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REVIEW ARTICLE

Interpreting the lipidome: bioinformatic approaches to embrace the complexity

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Abstract

Background Improvements in mass spectrometry (MS) technologies coupled with bioinformatics developments have allowed considerable advancement in the measurement and interpretation of lipidomics data in recent years. Since research areas employing lipidomics are rapidly increasing, there is a great need for bioinformatic tools that capture and utilize the complexity of the data. Currently, the diversity and complexity within the lipidome is often concealed by summing over or averaging individual lipids up to (sub)class-based descriptors, losing valuable information about biological function and interactions with other distinct lipids molecules, proteins and/or metabolites.

Aim of review To address this gap in knowledge, novel bioinformatics methods are needed to improve identification, quantification, integration and interpretation of lipidomics data. The purpose of this mini-review is to summarize exemplary methods to explore the complexity of the lipidome.

Key scientific concepts of review Here we describe six approaches that capture three core focus areas for lipidomics: (1) lipidome annotation including a resolvable database identifier, (2) interpretation via pathway- and enrichment-based methods, and (3) understanding complex interactions to emphasize specific steps in the analytical process and highlight challenges in analyses associated with the complexity of lipidome data.

Keywords Lipidomics · Bioinformatics · Lipid Identification · Ontologies · Pathway enrichment · Data integration

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1 Introduction

Due to the central role that lipids play in energy metabolism, cell structure, signaling, and protein function as well as technological advancements, current research programs ranging from biomedical to ecological applications have shown an increase in the analysis of lipids (O'Donnell et al., 2020). Lipidomics specifically is the large-scale study of the structure and function of all lipids in a sample, termed the lipidome. Studies employing lipidomics have rapidly increased over twofold in the last five years, since recent advances in mass spectrometry (MS) technologies together with bioinformatic developments are enabling researchers to routinely identify hundreds of unique molecular lipid species (Aimo et al., 2015; Fahy et al., 2019; Fahy et al., 2009; Ni et al., 2017; Ni et al., 2017a, b). However, understanding the biological relevance and effect on the function of other biomolecules (e.g., proteins) is a grand challenge in the field of lipidomics, given the diversity and complexity thereof

(Harayama & Riezman, 2018). Unraveling this challenge creates considerable opportunities to advance lipidomics research through computational approaches (Clair et al., 2019; Madrid-Gambin et al., 2019; Oresic, 2011; Slenter et al., 2018; van der Meer-Janssen et al., 2010).

The International Lipid Classification and National Nomenclature Committee referred to as LIPID MAPS (http://www.lipidmaps.org) (Fahy et al., 2009) categorizes lipids into eight categories, based on their chemical and biochemical properties. These lipid classes are further divided into more specific structural and chemical properties; currently there are over 40,000 lipid structures in this database. The complexity of the lipidome is often under-described by current lipidomics pipelines as most lipids cannot be fully structurally resolved and represent aggregated isobaric species, thereby losing valuable information affecting the biological interpretation. For example, incorporating quantitative data for individual complex glycerolipids or phospholipids is impossible in most pathway analysis steps, since the average values of the entire lipid subclass can only be plotted, not the individual lipids. This can result in the simplification of intrasubclass lipid dynamics and inaccurate interpretations (Fig. 1). Currently, class-based analyses can only confidently identify hundreds of lipids versus the thousands potentially present.

Embracing the diversity and complexity of the lipidome is essential to understand the specific biological functions and mechanisms within biological systems. In addition, inferring the interaction of the lipidome with the genome, proteome and metabolome is key to mechanistic understanding. While methods and tools in bioinformatics and computational biology have improved over the past few years, computational developments are at the center of the gap in deriving insights from lipidomics data. In this mini-review, we will highlight six methods that currently address these grand challenges in lipidomics and share opportunities to enhance lipidome characterization and underlying biological functions. Specifically, this is not a comprehensive review; but we detail a few approaches to identify novel lipids, enrichment analyses that bridge complex lipid species to aid in biological interpretation, and novel approaches to integrating lipids with biomolecules collected from other 'omics data types.

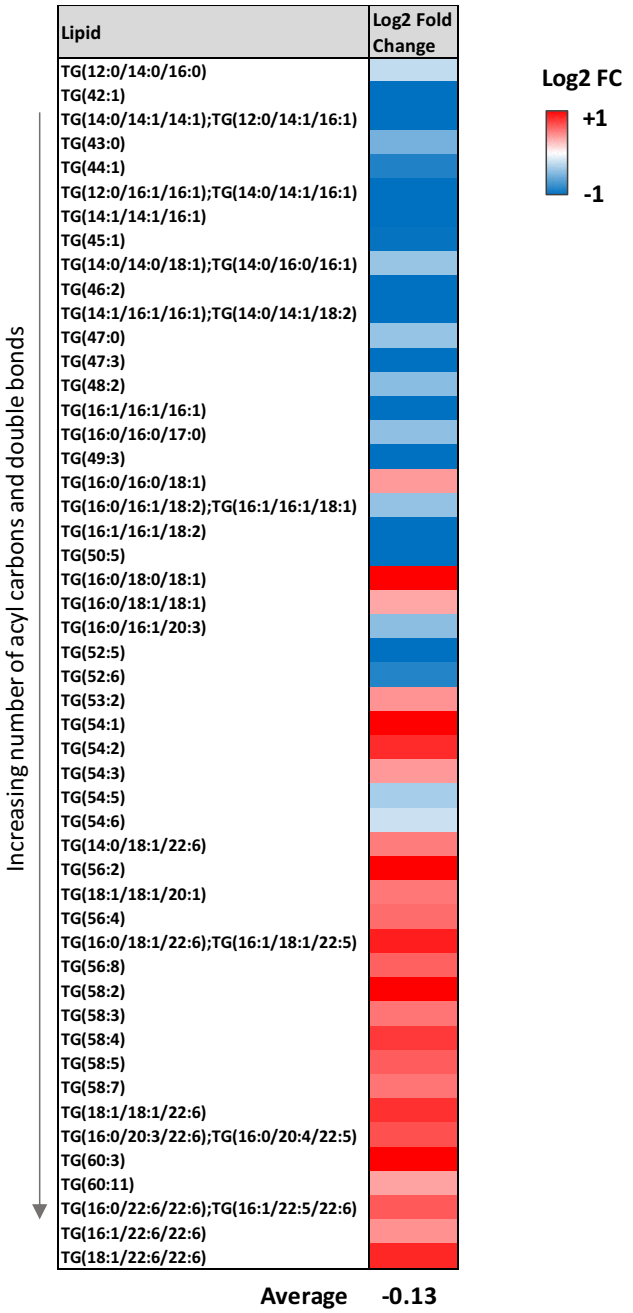

Fig. 1 Heatmap of $\log_2$ Fold-Changes (FC) for triacylglycerides (TG) identified in a virus-infected cell line study. Currently, most pathway analysis tools can only plot the value of the TG subclass rather than the individual lipids. In this example, the average value over identified TGs ($\log_2$FC of − 0.13) would have obscured the effect of the infection. In addition, by allowing only one value for the TG subclass to be plotted on a pathway map, information on TG depletion (blue FCs) and enrichment (red FCs) associated with fatty acid chain length and unsaturation state would have been lost

2 Lipidome characterization: advanced identification and knowledgebase

2.1 Exploring the diversity of natural lipidomes: a focus on high accuracy lipid identifications

Robust liquid chromatography–tandem mass spectrometry (LC–MS/MS) workflows to improve lipid identification in complex biological samples greatly expanded over the last 5 years (Ellis et al., 2018; Fahy et al., 2019; Hutchins, Russell, & Coon, 2018; Kyle et al., 2017; Misra & Mohapatra, 2019; Ni et al., 2017; Ni et al., 2017a, b; Peng et al.,

2020; Tsugawa et al., 2015). A review of current lipidomics identification software is available elsewhere (Misra & Mohapatra, 2019; O'Shea & Misra, 2020) including on the LIPID MAPS website (https://www.lipidmaps.org/resources/tools). However, automated analysis and subsequent identification of MS and MS/MS datasets often remain a bottle-neck in modern untargeted lipidomics. In the past few years, several approaches have been reported to improve identification (Bowden et al., 2018; Hutchins et al., 2018; Koelmel et al., 2017; Kyle et al., 2017; Kyle et al., 2016; Tsugawa et al., 2015; Tsugawa et al., 2020). One example software, LipidHunter, is an open source tool, which was developed to facilitate high-throughput identification of lipids from data-dependent LC–MS and shotgun datasets (Ni et al., 2017a, b). The tool performs lipid identification by assembling detected fragments to the matching precursor using a pre-defined fragmentation rules and white list of lipid building blocks (e.g., fatty acyl chains and phospholipid head groups). By utilizing this bottom-up approach, LipidHunter does not require structural or spectra databases, and can be easily adjusted to fit user-specific analytical conditions. LipidHunter provides information-rich tabular and graphical reports allowing users to trace back key identification steps and perform data quality control. The tool is freely available at https://github.com/SysMedOs/lipidhunter, and is equipped with a graphical user interface.

With increasing sensitivity and resolution of modern MS platforms the complexity and diversity of natural lipidomes, including low abundant modified lipid species, can now be investigated in much more detail. Lipids can be enzymatically and non-enzymatically modified by the introduction of small chemical groups. Similar to DNA (epigenetics) and proteins [Post Translational Modifications (PTMs)], modifications of lipids via enzymatic and non-enzymatic reactions including oxidation, nitration, sulfation and halogenation exist. These modifications are required to regulate complex biological function, thereby creating a new level of lipidome complexity (the epilipidome) (Ni et al., 2019). The LPPtiger software was specifically developed to support automated processing of shotgun and LC–MS/MS datasets for the identification of oxidized lipids (Ni et al., 2017a, b). LPPtiger includes three main algorithms—in silico prediction of oxidized lipids, their in silico fragmentation, and identification from MS/MS spectra using a multi-scoring approach. The in silico prediction is a unique feature of LPPtiger based on the implementation of metabolic networks covering enzymatic and non-enzymatic oxidations for ten main mammalian polyunsaturated fatty acids (PUFAs). The in silico fragmentation algorithm was developed based on the accurate assignment of more than 500 MS/MS spectra of oxidized lipids generated from chemically defined lipid standards via in vitro oxidation. Finally, the identification algorithm relies on a spectra matching approach (reverse dot plot based score to compare experimental and in silico predicted MS/MS spectra), as well as isotopic, rank (bottom-up), specificity, and fingerprint scores increasing specificity. LPPtiger provides unique solutions for high-throughput epilipidomics (Ni et al., 2019) with graphical interface, information-rich tabular and graphical reports and can be downloaded at https://bitbucket.org/SysMedOs/lpptiger.

2.2 Lipid databases: linking lipids with enzymes

Searchable databases for the field of lipidomics are relatively rare resources. In 2003, the LIPID MAPS committee was formed, establishing an internationally recognized classification system and curated lipid structure database of individual molecular lipid species. Besides gathering structural information and categorization, linking lipids with their biological function is of great need in the lipidomics community. SwissLipids (www.swisslipids.org) is an expert curated knowledge resource dedicated to lipids and their biology, designed to better integrate lipidomic data with biological knowledge (Aimo et al., 2015). This knowledgebase features a library with more than 590,000 known and theoretical lipid structures belonging to over 500 lipid classes, each enriched with information on lipid components, reactions, and enzymes, with supporting links to primary literature. All lipids are mapped to the chemical ontology database ChEBI (www.ebi.ac.uk/chebi/) (Hastings et al., 2016), which is used to describe lipid metabolism in the Rhea knowledgebase of biochemical reactions (www.rhea-db.org) (Morgat et al., 2020), and linked to the corresponding enzymes from the UniProt knowledgebase UniProtKB (www.uniprot.org) (The Uniprot Consortium 2021). SwissLipids features two distinct hierarchical lipid classifications—one that parallels the structural classification of LIPID MAPS, and one based on the shorthand notation for mass spectrometry (Liebisch et al., 2020; Liebisch et al., 2013) that maps lipid analytes to structures and biological knowledge such as enzymes (Table 1).

Users can browse these classifications, search for lipids (by lipid name, abbreviation, formula, SMILES, InChIKey, mass) and enzymes (using gene names and UniProtKB accessions), or use the ID mapping tool to map identifiers from reference resources like LIPID MAPS and HMDB to their corresponding structures in SwissLipids, reactions from Rhea, and enzymes from UniProtKB. The latest addition includes the expert curation of complex glycosphingolipids and their metabolism, using as a base the work done in SphinGOMAP (http://www.sphingomap.org/) (Merril, 2005). SwissLipids nomenclature is aligned with the Lipidomics Standards Initiative (LSI). All data is freely available for download and reuse under a CC-BY-4.0 license, with an API providing programmatic access.

Table 1 Lipid analytes can be linked to enzymes using the hierarchical classification

Structural hierarchical classification levels	Lipid class and sum composition known, individual fatty acids unknown	Lipid class and individual fatty acid composition known, *sn*-positions unknown	Lipid class and individual fatty acid composition known, *sn*-positions known	Lipid class and individual fatty acid composition with known position and geometry of double bonds, *sn*-positions known	Enzyme(s) that can catalyze this lipid
Examples for lipid shorthand notation	PC(38:4)	PC(18:0_20:4)	PC(18:0/20:4)	PC(18:0/20:4(5Z,8Z,11Z,14Z))	PLA2G4A PLA2G15

Like SwissLipids, UniProtKB now uses Rhea to describe enzymatic reactions (Morgat et al., 2020) and provides a rich source of lipid annotations which can be accessed interactively or programmatically to annotate lipidomics datasets or databases. Future iterations of SwissLipids will use UniProtKB as the basis to build and annotate more extensive lipid libraries covering all taxa in UniProtKB.

3 Interpretation via enrichment-based methods

The interpretation of sequence-based-omics (= genomics, transcriptomics, proteomics) greatly benefited from the sequence-function relationship that enables researchers and clinicians to relate specific sequences to ontological terms, pathways, and to their cellular localization. Lipids are classified into different categories, each with their own class and subclass hierarchy based on a common backbone structure, and can comprise of a variety of acyl chains. Their physicochemical properties depend on these parameters, which also determine the role they carry out in biological systems. The lipid nomenclature has evolved throughout recent years to become simpler and more representative of their chemical nature. Currently, the most updated lipid databases, namely LIPID MAPS and SwissLipids, utilize the same simplified nomenclature to describe lipids. In the majority of the cases, the lipid names alone contain essential details necessary to determine the chemical nature of the lipids (e.g., "common name" in LIPID MAPS and "abbreviation" in SwissLipids). Utilizing the information provided by lipid common names, two lipidomics enrichment tools, Lipid Mini-On (Clair et al., 2019) and LION/web (Molenaar et al., 2019) has recently been developed to aid users in the biological interpretation due to alterations in the lipidome.

The R based tool Lipid Mini-On reads this unifying nomenclature and generates structural ontology terms from these names (Clair et al., 2019). Currently, the applied text-mining algorithm can generate Lipid Ontology (LO) terms from molecules belonging to 7 lipid categories, over 42 main classes, and 109 subclasses of lipids. As Lipid Mini-On uses a text mining approach rather than a database approach, LO terms can be generated for an infinite number of lipids whether they are present in databases or not (as long as their naming follows the common lipid nomenclature established by LIPID MAPS). For LO enrichment analysis, Lipid-Mini-On contains a variety of common enrichment statistics including methods enabling to compare a query to a background list (e.g., DAVID's EASE score (Huang et al., 2007), Fisher's exact, binomial, and hypergeometric tests) or assessing the distribution of the lipids within a ranking lipids list (e.g., weighted Kolmogorov–Smirnov test). The tool has been complemented with the generation of publication-level figures representing

the proportion of the LO attributed to the different lipids (and subsets of lipids). Further, users can also identify redundant LO terms via the generation of interactive networks of these terms and their associated lipid species. Lipid-Mini-On is available both as an R package (Rodin: https://github.com/PNNL-Comp-Mass-Spec/Rodin) and as a simple and intuitive graphic user shiny interface (https://omicstools.pnnl.gov/shiny/lipid-mini-on/).

Whereas Lipid Mini-On generates a lipid ontology on-the-fly, a second enrichment tool, LION/web (Molenaar et al., 2019), makes use of a hard-wired Lipid Ontology (LION, http://bioportal.bioontology.org/ontologies/LION). LION links over 50,000 lipid species to the well-established LIPID MAPS-classification system (Fahy et al., 2009). In addition, LION associates lipid species to biophysical, chemical and cell biological features such as headgroup charge, membrane fluidity, intrinsic curvature, and predominant subcellular localization. As a result, LION-terms are defined lipid subsets that share properties of biological interest. Rather than to be used as a knowledge database, LION is designed specifically for enrichment purposes—the level of detail is high enough to obtain new biological insights on the one hand, and on the other hand sufficiently restricted to preserve enough statistical power.

The associated user-friendly online web-tool (LION/web, accessible at www.lipidontology.com) performs LION-term enrichment analysis on user-provided lipidomics datasets and generates publication-ready figures in just a few clicks. The web-tool is platform-independent, has great flexibility in the matching of several lipid nomenclature dialects to the ontology, and accepts a simple CSV-file as input. Enrichment analysis can be performed by either comparing hitlists with background lipidomes, or by assessing LION-term distributions over ranked lists of lipids (Molenaar et al., 2019).

A recent addition to LION/web is the LION–PCA heatmap module (heatmap.lipidontology.com). This module generates a heatmap containing the most dynamic LION-terms in a given dataset. These LION-terms are selected by performing enrichment analysis based on the loadings of a user provided number of principal components (describing the variation in the data) through Principal Components Analysis (PCA). The module offers built-in options for clustering and manual visualization. Importantly, while classical enrichment analyses typically compare only two conditions, LION–PCA heatmap is able to process datasets with more conditions, thereby offering an intuitive way to quickly browse through meaningful trends of a complete dataset (Fig. 2).

4 Understanding complex interactions: integration with other omics

4.1 Integrating lipidomics data with Pathway Models

Pathway analysis is routinely conducted for transcriptomics (Kutmon, Evelo, & Coort, 2014) and proteomics data separately, or by integrating this data with other 'omics datasets, e.g., metabolomics data, for which the usable set of tools is increasing (Jassal et al., 2020; Jewison et al., 2014; Luo et al., 2017; Zhou & Xia, 2018, 2019). Unfortunately the integration of lipidomics data is still lagging, which is primarily due to the difficulties in identifying the exact structure of lipids as well as missing biological knowledge in pathway databases. This issue is particularly true for complex lipids (e.g., glycerolipids, glycerophospholipids, sphingolipids), which are formed (and altered) through a network of proteins. Given this challenge, most pathway tools map the individual lipids to their main parent-term or a less specific subclass of lipids, however are unable to map higher structural resolution data generated from modern lipidomics methodologies. To address this need, in the last couple of years, multiple computation approaches have been developed to bridge lipidomics pathway mapping and the integration of other 'omics data (see review by Lam et al., 2020). Another approach to bridge this gap is the open source pathway database WikiPathways (O'Donnell et al., 2019; Slenter et al., 2018). WikiPathways for lipidomics started with the conversion of nine highly curated originally *Mus musculus* (mouse) pathways (Dennis et al., 2010) to their *Homo sapiens* (human) counterpart by homology mapping. Several of these pathways were updated with previously unknown signaling functions, e.g. anti-inflammatory effects of eicosanoids (which were previously though to be pro-inflammatory only) (Dennis & Norris, 2015). WikiPathways data for lipids is accessible for both mouse and human species as bulk data download for pathway analysis and -omics integration, e.g., GUI and automation in PathVisio (Ellis et al., 2018; Kutmon et al., 2015) and Cytoscape (Shannon et al., 2003). Furthermore, the pathways are open for community curation and species extension, and are available at the Lipids Portal (http://lipids.wikipathways.org) (Martens et al., 2021), which will expand over time as new knowledge is generated.

4.2 Modeling lipid metabolism in health and disease

Lipids have many key structural and functional roles, including being crucial for energy storage, as parts of cellular membranes and particles (e.g., lipoproteins,

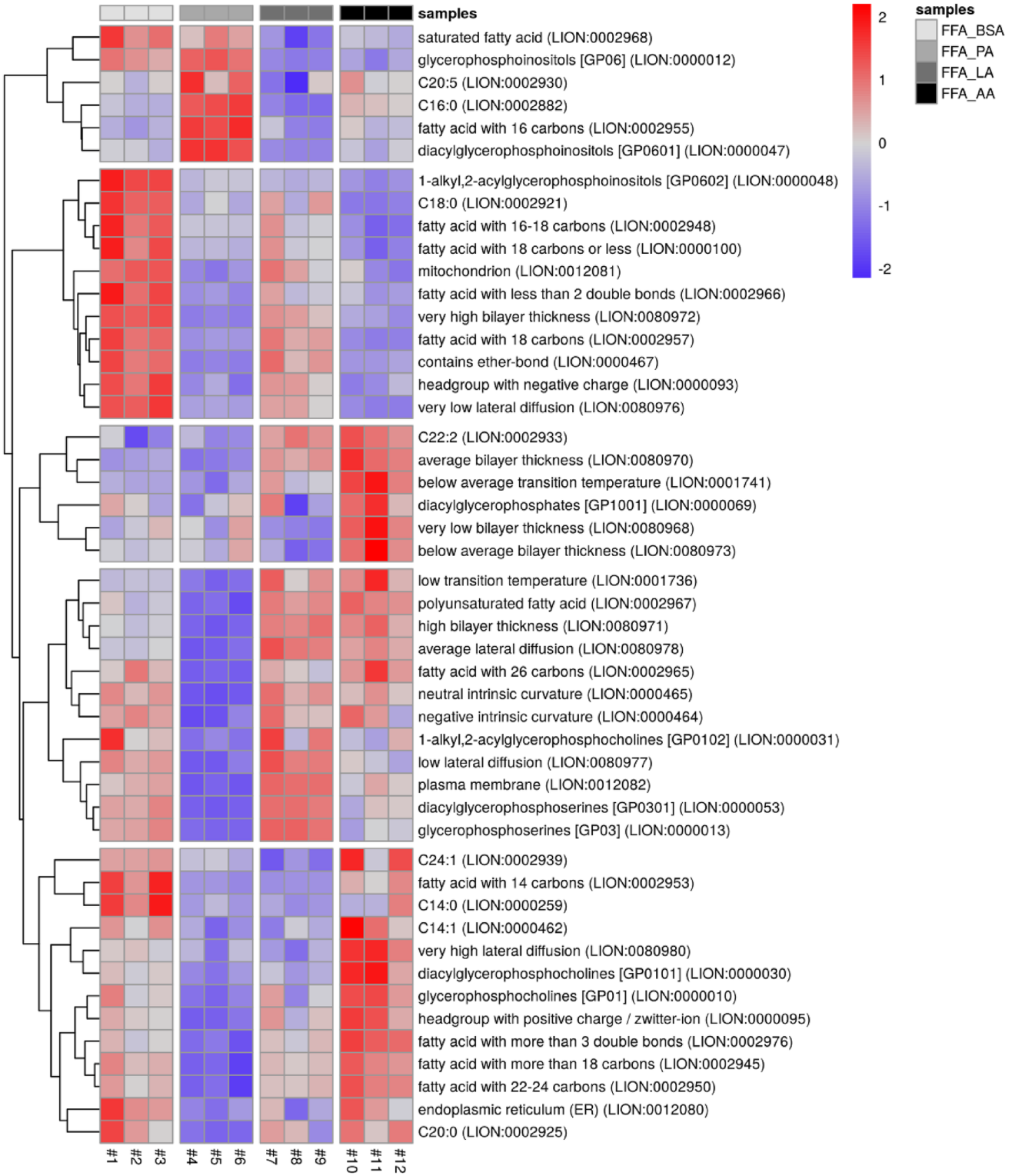

Fig. 2 PCA–LION heatmap of a previously published lipidomics dataset containing the lipid profiles of CHO-k1 cells, incubated with different free fatty acids (FFA): palmitic acid (PA), linoleic acid (LA), arachidonic acid (AA), or control (BSA) (Molenaar et al., 2019). Heatmap colors (from blue to red) indicate the mean z-score of a given LION-term per sample. Enriched LION-terms (displayed at the right side) are grouped into five clusters by hierarchical clustering. Samples are sorted by sample group (control, incubated with palmitic acid, linoleic acid, and arachidonic acid) and labeled with their unique sample number at the bottom of the heatmap

exosomes), as signaling molecules, and as mediators in central metabolic pathways. Given such important roles, lipid metabolism is tightly regulated and lipid-related disturbances characterize many common diseases (Hyotylainen & Oresic, 2014). Such a functional diversity presents specific challenges for the analysis and interpretation of lipid-related data, such as from lipidomic analysis (Alves et al., 2021).

Lipidomic profiles measured in biological samples reflect bulk amounts of lipids, with high underlying spatial complexity. In the case of plasma samples, lipid profiles depend on the composition of lipoprotein particles (e.g., VLDL, HDL, LDL), while in cell or tissue samples for example, lipidomic profiles also reflect compositions of cellular membranes. Since lipid structures are tightly homeostatically regulated in order to maintain key biophysical properties such as membrane fluidity, lipids are also highly co-regulated. In lipidomics data, this regulation is observed as a high degree of associations between and within different classes of lipids. Lipids should therefore be analyzed as ensembles, e.g. by various multivariate methods, in order to capture underlying co-regulation of individual lipids captured within the data. Additionally, there is increasing interest in using molecular simulations (e.g. molecular dynamics) to study the lipidomes in their spatial and dynamic context (Ingolfsson et al., 2017; Pietilainen et al., 2011; Wassenaar et al., 2015).

With the advent of genome-scale metabolic reconstructions for many species including human, Genome-Scale Metabolic Modeling (GSMMs) have been increasingly used to study lipid metabolism, including in human health and disease (Hyotylainen et al., 2016; Nielsen, 2009; Thiele et al., 2013). GSMMs are used for constraint-based modelling, integrating genetic and biochemical information about specific compartment/cell/tissue/organism within a computational framework, which can be used to decipher the metabolic genotype–phenotype relationship of an organism (Brunk et al., 2018). The most common input for GSMM is transcriptomic data, which helps link metabolic genes with the specific pathways, and models can be further constrained, for example, by metabolomics/lipidomics, proteomics and dietary intake data. Given the spatial and functional complexity of lipid metabolism, GSMM approaches to study lipids have so far lagged behind on the modeling of central metabolic pathways relevant for lipid interactions. Nevertheless, studies and model building including lipids by using GSMMs are increasing (Alves et al., 2021), e.g., for adipocytes (Mardinoglu et al., 2013), peripheral blood mononuclear cells (PBMCs; Sen et al., 2020), and hepatocytes (Mardinoglu et al., 2014). For example, GSMM was applied in a recent study of PBMCs from children who later progressed to type 1 diabetes (T1D) and from nondiabetic controls (Sen et al., 2020). While altered lipid, including sphingolipid metabolism as examined by serum lipidomics, is a common metabolic feature preceding the onset of T1D, here GSMM helped to identify specific ceramide pathways in immune cells that are specifically associated with progression to T1D (Johnson et al., 2019; Oresic et al., 2008).

5 Conclusions

Understanding the biological relevance of the vast diversity of lipids is a fundamental challenge in lipidomics (Harayama & Riezman, 2018). The past 5 years has seen a rapid rise and advancement of lipidomics based software tools. These tools, as highlighted above, (1) improve the resolution and confidence of lipid identifications, (2) enable scientists to link identified lipids with biological knowledge through the use of databases and enrichment tools, and (3) integrate lipidomics data with other classes of molecules for a cellular and molecular understanding of lipid homeostasis and metabolism.

As high accuracy and high throughput MS technologies continue to improve and enable a more comprehensive characterization of the lipidome, both in assigning the exact structure and the coverage of lipids, computational and bioinformatics approaches will increasingly to be required to fully embrace and utilize the power of data generated from lipidomics analyses. Some of the greatest needs include (1) developing a false discovery rate (FDR) for confidently identifying lipids such that metrics of confidence can be universally applied, (2) tools that map and integrate novel accurate lipid structures with other cellular molecules, and (3) identifier mappings to enable more robust integration of lipidomics data with pathways (Waagmeester et al., 2020) and databases (van Iersel et al., 2010).

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Declarations

Conflict of interest All authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval This review is a retrospective analysis of existing tools and databases and therefore approval by the Research Ethics Committees were not applicable.

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