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Sarantos Kostidis, Elena Sánchez-López and Martin Giera

Abstract

Despite being a relatively new addition to the Omics' landscape, lipidomics is increasingly being recognized as an important tool for the identification of druggable targets and biochemical markers. In this review we present recent advances of lipid analysis in drug discovery and development. We cover current state of the art technologies which are constantly evolving to meet demands in terms of sensitivity and selectivity. A careful selection of important examples is then provided, illustrating the versatility of lipidomics analysis in the drug discovery and development process. Integration of lipidomics with other omics', stem-cell technologies, and metabolic flux analysis will open new avenues for deciphering pathophysiological mechanisms and the discovery of novel targets and biomarkers.

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Introduction: Lipid metabolism as druggable axis

Lipid metabolism has early on been adopted as druggable axis in various diseases [1]. Key examples include, cholesterol lowering therapies (e.g. statins as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors), ergosterol biosynthesis as anti-fungal drug target (e.g. azole antifungals) as well as eicosanoid metabolism as key target of non-steroidal anti-inflammatory drugs (NSAID) (e.g. ibuprofen, diclofenac). In parallel with the development of advanced lipidomics technologies and integration with other Omics' has lipid metabolism emerged as therapeutic avenue in various pathologies, including anti-viral therapies [2], non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steato-hepatitis (NASH) [3] (chronic) inflammation [4] and cancer [5]. Recently, the combination of metabolomics/lipidomics with induced pluripotent stem cell (iPSC) technologies has led to significant discoveries particularly in the field of neurodegenerative diseases as for example, Alzheimer's disease, multiple sclerosis and Parkinson's disease [6–8]. In most cases, the identified targets include the interaction of lipids with proteins or nuclear receptors. Notable enzyme-related examples are stearoyl CoA desaturase 1 (SCD1), dehydrocholesterol reductase 24 (DHCR24) [9], prostaglandin dehydrogenase (PGDH) [10], mechanistic target of rapamycin (mTOR) and inhibition of lipogenesis (e.g. ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS)) [11]. Within the superfamily of nuclear receptors, peroxisome proliferator-activated receptors (PPARs) [12], liver X receptors (LXRs) [13] and the sterol response element binding proteins (SREBPs) [14] have taken center stage. Importantly, several of these targets are not solely utilized to block disease relevant pathways but also to control accumulation of bioactive metabolites in order to leverage their selective accumulation [15] as therapeutic means. Recent examples include, targeting the breakdown of prostaglandin E2 (PGE_2) as tissue regenerative therapy by inhibiting PGDH [10] or blocking DHCR24 to boost the accumulation of the selective LXR agonist desmosterol without simultaneous SREBP1c activation [9]. As particularly lipids harbor diverse signaling functions and bioactivities, their detailed mapping and analysis is crucial to the future success of such therapeutic concepts and our understanding of pathophysiological mechanisms.

Here, we will give a brief summary of lipidomics technologies and present recent examples on how lipidomics can assist in drug discovery and development by shedding light on molecular mechanisms, marker development of physiological response (PR) and target engagement (TE) as well as assisting mode of action (MoA) investigations.

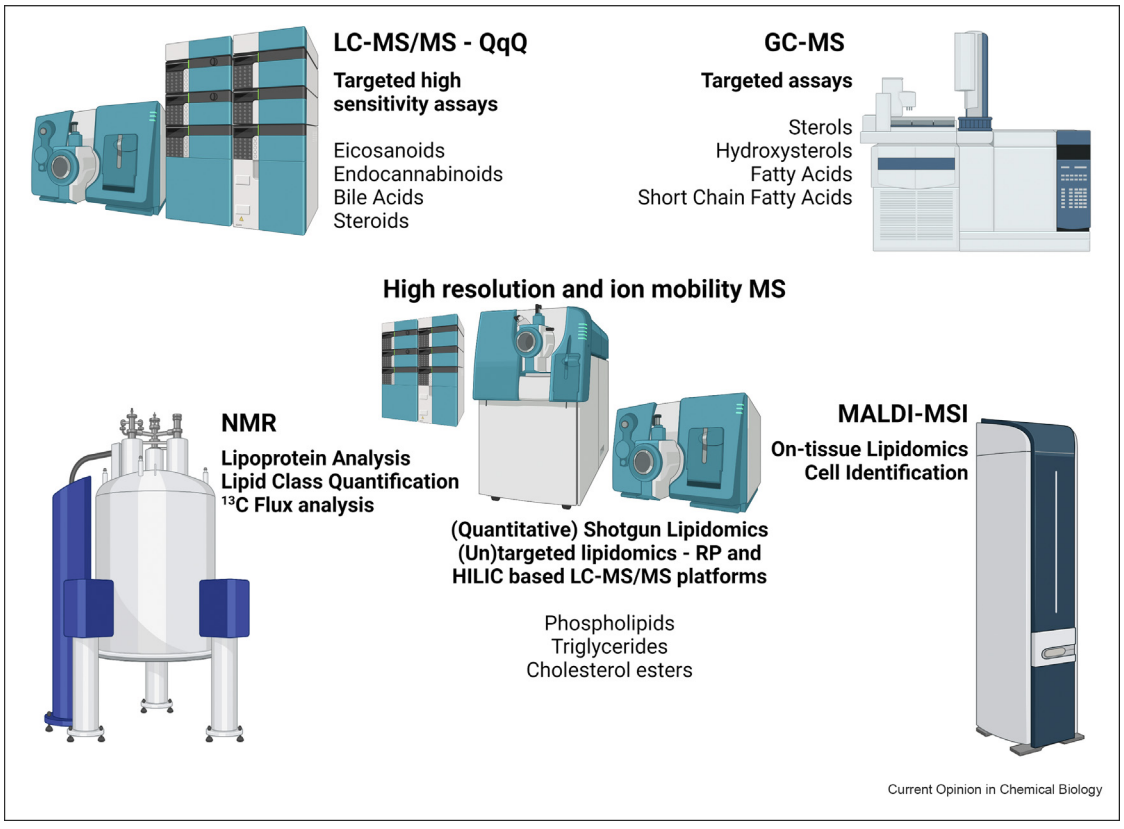
Lipidomics technologies

Although lipidomics could be seen as "just" a sub-field of metabolomics, a clear distinction between the two disciplines is made. The main reasons for this lie in the technological requirements and nature of investigated metabolites. While metabolomics has for many years focused on water-soluble metabolites, lipidomics analysis is targeting the water-insoluble matter. Moreover, lipidomics deals with biochemically and structurally highly intertwined molecules, a fact that is reflected in the lipid building block concept and extensive lipid classification systems. Jointly, these characteristics have led to lipidomics specific developments, from analytical methods to informatics approaches. Early efforts to study lipid metabolism mainly relied on thin layer chromatography and radioactively labeled compounds. Yet, as for metabolic research, lipidomics benefited from the advent of advanced separation technologies combined with high sensitivity mass spectrometry (MS) [16]. Gas chromatography–MS (GC–MS) was early on adapted to study sterols and fatty acids [17]. However, the analysis of more complex lipids such as, phospholipids, ceramides and oxylipids was facilitated by highly sensitive and selective liquid chromatography tandem mass spectrometry (LC-MS/MS) assays which mainly evolved during the last decade(s) [18]. Today, targeted and untargeted lipidomics approaches are being distinguished. Targeted approaches predominantly involve the quantification of a given set of lipids by triple quadrupole (QqQ) MS analyzers operating in multiple reaction monitoring mode (MRM). Untargeted approaches on the other hand rely heavily on high-resolution MS systems for enhanced lipid identification and subsequent informatics analysis using databases such as MS-DIAL [19], METLIN [20], or *de novo* identification based on decision rules [21]. The choice of extraction solvent is particularly important in the quantitative analysis of lipids. While the biphasic Bligh and Dyer or Folch extractions are traditionally used, monophasic solvent extraction appears as a more straightforward and cost effective approach. However, the downside of most monophasic solvents is that they do not allow for removal of salts and other polar metabolites that may interfere with lipid analysis. For details please refer to the study by Horing et al. [22]. In addition, the use of internal standards is strongly recommended to achieve reliable quantification [16]. With regard to separation methods, reversed phase (RP) is mainly used to separate lipids based on their side chain composition, whereas, hydrophilic interaction chromatography (HILIC) [23] provides lipid class separations [24]. The latter is also feasible with gas phase separations as for example differential mobility spectrometry (DMS) which has significantly boosted the applicability of quantitative shotgun lipidomics [25]. An overview of state-of-the-art assays for several lipid classes and their biological context is presented in Table 1 and Figure 1. Most of the mentioned methods are of medium throughput with typical run times for LC based targeted approaches of around 10–20 min per sample while DMS based shotgun lipidomics and untargeted analysis roughly take 20–30 min per sample. Other technologies applied in the field include nuclear magnetic resonance (NMR) spectroscopy-based lipoprotein profiling and stable isotope tracing [26,27]. While the latter has yet only limited applications for lipids, lipoprotein (sub)class analysis by NMR is gaining interest—also due to commercialized platforms like Bruker IVDr and Nightingale Health—as a high-throughput screening method with automatic data reporting of lipid (sub)particles and their associated lipid classes. Matrix-assisted laser desorption/ionization (MALDI) based mass spectrometry imaging (MSI) has recently entered the field, enabling on-tissue analysis of (complex) lipids. Importantly, lipid profiles have just recently been proven highly characteristic for individual cell types, a fact that might possibly assist in on-tissue cell typing [28,29]. Finally, there are significant developments in the structural analysis of lipids and some examples of in-depth profiling of lipid species are worth mentioning. Ozone-induced dissociation (OzID) is a technology developed for the mass spectrometric elucidation of double bond positions. This technique makes use of ozone's well described reactivity with carbon–carbon double bonds [30]. Electron impact excitation of ions from organics (EIEIO) is a technology that has recently been commercialized by Sciex and promises a complete structural elucidation of complex lipids based on MS fragmentation data [31]. Moreover, deep triglyceride profiling employing MS^3 analysis on QTrap systems has been shown to provide the complete fatty acid composition of complex triglycerides [32]. Altogether, researchers today possess a rich and highly sensitive tool set for lipid analysis allowing for a detailed mapping of molecular lipid changes. Consequently, these technologies and possibilities have been leveraged to assist drug discovery and development.

Lipidomics in drug discovery and development

Lipidomics technologies are well suited for both targeted and phenotypic drug discovery (TDD and PDD, respectively). Although TDD has been the most common approach in the pharmaceutical industry, recently target agnostic PDD is gaining ground, especially following advances in the development of cellular assays as surrogate disease models [51]. In the case of PDD, once desired phenotypes are observed and candidate drugs are established, detailed metabolic investigations aim to define a solid mechanistic understanding [51,52]. Subsequently, in both TDD and PDD, targeted lipidomics can be used for exploitation of disease models and to define TE markers as measurable molecular changes that reveal the degree of

Figure 1



State-of-the-art lipidomics technologies and assays.

interaction between drug and target. For example, it was shown recently how the chemical inhibition of 15-PGDH in aged muscle resulted in rejuvenation of muscle mass due to the increase of its substrate PGE_2, which in turn activated a signaling cascade upon binding to its receptor EP4 [10]. Other examples include the screening for antifungal drugs [53] and inhibitors of distal cholesterol biosynthesis [41,54]. Next to more widely established TE approaches [55], another *in vitro* strategy lies in chemical proteomics and the use of lipid probes in combination with stable isotope labeling using amino acids in cell culture (SILAC) and competition experiments [56]. However, these approaches are not yet routinely applied and the biggest pitfall of lipid probes is non-specific binding making it difficult to assign specific interaction partners. Nevertheless, several reports showing the successful application of lipid probes in combination with chemical proteomics have been published, e.g. cholesterol [57], 17-hydroxydocosahexaenoic acid [58].

It is important to note that TE markers based on pathway analysis do not necessarily reflect a quantitative measure of one enzyme–substrate interaction due to metabolic shifts and turnover by related enzymes. In this context, targeted and untargeted lipidomics are suitable for lipid biomarker discovery and pathway analysis, which in turn define PR markers of the (unspecific) physiological response caused by an intervention. A recent example of this approach from the field of neurodegenerating diseases is the mechanistic investigations of specialized neuronal cells from iPSCs, like astrocytes and microglia [6,59]. Ultimately, in the drug development process, MoA investigations aim at integrating all the information gathered from TE and PR determination, disease mechanism (e.g. pathway analysis), and the use of additional molecular biology tools in order to define a complete picture of a drug intervention [52]. A prominent example is the study of dose dependent anti-inflammatory/thrombotic effects of aspirin by using an eicosanoid profiling platform in combination with murine knockout models [60]. The authors explain how systemic versus platelet cyclooxygenase 1 inhibition influences the anti-thrombotic actions of aspirin. This data explains the different MoA of aspirin at high and low dosage and outlines why an increasing aspirin dosage or the combination with other drugs might lessen its antithrombotic actions.

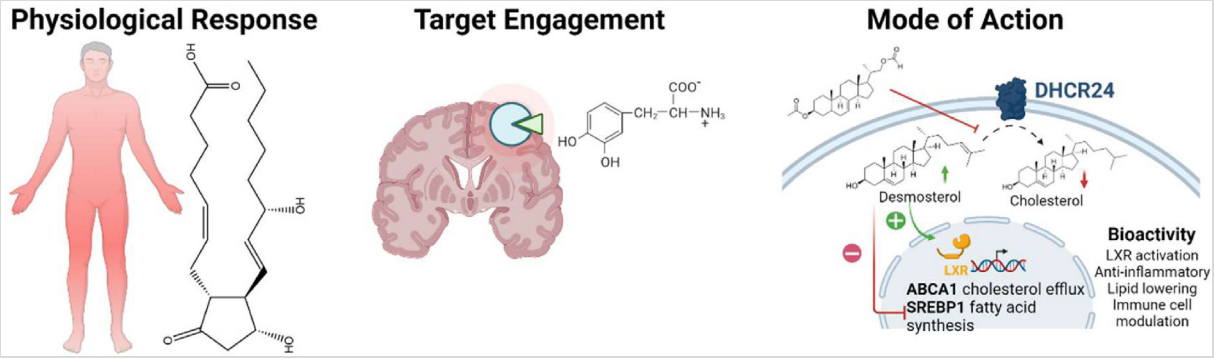
Table 1

Widely applied targeted lipidomics assays categorized per lipid class (IS: internal standard).

Lipid class	Biological context	Analytical technology	Analytical characteristics	Remarks	Reference
Acylcarnitines	Fatty acid metabolism	FIA-MS/MS DMS-MS/MS LC-MS/MS	Quantitative analysis, IS used, 20 to >100 analytes monitored	Flow-injection based analysis and LC based assays	[33,34]
Bile acids	Cholesterol metabolism Lipid digestion Microbiome Inflammation	LC-MS/MS	Quantitative analysis, IS used, typically 20–30 analytes monitored	Strong matrix effects, compound specific, labelled IS are mandatory, requires fasted plasma	[35,36]
Endocannabinoids	Neurotransmission Inflammation	LC-MS/MS	Quantitative analysis, IS used, typically 5–10 analytes monitored	Low concentration range, instability	[36,37]
Phospholipids (e.g. phosphatidylcholines, sphingomyelins)	Membrane lipids	Shotgun lipidomics LC-MS/MS NMR	Quantitative analysis, IS used, MS-based platforms cover >100 analytes, NMR solely used for class quantification	LC-MS/MS requires IS for quantification, prone to matrix effects, negative ESI mode for FA compositional analysis	[25,38,39]
Steroids	Immune system Stress Circadian rhythm	LC-MS/MS	Quantitative analysis, IS used, typically 10–20 analytes monitored	Low concentration range	[40]
Sterols (e.g. cholesterol, 24S-hydroxycholesterol)	Immune system Membrane integrity Signaling Neurodegeneration	GC–MS LC-IM-MS/MS	Quantitative analysis, IS used, typically 15–20 analytes, GC considered gold standard for analysis	High structural similarity, double bond isomers. Derivatization recommended for GC–MS analysis	[41,42]
Neutral lipids (e.g. triglycerides, cholesterol esters)	Lipid droplets Energy storage Liver pathologies	Shotgun lipidomics (MS/MS and MS^3) LC-MS/MS, NMR	Quantitative analysis, IS used, MS-based assays cover > 100 analytes. NMR only provides lipid class quantification	Highly complex, positional isomers	[25,32,43]
Oxylipids (e.g., eicosanoids, docosanoids, hydroxy fatty acids)	Signaling lipids Inflammation Immune system	LC-MS/MS	Quantitative analysis, IS used, typically around 100 analytes monitored	Low concentration, stereoisomers and enantiomers	[36,44]
Fatty acids and short chain fatty acids (SCFAs)	Lipid building blocks Microbiome Immune system	GC–MS LC-MS/MS	Quantitative analysis, IS used, typically 20–40 analytes monitored	For SCFAs, pentafluorobenzyl bromide derivatization is preferred to avoid undesired signal overlap (GC–MS). LC-MS allows for fast analysis of fatty acids obtaining comparable results to GC–MS	[45,46]
Lipoproteins	Lipid transport	LC-MS/MS NMR	Quantitative analysis, IS used in the case of LC-MS/MS, typically 10–20 analytes monitored, NMR covers >100 analytes	NMR preferred for broad coverage of sub-classes and simpler sample preparation workflow	[47,48]
Sphingolipids (e.g., ceramides, cerebrosides and gangliosides)	Biomarkers	LC-MS/MS	Quantitative analysis, IS used. 33 analytes monitored covering 7 different sphingolipid subcategories. Similar methods cover up to 13 different gangliosides.	Gangliosides require a dedicated method where a phenyl-hexyl column showed good performance	[49,50]

Table 2

Specific examples of lipid analysis assisting drug discovery and development.



Pathology and target	Drug example	Marker	Technology	Reference
Alzheimer's disease, activation of CYP46A1	Efavirenz	24-Hydroxycholesterol, PR and TE marker	Shotgun lipidomics and GC–MS based sterol analysis for MoA investigation of Efavirenz in Alzheimer's disease and use of 24-Hydroxycholesterol as PR and TE marker	[6,68,69]
Inflammation/regeneration, PGDH	Experimental inhibitors (ExI)	PGE_2 and metabolites, PR and TE marker	Targeted LC-MS/MS based eicosanoid analysis for MoA and TE investigations	[10]
Inflammation, DHCR24	ExI	Desmosterol, PR and TE marker, MoA	Shotgun lipidomics and targeted LC-MS/MS based oxylipid analysis for MoA investigation	[9]
Primary biliary cholangitis, FXR activation	Obeticholic acid	Bile acids, TE and PR marker	PR investigation using NMR based lipoprotein profiling	[70,71]
Inflammation/thrombosis, dose-dependent aspirin effects	Aspirin, MoA	Eicosanoids	LC-MS/MS based oxylipid analysis for MoA investigation	[60]
Asthma, leukotriene A4 hydrolase inhibitors	ExI	Leukotrienes	LC-MS/MS based leukotriene analysis as PR biomarker	[72]
Senolysis	ExI, senolytic drug development	Oxylipids, PR and MoA	Targeted LC-MS/MS based oxylipid analysis for sensolysis characterization and biomarker identification	[73]
Charcot–Marie–Tooth type 1A disease, sphingolipid metabolism	ExI	Sphingolipids and phospholipids	Untargeted LC-MS based lipidomics for target identification	[74,75]
Neurotoxic astrocytes, target identification, ELOVL1	ExI	Saturated lipids	Untargeted LC-MS based lipidomics and metabolomics for the characterization of neurotoxic astrocytes and identification of ELOVL1 as toxicity mediator	[59]
Parkinson's disease, SCD1	YTX-7739	Unsaturated fatty acids (fatty acid profiling), PR and TE marker	Untargeted LC-MS based lipidomics for target identification	[76,77]
Nonalcoholic steatohepatitis, ACC1/2 and DGAT2	PF-05221304, PF-06865571	Malonyl-CoA, Triglycerides, PR, TE and MoA	LC-MS based triglyceride profiling for MoA investigation	[63,78]
Nonalcoholic steatohepatitis, ACC1	IMA-1	Malonyl-CoA, PR, MoA	Untargeted LC-MS/MS based lipidomics for PR and MoA investigation of IMA-1	[64,65]

More examples on how lipidomics can assist drug discovery and development in defining markers for PR and TE as well as MoA investigations, are shown in Table 2.

Despite many successful examples of discoveries achieved by lipidomics technologies, some important considerations need to be made with regard to drug discovery and development. First, most pharmaceutical markers relate to a specific intervention by a candidate molecule or therapy while disease markers mostly reflect the (personalized) health status and disease progression. Thus, one of the main challenges arising from this fact is how far lipidomics signatures can be attributed to drug induced effects or to a general physiological response. In other words, since drug interaction markers are often observable during pathological events, it is not trivial to distinguish if the measured molecular changes are induced by the pharmaceutical intervention or due to a change in the physiological (health) state. This becomes even more complicated by the fact that lipid metabolism is highly intertwined [5] as proven by a plethora of known lipid binding proteins [56]. A second challenge lies in a candidate drug's selectivity, which may result in broad metabolic perturbances. For example, Wages et al. provided evidence about dozens of prescription drugs unexpectedly interfering with distal cholesterol biosynthesis [54]. More to that, blockade of a specific pathway may lead to accumulation of precursors and shortage of products which in turn might result in unwanted signalling/activation of related pathways (e.g. Aspirin-exacerbated respiratory disease where non-selective COX 1/2 inhibition has been linked to a diminishment of residual PGE_2 levels and exacerbated 5-lipoxygenase activity, for details please refer to the study by White et al. [61]). In the case of *in* vivo inhibition of 15-PGDH mentioned previously, there were no accompanied adverse effects, thus rendering 15-PGDH the first therapeutic avenue for the treatment of sarcopenia [10]. On the other hand, many promising candidates fail during clinical trials, for example, the majority of clinical trials of promising liver-directed ACC inhibitors (ACC1 and ACC2) aiming at NASH therapy have failed due to the induction of hyperlipidemia as an adverse effect in parallel with the desired inhibition of liver lipogenesis [62]. Mechanistic investigations and multi-omics analysis however, facilitated two recent independent discoveries that resolved hyperlipidemia and blocked NASH progression in humans. In the first case, a multi-drug therapy approach was used that simultaneously blocked NASH progression via liver ACC1/2 inhibition and resolved hyperlipidemia by inhibition of diacylglycerol acyltransferase 2 (DGAT2) [63]. In the second case, a new inhibitor, IMA-1 [64], was used to selectively induce only ACC1 degradation by 50% which proved sufficient to block NASH progression without exhibiting the side effect of hyperlipidemia [65].

Future trends for the application of lipidomics in drug discovery involve multi-omics integration [66] as well as, its combination with stem cell technologies and micro tissues/organoids. Particularly induced pluripotent stem cell (iPSC) technologies in combination with CRISPR gene editing allow studying otherwise elusive cell types (e.g. astrocytes, microglia) with disease relevant genetic modifications [6,67]. From an experimental point of view, these systems are very attractive as they are easier to standardize and should lead to better interpretable data when compared to in vivo experiments. To this end, iPSCs have already taken center stage in drug screening and lipidomics mapping of these systems is only a logical next step. First examples where lipid analysis/lipidomics helped to elucidate the MoA of drug candidates in combination with iPSC include Alzheimer's disease and multiple sclerosis [6,7]. Finally, the importance of metabolic flux analysis is steadily increasing and it will consequentially also be applied in lipidomics analysis to further aid in deciphering lipid metabolism and its druggable targets.

Conclusions and outlook

Omics' technologies have become an integral part of modern drug discovery and development. Several metabolomics and lipidomics technologies have advanced to useful biochemical tools. In the future, drug repurposing campaigns might also significantly benefit from metabolomics/lipidomics technologies as the metabolome (lipidome) is most closely related to the biological phenotype as it reflects the ultimate response of biological systems [79]. In turn, metabolic phenotyping of (registered) drugs and comparison with disease relevant metabolic disturbances might very well allow to deduce novel targets for registered drugs, aimed at normalizing (lipid) metabolism. A related approach has just recently been presented by Sauer and co-workers, matching drug induced metabolic alterations with induced overexpression profiles to elucidate drug target relations [80]. Moreover, as mentioned above stem cell and CRISPR technologies will further gain importance particularly in combination with lipidomic analysis. Other recent developments include spatial lipidomics analysis, integration of multi-omics data sets as well as metabolic (lipid) flux analysis. Multi-omics integration is a general trend within the omics' field and will eventually also include lipidomics [66,81]. With regards to drug development, multi-omics integration promises a deeper understanding of drug actions with potential impact on the prediction of orphan targets, drug repurposing as well as adverse effects. A related technology is Mendelian randomization which has recently been used to elucidate potentially novel cardio vascular disease related drug targets [82]. Ultimately, as metabolism is not a static process, metabolic flux analysis will consequently be developed for lipidomics analysis and

become a next cornerstone in our understanding of lipid metabolism and its druggable targets [83].

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Declaration of competing interest

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests: Martin Giera reports a relationship with Boehringer Ingelheim Pharma GmbH and Co KG Biberach that includes: consulting or advisory. Martin Giera has filed a patent application #P329036NL for DHCR24 inhibitors.

Data availability

No data were used for the research described in the article.

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