Lipidomics and Biomarker Discovery in Kidney Disease

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Summary: Technological advances in mass spectrometry–based lipidomic platforms have provided the opportunity for comprehensive profiling of lipids in biological samples and shown alterations in the lipidome that occur in metabolic disorders. A lipidomic approach serves as a powerful tool for biomarker discovery and gaining insight to molecular mechanisms of disease, especially when integrated with other -omics platforms (ie, transcriptomics, proteomics, and metabolomics) in the context of systems biology. In this review, we describe the workflow commonly applied to the conduct of lipidomic studies including important aspects of study design, sample preparation, biomarker identification and quantification, and data processing and analysis, as well as crucial considerations in clinical applications. We also review some recent studies of the application of lipidomic platforms that highlight the potential of lipid biomarkers and add to our understanding of the molecular basis of kidney disease.

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Lipids are the most abundant metabolites in the circulation and are an integral part of cell structure and function. Their alterations are linked with disease severity and outcome.[1,2] Historically, clinical and epidemiologic research on lipids has been limited to measurements of lipoproteins and total triglycerides. However, technological advances in modern mass-spectrometry–based lipidomic platforms have allowed identification and quantification of a large array of lipids.[3–5] Such advances provide opportunities to expand diagnostic capabilities through biomarker discovery efforts, identify novel alterations in involved pathways, and potentially identify targets amenable to intervention. The utility of large-scale lipidomic profiling has been shown in a number of systemic disorders,[3–8] and recent promising data have indicated that lipid profiling provides a powerful discovery tool in nephrology research as well.[2,8–11] In this article, we review the processes of lipid identification and quantification with lipidomic platforms, discuss the steps that commonly are applied in biomarker discovery efforts, and summarize some of the newly emerging findings on the role of lipids in clinical kidney-related studies.

CHALLENGES

Lipidomics has evolved tremendously in the past decade. However, despite this rapid growth, significant barriers remain to achieving the goal of a precise and comprehensive evaluation of the lipidome and its incorporation into metabolic pathways. The most significant challenge remains the extreme molecular heterogeneity of lipid compounds, which limits comprehensive characterization and quantification of the classes, multiple subclasses, and numerous species of biological lipids.[12] The desired balance between achieving precise quantification and capturing an ever-expanding lipidome determines the most appropriate platform for each lipidomic experiment.

The structural diversity that gives lipids their biological versatility and determines their chemical properties makes an all-inclusive method for their analysis difficult to achieve. Because standardized fragmentation spectra are lacking for lipids, pairing high mass accuracy with retention time is particularly helpful in the identification of lipid species. The current instrumentation (Orbitrap, ThermoFisher, Waltham,

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MA and Fourier transform)[13] provides adequate mass resolution and accuracy, but challenges remain in separating lipid classes using hydrophobicity-based criteria. This is complicated further by similar hydrophobicity characteristics of some classes, and recent efforts have used serial chromatographic steps to achieve compound separation and increase coverage of the lipidome.[14,15] Currently, the most popular methods for high-throughput lipid analysis are limited in their ability to determine the precise arrangement of fatty acid substituents attached to the lipid-head group and the positions of any double bonds in the structure. Several techniques using alkali metal adducts[16] and ozone-induced dissociation[17] have been used with mixed results and are complicated by very low ion abundance and the generation of complex data, which can compromise results. Recent experiments using ion-mobility mass spectrometry have shown promising results, and this technology remains at the forefront of method development.[18]

There are additional obstacles in data processing for lipidomic analyses because of the absence of robust, platform-independent software for conversion of raw data into quantifiable biological information for analysis. This bioinformatic deficit is exacerbated further by the inability to comprehensively integrate lipidomic data with molecular profiles from other types of -omic analyses, which would allow us to validate the lipidomic data through investigation of other -omic modalities and identify pathways or lipids requiring further study to enhance our understanding of metabolic regulation. Our current measurements and analyses provide a snapshot of the lipidome at a given point in time without identifying the determinants of the lipid profile and raising questions about the role of enzymatic activity and genetic regulation. These aspects can be explored further with experiments looking at stable isotope monitoring in flux analysis to determine reaction rates in biochemical pathways and advance our understanding of both human health and disease.

LIPIDOMICS WORKFLOW

Lipidomics workflow may differ from study to study based on the aims and scopes of each project. The steps summarized in Figure 1 are followed in most studies.

Study Design

At the beginning of each lipidomics study, a decision must be made regarding which platform (untargeted or targeted) is most appropriate to answer the study question. Furthermore, application of biological and technical replicates and a plan for quality control and assurance are important study pillars that need to be addressed in the design and largely are determined by the study's questions and aims.

Untargeted and targeted platforms

Lipidomic studies are broadly classified as untargeted or targeted. Table 1 summarizes these two approaches to lipidomics and their most important characteristics.

The untargeted approach is a hypothesis-generating platform that provides a snapshot of all identifiable lipid species in the biosample. In an untargeted analysis, the goal is to identify as many lipids as possible to determine differentially regulated lipid panels. Analysis using an untargeted platform is viewed as the discovery phase, and the differentially regulated lipids identified then comprise a panel of candidate biomarkers, which is required for confirmatory downstream studies using targeted platforms. During sample preparation, chromatographic separation, and mass spectrometric detection there may be preferential detection of certain lipids and signal loss for others, which make the method susceptible to systematic and methodologic bias. Measurements are semiquantitative and are based on the relative abundance of peak intensities because absolute quantification is not feasible. When using untargeted platforms, more rigorous quality control is required to ensure high-quality data. Adequate quality control includes continuous instrument maintenance and calibration and application of various pooled samples, internal standards, and technical replicates. Internal standards are isotopically labeled analogs of the target analytes, which are added to the samples to compensate for losses during sample preparation and ionization, and help with the normalization process. In untargeted platforms, generally no more than one or two internal standards are used per lipid class because of the unavailability of internal standards for each endogenous lipid and prohibitive costs upon availability.

Conversely, the targeted approach is a hypothesis-driven platform. In targeted analyses, the goal is to

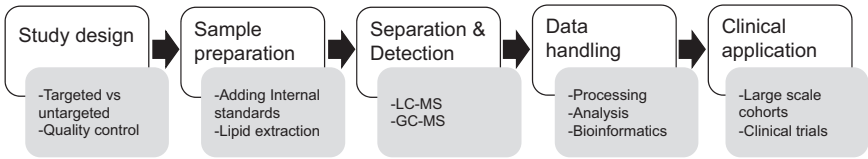

Figure 1. Summary of typical workflow when using lipidomic platforms for biomarker discovery. GC-MS, gas chromatography–mass spectrometry.