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Recent advances towards mass spectrometry-based clinical lipidomics

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Abstract

The objective of this review is to provide a comprehensive summary of the latest methodological advancements and emerging patterns in utilizing lipidomics in clinical research.In this review, we assess the recent advancements in lipidomics methodologies that exhibit high levels of selectivity and sensitivity, capable of generating numerous molecular lipid species from limited quantities of biological matrices.

The reviewed studies demonstrate that molecular lipid signatures offer new opportunities for precision medicine by providing sensitive diagnostic tools for disease prediction and monitoring. Moreover, the latest innovations in microsampling techniques have the potential to make a substantial contribution to clinical lipidomics. The review also shows that more work is needed to harmonize results across diverse lipidomics platforms and avoid significant errors in analysis and reporting. The increased implementation of internal standards and standard reference materials in analytical workflows will aid in this direction.

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Keywords

Biomarkers, Human health, Lipidomics, Mass spectrometry, Microsampling.

Key points

- Microsampling techniques, including DBS, PSC, and VAMS, hold significant promise for contributing to the field of lipidomics
- While non-targeted high-resolution methods have proven to be valuable in discovery research including in identifying novel molecular lipids, targeted methods present significant and important advantages in terms of reduced setup costs and easier maintenance. This makes them more suitable for use in routine clinical settings.
- Molecular lipid signatures offer new opportunities for precision medicine by providing sensitive diagnostic capacity for disease prediction and monitoring

Introduction

Molecular lipids are one of the major cellular components that are essential for energy storage, membrane stability, and signaling. Although structurally diverse, membrane lipids are primarily characterized by having a polar head group that is linked to a hydrophobic tail composed of one or more acyl chains. As molecular lipids are incorporated into lipoproteins, they are extensively disseminated throughout the body's tissues via the circulatory system and serve as a reflection of an individual's metabolic state and thus have a crucial pathophysiological relevance. Currently, however, only a few lipids, in addition to the traditional clinical lipids such as cholesterol, triacylglycerols, and lipoproteins are used in clinical practice. Among the various biological compartments, plasma or serum molecular lipids have received the most extensive research attention due to their easy accessibility relative to other biological matrices. While blood-based lipidomics is a promising approach for clinical research, standardized sample collection, storage, and subsequent analytical methodologies and quality assurance/control measures continue to pose challenges. Additionally, analyzing low-abundance lipid mediators in plasma or serum necessitates distinct analytical sample preparation, separation, and detection methods.

Over the past two decades, the analytical development in mass spectrometry (MS)-based lipidomics has resulted in highly specific, sensitive, and robust methods.

This is largely due to techniques based on mass accurate and selective detection (high resolution) as well as applications of quantitative mass spectrometry including isotope dilution. The current analytical approaches for lipidomics comprise non-targeted high resolution mass spectrometry (HRMS), targeted lipidomics using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), and direct infusion-based shotgun lipidomics. Combinations of these analytical techniques have together enabled the identification of thousands of molecular lipids in circulation and there exists a great demand for global lipidomics methods, but also for specialized targeted methods for regulatory lipid mediators, that can be used to improve the diagnosis prediction and treatment of diseases. The primary objective of this short review is to explore the landscape of molecular lipid profiling in pre-clinical and clinical studies. Specifically, the review will focus on the use of MS-based lipidomics techniques. The scope of analysis will be limited to studies published within the time frame of 2020–2023, ensuring an up-to-date examination of the field. By synthesizing the findings from this select time period, the review aims to provide a comprehensive understanding of the advancements, trends, and insights gained through MS-based lipidomics approaches in the study of molecular lipids in (pre-)clinical settings.

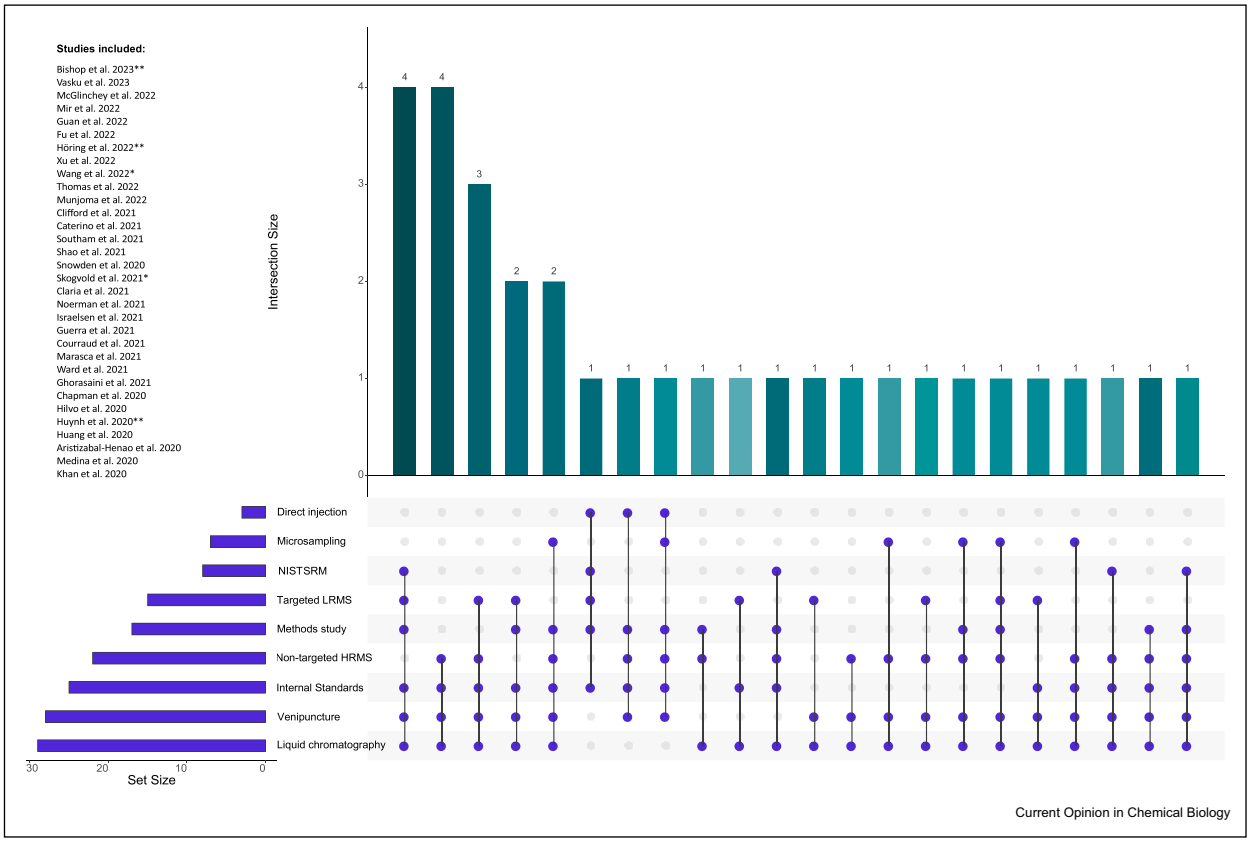
Analytical strategies for mass spectrometry-based lipidomics

The compiled analytical methods for lipidomics are presented in Figure 1, which encompasses original articles published between 2020 and 2023. This figure provides an overview of the current trends and advancements in MS-based lipidomics applicable to biological and clinical samples.

Sample collection

In many lipidomic studies, biological samples are often collected from plasma and serum, and to a lesser extent, urine. Studies have shown that a variety of factors can influence the subsequent lipidomics analysis, including the immediate separation of serum or plasma, the temperature and length of storage prior to processing, the transit time of shipping, and the number of freeze–thaw cycles [1*]. So far, consensus exists that samples should ideally be collected in plain EDTA tubes and not in heparin-containing tubes, that serum or plasma must be separated immediately, and that the samples must be stored at −80 °C. Applying such standardized sample

Figure 1



Summary of intersections and overlap across the commonly used analytical approaches for lipidomics from original articles between 2020 and 2023.

collection procedures, the lipidomics analysis has been shown to be the most stable [1*]. Other sample collection information, such as date and time of collection, location, and date and time of transport can aid in normalizing potential bias arising due to an overall sample handling variability.

Microsampling

Economic and logistical challenges associated with the classic sample collection (venipuncture) have been obstacles for large-scale clinical studies. However, recent methodological developments in microsampling techniques have the potential to overcome these challenges. From this perspective, dried blood spot (DBS) sampling has been extensively used, especially among newborn screening programs where DBS are collected a few days after birth. The use of DBS in the context of metabolomics and lipidomics has been explored [2-4*] and to some degree hampered due to variability in volumetric amounts [5,6], and also because the number of recovered annotated molecular lipids from DBS has generally been modest [7,8]. However, the novel DBS systems that allow volumetric collection of blood overcome several of the pre-analytical issues associated with conventional DBS sampling [6].

Other types of devices for quantitative microsample collection and related technologies are emerging in the clinical lipidomics toolbox because they offer less invasive and more accessible alternatives. Such devices can be used point-of-care or home-sampling. In a recent study, plasma separation cards (PSC) were compared with classic wet plasma and DBS for comprehensive lipidomics profiling [8*]. The authors reported strong correlations between lipidomics profiles obtained via classic wet plasma and PSCs, but to a lesser extent with DBS. Also, the molecular lipid recovery and stability were found to be comparable between the PSC and DBS samples, with 60% of molecular lipids showing acceptable stability at room temperature after 28 days [8*]. Another microsampling technique is volumetric absorptive microsampling (VAMS). When compared with DBS for lipidomics, VAMS significantly outperformed DBS both in terms of extraction recovery and the number of lipids detected [5]. Among the lipidomics studies surveyed in this review, 20% incorporated microsampling techniques as novel approaches in lipidomics, with the predominant techniques being DBS, PSC, and VAMS (Figure 2a).

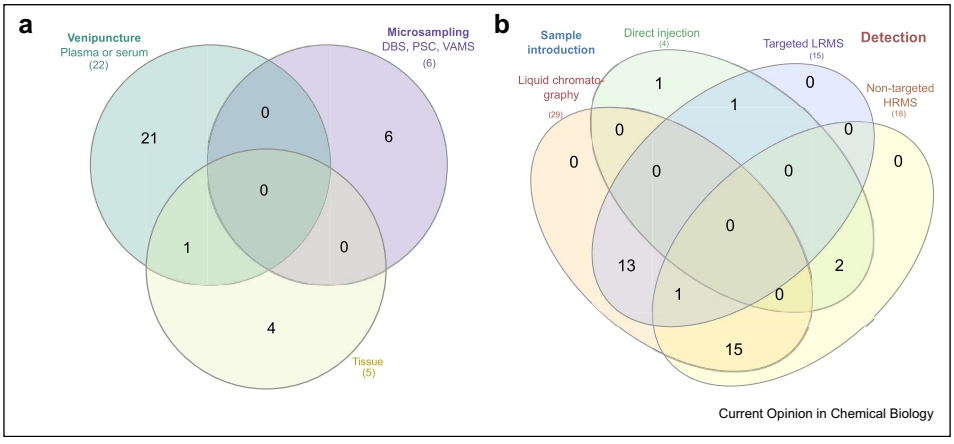
Sample preparation

The most widely used approaches to extract molecular lipids are two-phase extractions or using a methyl tert-butyl ether (MTBE)/methanol/water mixture. Although one-phase lipid extractions and automated developments have recently been suggested to offset the disadvantages of manual two-phase extraction [9–11], there are also studies which suggest that the use one-phase extractions should be limited to the polar lipid classes [12**].

Non-targeted lipidomics for discovery of molecular lipids in preclinical studies

Most of the methods reported for global lipidomics are based on HRMS, including time-of-flight and Orbitrap mass analyzers, providing reliable identification and quantification [13]. By addition of ion mobility as fourth dimension, the separation and identification of the lipids can be further improved [14]. These methodologies have facilitated the identification of numerous molecular lipids in plasma and serum, thereby serving as preliminary stages in the integration of lipidomics into clinical practice (Figure 2b). These comprehensive techniques can be further refined and transformed into rapid high-throughput routine assays, enabling their application in routine clinical analysis. New algorithms for processing non-targeted data are becoming

Figure 2



Venn diagram showing the intersection of (**a**) sample collection and sample types; and (**b**) chromatography and mass spectrometry approaches as reviewed.

increasingly available together with workflows for feature-based molecular networking that enable molecular visualization and increased annotation of a large part of the chemical space in non-targeted MS data [15,16].

Targeted lipidomics for translation to the clinic

Targeted lipidomics, employing low-resolution MS (LRMS) and multiple reaction monitoring (MRM) detection, is generally aimed at the identification and quantification of a predefined subset of lipids and is most applicable when addressing specific hypotheses [17,18]. Targeted lipidomics is becoming increasingly important in lipidomics field and can offer comparable molecular lipid coverage to that of non-targeted global lipidomics [19–21]. Targeted lipidomics methods are becoming more frequently used (Figure 2b) and have been demonstrated to enable deep lipid phenotyping that can resolve lipid isomers at the structural level [22,23]. Given that non-targeted HRMS methods are still far from being implemented in clinical routine, targeted LC-MS/MS based lipidomics methods that meet regulatory compliance can be more readily translated to the clinic. Hence, they will be the preferred method for the clinical translation of global lipidomics.

QA/QC

Standardized QA/QC is critical to ensure that comparable results are obtained regardless of the diverse range of sample collection, sample preparation, and instrumental mass spectrometry-based detection are obtained. Studies that have assessed the conformity using similar instrumental setups and methods applied to the same sample set in two different laboratories led to comparable results for the measured plasma lipids [24]. Using a similar methodological setup but using a targeted QTRAP instrument and a non-targeted Orbitrap, differences in both lipid class coverage and data variability were reported [25]. The use of standard reference materials, calibration, and internal standards offers means of controlling and avoiding severe errors in the analytical procedure [20,26]. However, recent efforts to assess this among well-established laboratories show that only about a third of them use standardized reference materials regularly and with different purpose; some laboratories use the reference materials as a long-term reference QC samples, whereas others utilize them for cross-platform evaluations or interlaboratory studies [27*]. Similarly, our review indicates that internal standards were employed in the majority (75%) of studies examined in the lipidomics workflow. However, a standard reference material such as NIST was used in only 20% of the studies.

By comparing several different mass spectrometry platforms (reverse phase, MRM and non-targeted direct injection), the normalization to standardized reference materials was found to significantly improve the agreement in lipid concentrations between different methods [24]. Given the newly established collaborations for harmonizing lipidomics such as LIPID MAPS, EpiLipidNET, Lipidomics-Standards-Initiative (LSI), and the Clinical Lipidomics Interest Group methods for standard reporting are anticipated to improve over the years to come.

Progress toward clinical lipidomics

Molecular lipid measurements in clinical settings are limited to total triacylglycerols, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol, leaving a plethora of information (wide range of lipid species) unexplored in the biological samples. The clinical lipidomics approach aims to advance our understanding of disease mechanisms and aids in the diagnosis, prognosis, and risk stratification of disease [28–37]. However, currently clinical lipidomics is primarily restricted to the analysis of population or pre-clinical sample sets in scientific research settings. Table 1 presents an overview of pre-clinical lipidomics studies investigating the relationships between lipids or lipid classes and diverse disease outcomes and their findings. Here we highlight the current state of mass spectrometry based clinical lipidomics and recent efforts to translate lipidomics based research protocols to the clinical setting.

Lipidomics for early disease detection and diagnosis

Dysregulated lipid metabolism is one of the hallmarks of cardiovascular disease (CVD). For example, lipid species classes of phosphatidylcholines, alkenylphosphatidylcholines, phosphatidylinositols, diacylglycerols, triacylglycerols, ceramides, and sphingomyelins have been implicated in the prediction of CVD events [38]. More recently, Hilvo et al. [39] developed a predictive model that combines the four ceramides (Cer(d18:1/16:0), Cer(d18:1/18:0), Cer (d18:1/24:1), Cer(d18:1/24:0)) and three phosphatidylcholines ((PC(16:0/22:5), PC(14:0/22:6), PC(16:0/16:0)) into a score (CERT2) in the prediction of CVD events in patients with atherosclerotic coronary heart disease. The CERT2 score efficiently predicted CVD mortality (hazard ratios ranging from 1.44 to 1.69), and the inclusion of troponin T (a non-lipidomic measurement) increased the model's efficacy. The model was validated in three independent cohorts from Europe and Australia, including the Western Norway Coronary Angiography Cohort, the LIPID trial, and the Langzeiterfolge der Kardiologischen Anschlussheilbehandlung. Quest Diagnostics licensed this technology for future clinical test development [28].

Recently, Ottosson et al. [40] measured 184 lipids in baseline plasma samples from the Malmö Food and Cancer Cohort (n = 3865). In the follow-up period, 13.86% developed CVD. They utilized plasma lipid patterns (n = 8) to predict the CVD and claimed that

Table 1

Overview of pre-clinical lipidomics ltudies on associations of lipids/lipid classes with disease outcomes.

Author	N lipid classes	N molecular lipids	Main outcome	Top lipids/classes associated with outcome	# Reference
Wang et al., 2022	10	300	Lung cancer	LPCs 16:0, 18:0, and 20:4; PCs 16:0-18:1, 16:0-18:2, 18:0-18:1, 18:0-18:2, and 16:0-22:6; and TAGs 16:0-18:1-18:1	[30]
Clifford et al., 2021	13	636	NAFLD	TAG	[31]
Caterino et al., 2021	6	483	COVID-19	TG (20:3/34:3), TG(18:0/30:1), TG(20:2/32:1), TG(18:1/35:3), TG(17:1/36:5), TG(16:1/38:3), TG(18:2/36:5), TG(17:2/36:2), TG(18:1/34:3), TG(16:0/33:1), TG(16:0/40:8)	[32]
Shao et al., 2021	5	98	Psoriasis and pityriasis rubra pilaris	PCs 30:2, 32:0, and 30:1, and PEs 42:0 and 42:1 PE, α-linolenic acid (ALA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and derivatives of linoleic acid (LA) (13-HOTrE), arachidonic acid (AA) (8- and 9-HETE), and oxidized-DHA products (11-, 13-, 7-, 10-, 8-, 20-HDoHE)	[33]
Claria et al., 2021	13	223	Acutely decompensated cirrhosis	CE (16:0), CE (18:3), PC to LPC ratio, PUFAs, MUFAs	[34]
Noerman et al., 2021	n.a.	17 182 features	coronary artery disease	plasmalogens PC(O-16:0/18:2) and PC(O-16:1/18:2)	[35]
Israelsen et al., 2021	14	316	ALD and NAFLD	FFAs, LPCs, TAGs	[36]
Guerra et al., 2021	11	146	Phenylketonuria	PCs containing PUFA	[37]
Mundra et al., 2018	24	342	Cardiovascular events	sphingolipids, ether lipids, and omega-6 fatty acids	[38]
Hilvo et al., 2020	24	342	Coronary artery disease	CERs and PCs	[39]
Ottoson et al., 2021	10	184	Coronary artery disease	SM (34:1), DAG (18:1/18:3)	[40]
Lauber et al., 2022	10	184	Diabetes and cardiovascular disease	TAGs, DAGs, PC-O	[41]
Hyuynh et al., 2020	32	569	Alzheimer's disease	ether lipids, sphingolipids such as GM3 ganglioside, and PEs and TAGs	[42]
Wolrab et al., 2022	4	168	Pancreatic cancer	SMs, CERs, and LPCs	[43]
McGlinchey et al., 2022	12	176	NAFLD	TG, LPC, PE, PC, SM, and DG	[44]
Ooi et al., 2021			NAFLD	TAGs, DAGs and sphingolipids including ceramide, dihydroceramide, hexosyl-ceramide and GM3 ganglioside	[45]
Thomas et al., 2022	12	176	TBI	LPC(18:2), LPC(20:5), two isomers of O-PC(34:2), O-PC(34:3), O-PC(36:3), SM(40:1), SM(40:2)	[47]
Miller et al., 2021	1	71	Concussion	PCaeC36:0, PCaaC42:6, PCaeC36:2, PCaaC32:0	[48]
Baloni et al., 2022	2	112	Alzheimer's disease	SM (d34:1)/SM (d43:1) ratio	[49]
Cadby et al., 2022	32	596	Coronary artery disease	PEs, DAGs, TAGs, LPEs, dhCERs, SMs, PCs	[51]
Mir et al., 2022	25	480	BMI, birth weight	AC, CE, TG, PC, PE, PI, SM, and Cer	[52]

Abbreviations: NAFLD, non-alcoholic fatty liver disease; TBI, traumatic brain injury; BMI, body mass index; na, not applicable.

traditional lipid measurements are insufficient for characterizing dyslipidemia. Utilizing the lipid signature, Ottosson et al. were able to predict cardiovascular disease up to 20 years prior to the disease, suggesting that identification of hidden dyslipidemia could encourage early preventive interventions [40]. Similarly, Lauber et al. utilized multiple lipid panels in the same population cohort to predict the beginning of type 2 diabetes mellitus or CVD several years before the disease occurred [41].

Alzheimer's disease (AD) has been linked to sphingomyelin and ceramide metabolic dysregulation. Huynh et al. performed a comprehensive lipidomics analysis of AD in their study, evaluating a total of 5733 samples from two separate and multicenter cohorts [42**]. There were 1912 people in the cohorts: 1112 from the Australian Imaging, Biomarkers, and Lifestyle (AIBL) cohort and 800 from the Alzheimer's Disease Neuroimaging Initiative (ADNI) baseline cohort. The authors used a fixed-effects model to identify 197 lipids and 11 classes that were linked with AD in both cohorts, with the lipid classes primarily from the sphingolipid classes. The disease classification model was improved by adding 10 lipid species on top of the basic model, which included age, gender, BMI, and the APOE 4 gene count [42**].

Lipidomics has also gained prominence in cancer diagnosis. Wang et al. for example, show that MS based clinical lipidomics provides an appropriate platform for the highly sensitive detection of early-stage non-small cell lung cancer (NSCLC) [30]. They identified a panel of nine lipids (three lysophosphatidylcholine, five phosphatidylcholines, and one triacylglycerol species) that can be used to detect NSCLC and it is likely that this strategy might be replicated in the diagnosis and prognosis of other cancers. In a recent study [43], Wolrab et al. found that serum lipids (sphingomyelins, ceramides, and lysophosphatidylcholines) were more accurate predictors of pancreatic ductal adenocarcinoma (PDAC) compared to traditional diagnostic techniques such as carbohydrate antigen 19-9. The study involved three laboratories in the Czech Republic, Germany, and Singapore, using various MS-based procedures including RP-UHPLC/MS, MALDI-MS, shotgun LR-MS, shotgun HR-MS, and UHPSFC/MS. Samples were collected from hospitals in Brno, Prague, Olomouc, and Pilsen, with each laboratory independently conducting sample preparation and data processing. Despite the heterogeneity, the lipid markers demonstrated over 90% accuracy in predicting pancreatic cancer. This suggests that lipidomic profiling in human serum could serve as a reliable, high-throughput method for screening clinical samples and can be replicated in any lab experienced in quantitative lipidomic analysis.

Similarly, considering the importance of dysregulated lipid metabolism in the development of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), various studies were conducted to evaluate the feasibility of employing circulating lipids as biomarkers for non-invasive NAFLD diagnosis [44–46] (Table 1).

Lipidomics for predicting treatment response and prognosis

Lipidomics analysis of human population cohorts has shown novel links between circulatory lipid levels and traumatic brain injury [47,48]. Thomas et al. discovered a link between the blood lipidome and acute traumatic brain injury, as well as a link between injury severity and patient outcome. To discover molecules linked with TBI severity and patient outcomes, Thomas et al. [47] examined the blood lipidome (including the metabolome) of 716 TBI patients and non-TBI comparison individuals. Lysophosphatidylcholines, ether phosphatidylcholines, and sphingomyelins were discovered to be closely related to TBI patient outcomes and to be among the strongest indicators of disease severity. They validated these findings in an independent dataset of 558 patients. These findings apply to TBI across the severity spectrum, including sports-related concussions, implying that lipidomics may facilitate assessment of severity of TBI and patient outcome prediction.

More recently, Baloni et al. examined human in vivo and post-mortem brain data to characterize the sphingomyelin pathway to AD etiology [49]. Using a multi-omics approach, they identified lipid-related changes in Alzheimer's etiology. They also performed metabolite genome-wide association studies to identify potential drug targets, suggesting that modulators of sphingosine-1-phosphate metabolism could be effective treatments for Alzheimer's disease. This discovery could help speed up the prediction of the disease and the development of effective treatments for Alzheimer's, which are now being explored in clinical studies [49,50].

Lipidomics for molecular phenotyping

Cadby et al. performed a genome-wide association analysis of 596 lipid species from 33 classes and discovered several genes involved in lipid metabolism related with coronary heart disease risk in a discovery cohort of 4492 individuals [51]. The findings were subsequently verified using a meta-analysis on two different validation cohorts of 670 and 895 European relatives, respectively. Importantly, a total of 186 genetic loci for coronary artery disease risk were associated to 134 lipid endophenotypes. Cadby et al. also investigated the genetic relationships between loci and coronary artery disease in 456 486 UK Biobank participants, respectively, regardless of clinical lipid readings. We anticipate that this integrative strategy (genomics technologies combined with the high-dimensional lipidome) will be able to identify major risk factors for CVD events, paving the way for personalized healthcare in clinical settings.

Mir et al. used population-based plasma lipidomics to examine the impact of obesity on circulating lipid levels in a mother-offspring cohort study [52]. They discovered that pre-pregnancy BMI altered lipids that differed considerably between maternal and fetal circulation, highlighting the need of managing obesity before pregnancy. Here, they evaluated 480 lipid species in 2491 plasma samples taken at four time points. Mothers were sampled during pregnancy (n = 752) and 4–5 years after giving birth (n = 650), while their kids were sampled at birth (n = 751) and 6 years of age (n = 338). Linear regression models were used to investigate pregnancy and age-related changes in plasma lipid profiles, as well as their relationship with obesity risk. The findings were confirmed using an independent birth cohort (n = 1935). This study provides a significant resource for future research aimed at developing early nutritional treatments to improve the metabolic health of both mothers and children.

Conclusion

Over the past two decades, we have seen a rapid expansion of LRMS and HRMS methods for lipidomics profiling. The application of these techniques has revealed the staggering diversity and large numbers of circulating molecular lipids and mediator lipids. Currently, 25 000 structural lipids have been curated in the flagship LIPID MAPS database. Going further by combining targeted and non-targeted methods with enantiomer specific separation will likely reveal even more molecular lipids. While the instrumental systems for lipidomics have undergone a revolution; there are now more reliable, accurate, and sensitive analytical methods and detection systems, less development has been occurred in the sample collection and subsequent extraction procedures. Although microsampling techniques, such as DBS, PSC, and VAMS, are in the early stages of development, further technical advancement is needed for them to evolve as the tool of choice for lipidomics in both point-of-care and self-sampling settings. Lastly, lipidomics has matured significantly in recent years, with several major advances in the field, particularly with respect to throughput, selectivity, sensitivity, and reproducibility. This will enable lipidomics to provide a more in-depth understanding of the pathogenesis of disease, with the potential to translate into validated methods to support precision medicine.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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* of special interest
** of outstanding interest

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