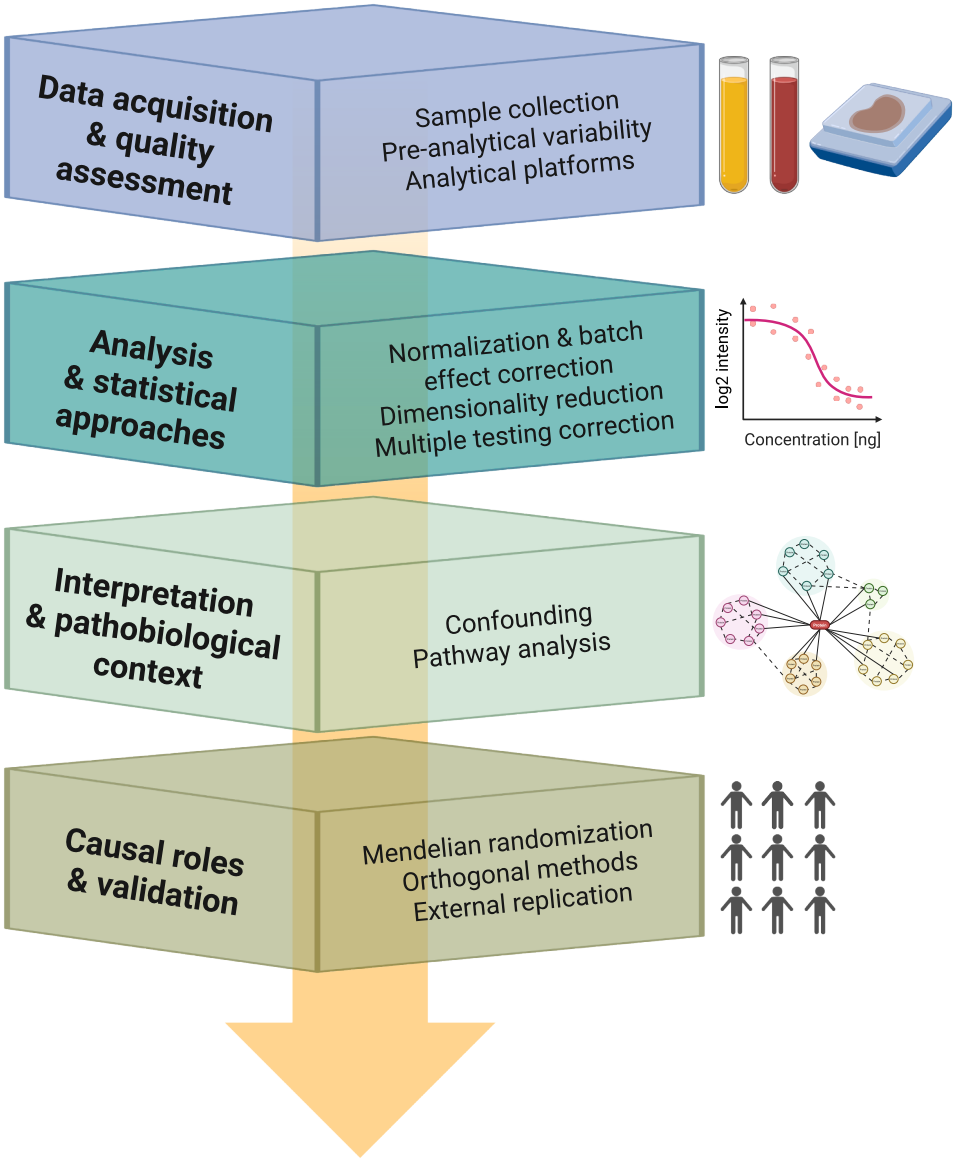

Figure 1. General workflow and considerations for interpreting metabolomics and proteomics studies in kidney disease research. Created with BioRender.com.

How are high-dimensional omics measurements analyzed?

An essential step to prepare measurement values for statistical analysis is normalization and scaling, which ensures that raw metabolite or protein values reflect accurate estimates of the underlying associations being observed. Common strategies include log transformation, median centering, and quantile normalization, all of which help to improve comparability and reduce the impact of extreme values.[16] Given the large number of variables in omics studies, dimensionality reduction techniques such as principal component analysis or clustering methods such as hierarchical clustering or non-negative matrix factorization can be applied to reduce noise, visualize patterns, and identify underlying data structures.[17] Common tools for differential analysis include limma and partial least squares discriminant analysis, which are typically used to identify candidate metabolites or proteins and are often used with predefined significance criteria, such as an adjusted P value or a fold-change threshold.[18] In all metabolomics and proteomics studies, multiple hypothesis testing should be explicitly addressed. To attenuate the risk of false discovery, statistical corrections such as false discovery rate adjustment or Bonferroni correction are commonly applied.[19] However, especially Bonferroni correction may be overly conservative for identification of biologic signals given the considerable intercorrelation between many analytes.

Table 1. Comparison of Metabolomics and Proteomics Technologies

Method	Type of Analyte	Throughput	Coverage	Sensitivity	Sample Preparation	Potential Applications	Important Considerations
Mass spectrometry	Proteins, metabolites	Moderate to high	~1,000s of analytes (metabolites or proteins)	High	Can be complex (especially for proteins); typically requires upfront separation technique (see below)	Broad profiling based on mass-to-charge ratio; can be used for targeted or untargeted applications	Different instrument types, ie, time of flight, quadrupole, and ion trap, have different advantages/disadvantages in mass accuracy, dynamic range, data complexity; generally, provide relative quantitation
Liquid chromatography–mass spectrometry					Liquid chromatography	Most common approach for separation of wide range of analytes	
Gas chromatography–mass spectrometry					Gas chromatography	Can be preferable for separation of volatile, nonpolar analytes	
Nuclear magnetic resonance spectroscopy	Metabolites	Low to moderate	~50-100 metabolites	Moderate	Minimal; no separation needed	Analyte identification based on magnetic properties of select atomic nuclei	Lower sensitivity, fewer detectable metabolites; can provide absolute quantitation
Affinity-based assays (eg, Olink, SomaScan)	Proteins	High	~1,000s of proteins	High	Minimal	High-throughput protein profiling using affinity reagents	Relative quantitation only; epitopes for most reagents not established

Is potential confounding addressed?

Age, sex, medication use, comorbidities (particularly diabetes and obesity for metabolomics), and kidney function can significantly influence metabolite and protein levels in blood. Estimated glomerular filtration rate (eGFR) is strongly associated with many metabolite and protein levels, and adjusting for baseline differences in eGFR is critical for any assessment of biomarker performance in kidney disease. Critically, adjusting for eGFR (and proteinuria) does not eliminate the risk of confounding by differences of kidney function, for example for analytes that undergo kidney metabolism, tubular reabsorption, and tubular secretion. By contrast, adjustment for eGFR has the potential to abrogate the association for circulating metabolites and proteins that play a causal, injurious role in kidney disease pathogenesis. Therefore, the risk of confounding and how it is addressed may depend on the purpose of the study. In patients with advanced CKD, additional factors such as dialysis modality (eg, hemodialysis vs peritoneal dialysis), residual kidney function, and sampling time (eg, pre- vs postdialysis) can also substantially influence circulating metabolite and protein levels, and should be carefully considered.

Are the metabolite or protein hits correctly assigned?

Whether the assignment of a given metabolite or protein identity is correct may require confirmation. Although beyond the scope of this review, this can sometimes be addressed by assaying commercial standards with the same methodology used to generate the data; many established metabolomics and proteomics platforms have already done this for a substantial number of analytes. In addition, an orthogonal method such as enzyme-linked immunosorbent assay (ELISA) for select proteins can be used to confirm findings. However, the discordance between the proteomic platform and a validated ELISA does not mean that the proteomic measurement is incorrect, as different methodologies may capture different isoforms of the same protein or the same protein with different post-translational modifications. Finally, because genome-wide association studies have been conducted in large cohorts profiled using Olink and SomaLogic, published cis-pQTLs for several hundred proteins measured by these platforms provide strong evidence that the protein identities are accurate (a cis-pQTL is a genetic polymorphism within a gene locus that is associated with blood levels of the encoded protein).

Are the findings validated?

For biomarker studies, validation of the metabolite or protein association with the outcome of interest in an external dataset is paramount to ensure robustness and generalizability. Nonoverlap in metabolome or proteome coverage across different platforms, or as a given platform expands coverage over different iterations, poses challenges and has been a barrier to the kinds of

large-scale meta-analyses that have been employed in genome-wide association studies.

For the pursuit of biologic insights, validation may involve a range of approaches to support biologic plausibility and causal associations. Pathway enrichment approaches, such as Ingenuity Pathway Analysis or gene set enrichment analysis, can place findings in pathobiological context; however, limitations of these approaches include potential bias owing to incomplete platform coverage and reliance on predefined gene sets that may not fully capture novel or context-specific biological mechanisms.[20] In addition, metabolites and proteins in blood can come from different tissues and are impacted by multiple factors—using blood-based metabolome or proteome snapshots to infer intraorgan biology warrants caution. In some cases, investigators may choose to correlate blood-based findings with tissue-based profiling of metabolites, proteins, or related genes. Direct assessment of renal arteriovenous gradients for circulating metabolites and proteins can provide insight into what molecules may be cleared or synthesized by the kidney.[21,22] To further disentangle causality from correlation, Mendelian randomization has emerged as a helpful tool, leveraging genetic variants as instrumental variables to infer causal relationships between circulating proteins or metabolites and outcomes of interest.[23-25] However, potential limitations, such as the use of genetic instruments with limited explanatory power or bias owing to horizontal pleiotropy, should be carefully considered when interpreting results.[26]

Recent Example of a Proteomics Study in Nephrology Research

In a recent study, Schmidt et al[27] investigated plasma biomarkers of acute tubular injury (ATI), a key histopathologic feature of acute kidney injury and an important predictor of future kidney disease progression. The primary purpose of the study was to identify potential candidate biomarkers for the noninvasive assessment of ATI, which could facilitate earlier detection and improve risk stratification in kidney disease. The study employed the SomaScan platform to identify plasma proteins associated with ATI severity in a cohort of patients (n = 434) with biopsy-confirmed kidney disease. Data quality was addressed through several measures, and the authors provided details on processing pipelines, sample storage, and coefficients of variation. This study did not explicitly apply high-dimensionality reduction techniques such as principal component analysis or clustering. However, the data were processed using $\log_2$ transformation and a structured pipeline for data preparation and normalization. Statistical analyses were conducted using multivariable regression models to assess associations between plasma biomarkers and ATI severity. The study accounted for key confounders, including age, sex, race, and eGFR, and addressed multiple testing by applying Bonferroni correction. For the identified protein hits, the study did not perform orthogonal validation using independent methods such as ELISA. However, select blood proteome findings were cross-referenced with kidney tissue expression data, including regional transcriptomics and proteomics. The study also employed a multicohort validation approach, with demonstration that the biomarkers discovered in the initial biopsy cohort were associated with incident acute kidney injury in external epidemiologic studies. Taken together, this integrative framework demonstrates the potential of proteomics approaches in nominating new markers of kidney disease histopathology.

Summary

Metabolomics and proteomics approaches have emerged as powerful tools in nephrology research. However, careful analysis and interpretation of these high-dimensional datasets, guided by the overarching purpose of the given study, remain essential to ensure clinical and biological relevance.

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