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REVIEW

An Introduction to Lipidomics: From Laboratory to Clinic

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ABSTRACT

Lipids are naturally occurring compounds that are insoluble in water but soluble in nonpolar solvents. The early milestones in lipid research were put forward in 19th century and many progresses awarded with Nobel Prizes were made in 20th century. Although it is possible to characterize the function of each lipid by individual analysis, lipids should be analyzed collectively to understand their complexity and collaboration in membrane organization, vesicle trafficking and energy metabolism. Therefore, the term 'lipidome' has come into use to denote the whole lipid species found in a biological system. Lipidomics is the study of this entire spectrum of lipids in terms of quantification and characterization dedicated to the understanding of the contribution or the relationship of lipids with cellular physiology and pathology. Lipidomics is assumed as one of the farthest points that have been reached by the central dogma of biology after implementation of genomics, transcriptomics, proteomics, and metabolomics. Regulation of lipid metabolism is essential for maintenance of health; therefore dysregulation of this system may play an important role in many human diseases such as neurodegenerative diseases, diabetes, cardiovascular diseases, cancer and infectious diseases. In a classical lipidomics experiment, lipid extract is first separated by liquid or gas chromatography and then delivered to mass spectrometer, ionized, vaporized and the resulting ions are sorted according to their mass to charge ratio in the mass analyzer. Last but not least, the acquired data is processed using dedicated bioinformatics tools.

Key words: Lipid, lipidome, lipidomics, extraction, chromatography, mass spectrometry, bioinformatics

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LIPIDS IN CELLULAR FUNCTION

Lipids are naturally occurring compounds that are insoluble in water but soluble in nonpolar solvents. They involve an enormous number of chemically distinct molecules (Table 1) that can be hydrophobic or amphipathic. Amphipathic lipids are important structural constituents of cell membrane that is constructed by phospholipids, sphingolipids and cholesterol. They have diverse functions in maintaining membrane architecture and in contributing to membrane dynamics during the life span of various cells [1]. Triglycerides and fatty acids are important elements of energy metabolism.It has been well established that lipids had an enormous diversity in structure and their function has gone far beyond cellular membrane structure and energy storage. Some lipid molecules such as fatty acids, eicosanoids, phospholipids (phosphatidylinositol-3-P) and ceramides, sphingosine, sphingosine-1-phosphate (S1P) and cholesterol have been recently designated as key mediators in certain signal transduction pathways [2]. Some of these include cell growth, proliferation, differentiation, survival, apoptosis, autophagy and inflammation.Understanding how this complex network of lipids and lipid homeostasis being maintained in the cell will be a great challenge in classical lipidology that needs collaboration with lipidomics. Lipidomic tools have provided an enormous data on lipid species mediating all these pathways at the membrane, cytoplasm or nuclear interface.

Lipidomics

The early milestones in lipid research were put forward in 19th century and many progresses awarded with Nobel Prizes were made in 20th century [3]. Lipidomics is assumed as one of the farthest points

Table 1. Classification of lipids by International Lipid Classification and Nomenclature Committee of LIPID MAPS consortium (LIPID MAPS® Lipidomics Gateway http://www.lipidmaps.org/data/classification/LM_classification_exp.php)

Fatty Acyls [FA]	Fatty Acids and Conjugates [FA01] Octadecanoids [FA02] Eicosanoids [FA03] Docosanoids [FA04] Fatty alcohols [FA05] Fatty aldehydes [FA06] Fatty esters [FA07]	Fatty amides [FA08] Fatty nitriles [FA09] Fatty ethers [FA10] Hydrocarbons [FA11] Oxygenated hydrocarbons [FA12] Fatty acyl glycosides [FA13] Other Fatty Acyls [FA00]
Glycerolipids [GL]	Monoradylglycerols [GL01] Diradylglycerols [GL02] Triradylglycerols [GL03]	Glycosylmonoradylglycerols [GL04] Glycosyldiradylglycerols [GL05] Other Glycerolipids [GL00]
Glycerophospholipids [GP]	Glycerophosphocholines [GP01] Glycerophosphoethanolamines [GP02] Glycerophosphoserines [GP03] Glycerophosphoglycerols [GP04] Glycerophosphoglycerophosphates [GP05] Glycerophosphoinositols [GP06] Glycerophosphoinositol monophosphates [GP07] Glycerophosphoinositol bisphosphates [GP08] Glycerophosphoinositol trisphosphates [GP09] Glycerophosphates [GP10] Glyceropyrophosphates [GP11]	Glycerophosphoglycerophosphoglycerols [GP12] CDP-Glycerols [GP13] Glycosylglycerophospholipids [GP14] Glycerophosphoinositolglycans [GP15] Glycerophosphonocholines [GP16] Glycerophosphonoethanolamines [GP17] Di-glycerol tetraether phospholipids (caldarchaeols) [GP18] Glycerol-nonitol tetraether phospholipids [GP19] Oxidized glycerophospholipids [GP20] Other Glycerophospholipids [GP00]
Sphingolipids [SP]	Sphingoid bases [SP01] Ceramides [SP02] Phosphosphingolipids [SP03] Phosphonosphingolipids [SP04] Neutral glycosphingolipids [SP05]	Acidic glycosphingolipids [SP06] Basic glycosphingolipids [SP07] Amphoteric glycosphingolipids [SP08] Arsenosphingolipids [SP09] Other Sphingolipids [SP00]
Sterol Lipids [ST]	Sterols [ST01] Steroids [ST02] Secosteroids [ST03]	Bile acids and derivatives [ST04] Steroid conjugates [ST05] Other Sterol lipids [ST00]
Prenol Lipids [PR]	Isoprenoids [PR01] Quinones and hydroquinones [PR02] Polyprenols [PR03]	Hopanoids [PR04] Other Prenol lipids [PR00]
Saccharolipids [SL]	Acylaminosugars [SL01] Acylaminosugar glycans [SL02] Acyltrehaloses [SL03]	Acyltrehalose glycans [SL04] Other acyl sugars [SL05] Other Saccharolipids [SL00]
Polyketides [PK]	Linear polyketides [PK01] Halogenated acetogenins [PK02] Annonaceae acetogenins [PK03] Macrolides and lactone polyketides [PK04] Ansamycins and related polyketides [PK05] Polyenes [PK06] Linear tetracyclines [PK07] Angucyclines [PK08]	Polyether antibiotics [PK09] Aflatoxins and related substances [PK10] Cytochalasins [PK11] Flavonoids [PK12] Aromatic polyketides [PK13] Non-ribosomal peptide/polyketide hybrids [PK14] Other Polyketides [PK00]

that have been reached by the central dogma of biology after implementation of genomics, transcriptomics, proteomics, and metabolomics. Number of publications in Web of Science with a keyword 'lipidomics' has increased gradually in each year (Figure 1).

Although it is possible to characterize the function of each lipid by individual analysis, lipids should be analyzed collectively to understand their complexity and collaboration in membrane organization, vesicle trafficking and energy metabolism. Therefore, the term 'lipidome' has come into use to

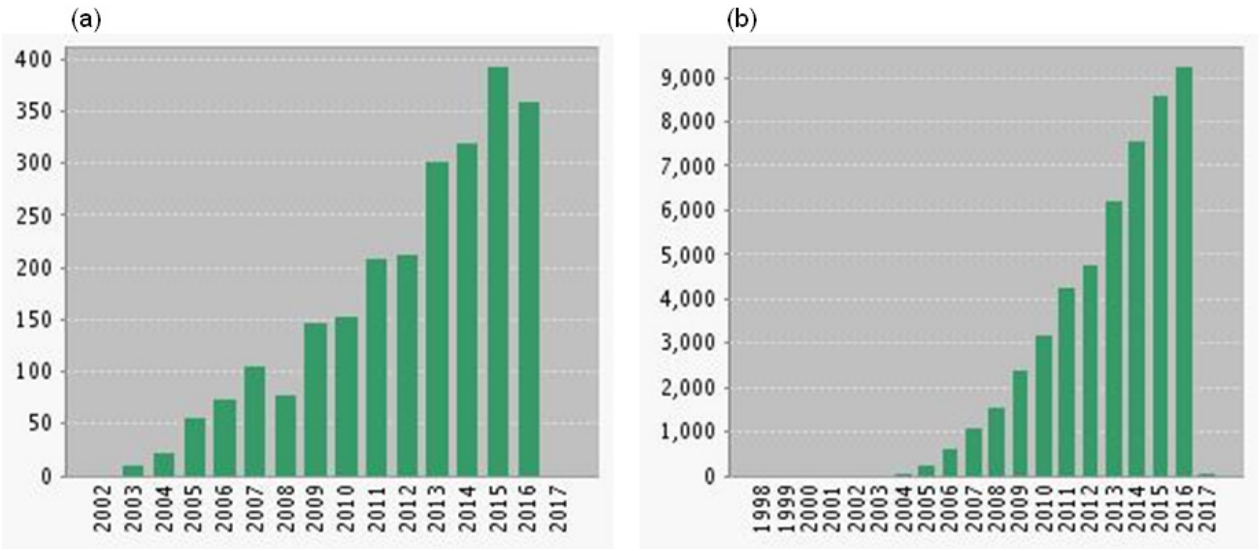

Figure 1. Number of (a) published papers on "lipidomics" in the years 2003-2016, (b) citations per year in Web of Science (accessed 04.01.2017)

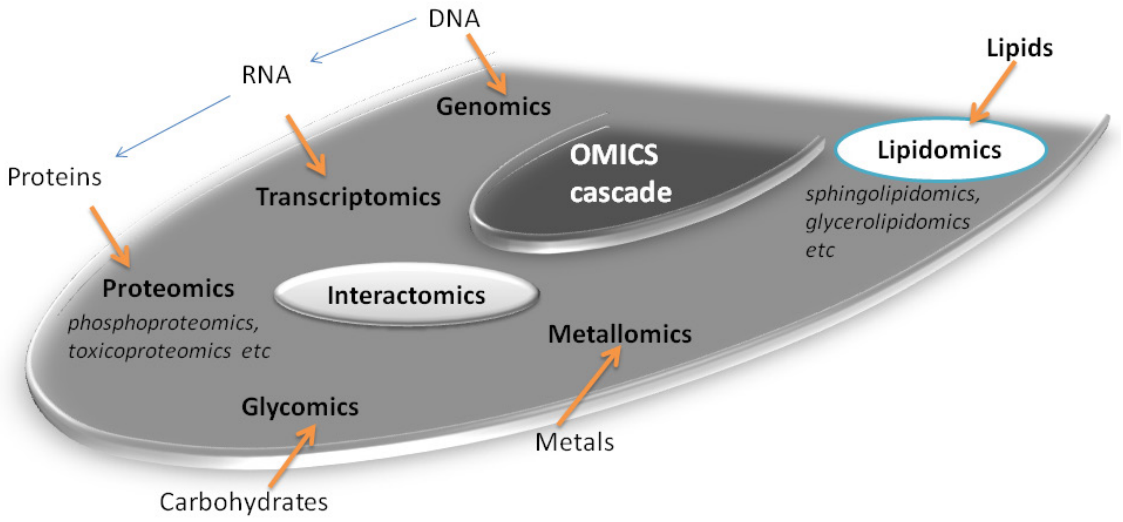

Figure 2. The "-omics" cascade

denote the whole lipid species found in a biological system [4]. Lipidomics is the study of this entire spectrum of lipids in terms of quantification and characterization dedicated to the understanding of the contribution of or the relationship of lipids with cellular physiology and pathology. Lipidomics is indeed a branch of metabolomics and is a systems-based study of all lipids, the molecules with which they interact, and their function within the cell [5].The application of the field is growing rapidly and the ultimate goal is to evolve an integrated omics picture -the interactome- of the genes, transcripts, proteins, and metabolites to describe cellular functioning as a whole (Figure 2).

METHODS IN LIPIDOMICS

Lipids are designated as hydrophobic molecules, insoluble in water but soluble in organic solvents. This definition is insufficient because complex glycolipids are soluble in water besides many other hydrophilic lipid metabolites [6].According to the International Lipid Classification and Nomenclature Committee of LIPID MAPS consortium, lipids have been classified into eight classes based on their structural (either hydrophobic or hydrophilic) and biosynthetic (ketoacyl or isoprene subunits) characteristics (Table 1) [7].There is a high complexity of lipidomic samples, either from cells or tissues.

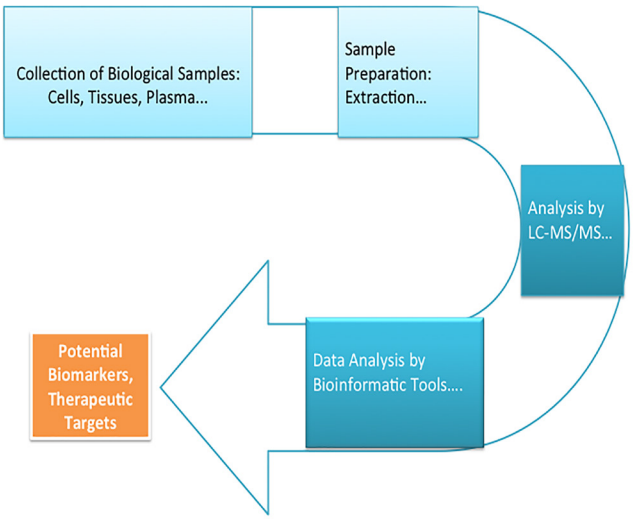

Figure 3. An exemplary working scheme in lipidomics

Eg. considering phospholipids; there is a variety of molecules depending on alcohol (either glycerol or sphingosine), fatty acids (number of carbons, number of double bonds, cis or trans forms) and polar heads (phosphatidic acid, choline, ethanolamine, serine, inositol), carbohydrates (glucose, galactose, sialic acid etc).Methodological difficulties are possibly the reason why lipid and lipidomic research has been neglected for many years when compared to other -omics. However, since the developments in instrument technology and computer science preceded each other, lipid world has become to be discovered by enthusiastic researchers. Thin layer chromatography has been the method of choice in lipid studies for its simple and cheap application for many years. Subsequently, gas chromatography (GC) has become available to analyze fatty acids from complex mixtures [8].In order to gain insight into the unique functions of lipids in biological systems, accurate and precise determination of lipids is a prerequisite. This challenging task mainly relies on molecular lipidomics consisting of multi-dimensional strategies and state-of-art workflows. In this context, liquid chromatography (LC) or GC hyphenated to mass spectrometry (MS), LC/MS or GC/MS is extensively adopted for lipidomic analyses. Then, obtained complex data are interpreted by use of bioinformatics tools because identification and quantitation of lipids of interest in biological samples require not only relevant instrumentation but also comprehensive database search. Generally, dedicated software programs help in lipid profiling by probing the mass to charge (m/z) of parent- and fragment-ions against an evolving database. Bioinformatics is helpful in processing raw data deduced from lipidomic research for further analysis, for statistical analysis, signal pathway analysis, data modeling [9].Working scheme contains steps including extraction of lipids from biological samples (cells, tissue, and fluids) and sample preparation for MS analysis (Figure 3). In general, lipids are extracted from the sample of interest with a solvent system including appropriate organic solvents (liquid phase extraction) before separation and peak detection. The extraction step should be successive enough to identify and profile lipids. The most popular solvent systems for lipid profiling were almost developed 50 years ago by Folch, Lees, and Sloane Stanley [10] and Bligh and Dyer [11].These involve a mixture of chloroform and methanol (MeOH) (in different ratios) to partition lipids. MeOH disrupts the hydrogen bonding networks or electrostatic forces between lipids and proteins. Then nonpolar chloroform is added to form a two-phase system and partitioning of lipids become possible. There is another type of lipid extraction named methyl-tert-butyl ether extraction with the advantage of portioning lipids at the upper layer of the two-phase solvent system [12].There is also a solid-phase extraction system for lipid analysis [13]. It uses stationary materials, such as bonded silica gel with $-CN$, $-NH_2$, or diol groups, in combination with different elution solvents for lipids separation. Solid-phase extraction is simple, reduces solvent cost and is preferred mostly in targeted lipidomic studies.In a classical lipidomics experiment lipid extract is first separated by liquid chromatography and then delivered to mass spectrometer, ionized, vaporized and the resulting fragment ions are sorted according to their mass to charge ratio (m/z) in the mass analyzer [14]. Some of the recent technological developments that made the MS as main method of choice in detecting biomolecules are increased sensitivity, higher mass accuracy, higher scan speeds, and the ability to acquire in both positive and negative modes in one run.Since 1990s the shotgun lipidomics with soft ionization techniques such as electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) has become popular [8]. The sample is directly injected into the instrument without pre-separation in shotgun lipidomics that is a fast way of identifying and quantifying as many lipid species as possible from biological samples [15]. Although shotgun lipidomics needs a shorter analysis time as an advantage, it may be hard to

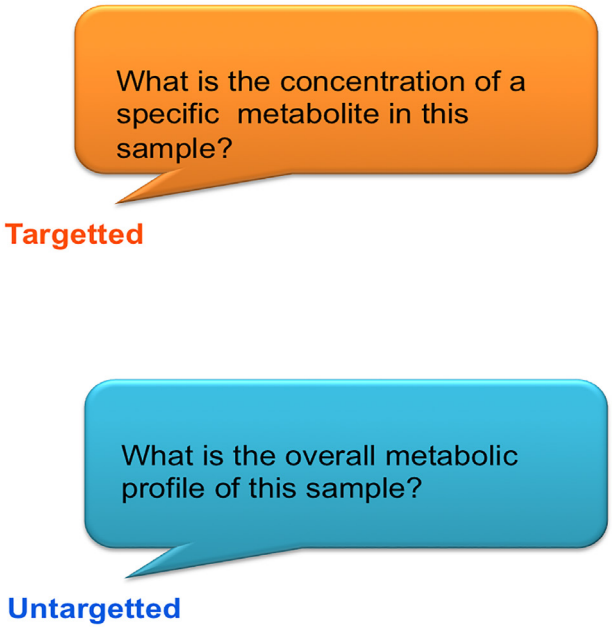

Figure 4. Targetted vs untargetted approaches in lipidomics

discriminate between isomeric lipids by shotgun lipidomics [16].

MS based analysis technology can be classified into two approaches (Figure 4) as, i) untargeted approach/ global lipidomic analysis ii) targeted lipidomic approach. There are various shotgun lipidomics-based platforms for analyzing diverse pathways involving lipid metabolism and signal transduction by using untargeted approach [17]. On the other hand LC-MS and LC-MS/MS based methods are utilized by targeted approach for identifying one or a few lipid classes of interest [18].

LIPIDS IN HUMAN DISEASES: BIOMARKERS AND TARGETS FOR THERAPY

Regulation of lipid metabolism is essential for maintenance of health; therefore de-regulation or dysregulation of this system may play an important role in many human diseases such as neurodegenerative diseases, diabetes, obesity, cardiovascular diseases (CVD), cancer and infectious diseases [19].

The discovery and identification of unique lipid biomarkers carry the potential to discriminate individuals at risk for a specific disease from healthy individuals. This will enable clinicians to diagnose diseases earlier, and facilitate development of personalized treatments. Some diseases or disease-related conditions are selected herein and will be described below for their potential in lipidomic research.

i) Cardiovascular Diseases:

Understanding the lipid species in the cardiac tissue may help to understand the pathophysiology of CVD and to suggest better biomarkers as well as novel therapeutics for CVD [20-21]. In the future, use of lipidomic-based measurements applied in clinical practice, may be expected to serve as a useful tool in prediction of CVD risk [22], and molecular lipid signatures for CVD outperforming the conventional biochemical tests will appear in the literature [23].A recent study investigating serum lipid profile in coronary heart disease (CHD) patients has reported promising findings. Four metabolites; phosphatidylethanolamine (16:1), aldosterone, 2-acyl-sn-glycero-3-phosphocholine, and alpha-tocopheronic acid, were suggested as potential markers that distinguished CHD patients from normal subjects [24]. Glycerolipid and glycerophospholipid metabolism, and steroid hormone biosynthesis networks were found to be acutely perturbed in CHD patients in this study which provided lipidomic data for a better understanding of the disease.The obesity-related issues continue to gain more attention worldwide, and lipidomics may contribute to the field. For example, Syme et al [25] have utilized targeted serum lipidomics in adolescents to establish a panel of glycerophosphocholines, and probed associations with classical risk factors of CVD including excess visceral fat, insulin resistance, and atherogenic dyslipidemia. The results have indicated notable associations between some glycerophosphocholines and classical risk factors, underlining the availability of glycerophosphocholines as indicators of obesity-related CVD risk [25].

ii) Cancer:

Fatty acids, glycerolipids, glycerophospholipids, sphingolipids, and sterol lipids have currently been investigated for their relation with cancer [26]. Ceramide and S1P are two biologically active lipids mediating the pathways involved in tumor development and progression [27]. Ceramide is a tumor-supressor lipid whereas S1P is a tumor promoting lipid (Figure 5). Defining the role of these bioactive lipids in signal transduction pathways

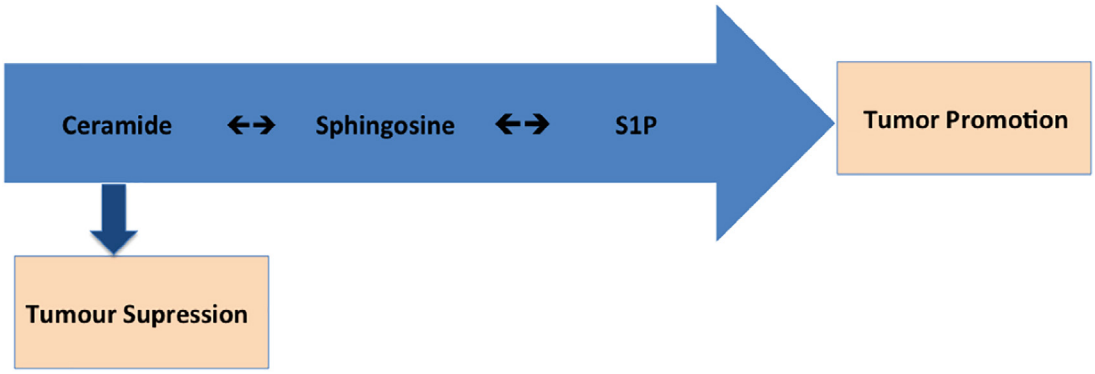

Figure 5. Role of bioactive sphingolipids in tumor development

has enabled researchers with new targets in designing better drugs in fight with cancer. In this respect ceramide analogs are shown to be beneficial in combination therapies against tumour development [28].

Promising applications of mass spectrometry (MS) based lipidomics analysis in various settings include hepatocellular carcinoma [29], intrahepatic cholangiocarcinoma [30], breast cancer [31], ovarian cancer [32], thyroid cancer [33] as well as prostate cancer [34].

iii) Rheumatoid arthritis:

Cardiovascular mortality is important for rheumatoid arthritis patients and finding novel lipid markers is an important task in predicting cardiovascular risk. In a study plasma lipidomic profiling revealed that dihydroceramides, lysophosphatidylinositol, phosphatidylserin classes were significantly associated to rheumatoid arthritis after adjusting for age, sex, body mass index, current smoking, systolic blood pressure and anti-hypertensive treatment in a binary logistic regression model [35].

iv) Neurodegenerative diseases

Lipid content is high in nervous tissue and this brings out many opportunities for lipidomic research that may offer novel insights into underlying pathology. Alzheimer's disease is the most common cause of dementia that has no concrete treatment for the time being [36]. Early studies provided data about perturbation of lipid metabolism in Alzheimer's disease and lipidomic research may have provided new biomarkers for early diagnosis and/or follow up [37].

v) Non-alcoholic fatty liver disease(NAFLD)

Hepatic lipid composition is altered specifically in nonalcoholic fatty liver disease (NAFLD). With the help of linear discriminant analysis, glycerophospholipids, sphingolipids, sterols, and various aqueous small molecular weight components had been analyzed in liver biopsy samples. The finding about a differentiation between nonalcoholic steatohepatitis and steatosis provided a promising platform to improve diagnostic and therapeutic intervention for NAFLD [38].

vi) Inflammation

Some studies have probed closely related pathological conditions in the same study design, and have come across a differential representation of lipid profile. For example, when endometriosis and endometrioma groups were evaluated, it has been observed that sphingolipids and phosphatidylcholines were more abundant in endometriosis patients while in the endometrioma group sphingolipids and phosphatidylcholines exhibiting different m/z as compared with endometriosis group were of high abundance [39].

Another example is cystic fibrosis that is a genetic disorder characterized by chronic pulmonary infection. It has recently been understood that alterations in ceramide metabolism led to excessive mucus production in alveolar epithelium in these patients [40]. Ceramide accumulation was observed in type-II pneumocytes before development of an infection in a rat model where inhibition of acid sphingomyelinase resulted with a decrease of ceramide concentrations and alleviation of infection in lungs [41]. This is a good example that lipidomics contributed to understanding the pathology and developing a new therapeutic approach.Lipidomics may serve as a powerful drug-screening tool, for example in idiopathic pulmonary fibrosis that is a fatal lung disease. A lipidomic study in an experimental model underpinned that treatment with a glucocorticoid (e.g. dexamethasone) may lead to lipotoxicity, hepatic lipidome alterations and fat accumulation in the liver, while another medication may

help to improve the disorder-related outcomes [42]. Lipidomic analysis of circulating polyunsaturated fatty acids revealed a proinflammatory state in acne vulgaris which is a common skin disease characterized by inflammation of the follicular portion of the pilosebaceous unit [43]. Lipidomic analysis enable clinician to suggest new therapeutic options, eg omega-3 fatty acids as adjuvant treatment in such clinical conditions.

vii) Infection

A recent study compared lipid profiles in antibiotic resistant and sensitive Staphylococcus aureus (S. aureus), and found that some lipid species important in the biosynthesis of major S. aureus membrane lipids and lipoteichoic acid were significantly different between resistant and sensitive isolates [44].Another recent study investigated lipidomic profile in Zika virus infected mosquito cells to provide potential targets for viral control in mosquitoes [45]. Thirteen lipids were identified being related with viral replication and these results provided further data in breaking the viral transmission cycle.

viii) Other

Substance abuse related studies also have begun to take advantage of lipidomics [46-47]. An experimental model in rats showed that cocaine-induced sensitization assay caused remodeling of specific phospholipids in brain tissue exhibiting region-specific manner, additionally an alteration in intensities of certain types of phospholipid species including phosphatidylethanolamines, phosphatidylserines and phosphatidylcholines in rat blood were noted [46].Another point of interest is the promising finding on cell lipid fingerprint image that may be of use to predict physio-pathological status of a cell. Bestard-Escalas et al. have employed matrix assisted laser/desorption ionization imaging techniques to identify lipid species changing concomitant to colonocyte differentiation in human colonoscopic sections [48]. This study highlighted strict patterns of distribution in lipid species (phosphatidylinositols or arachidonic acid-containing lipids) and related enzymes as a sign of existence of regulatory mechanisms coordinating the lipidome in line with physiological state of the cell.Some representative studies related with lipidomics research are summarized in Table 2.

INTERNATIONAL LIPIDOMICS INITIATIVES

There are three main international lipidomics initiatives;

1. The European Lipidomics Initiative, ELIVE, Europe (http://www.lipidomics.net) [49]. ELIVE is a project supported by European Commission aimed to mobilise and organise key stakeholders, researchers and end-users in the area of metabolomics, especially lipidomics research, and to further define this field of research in terms of participants, scientific contents and strengths.
2. The Lipid Mass Consortium, USA (www.lipidmaps.org) [50].

LIPIDMAP, Lipid Metabolites And Pathway Strategy, is a multi-centered research group founded in 2003. The project is supported by American National Institute of Health. Its aim is to characterize all major and most of the minor lipids found in mammalian cells. During the projects, many new methods on lipid analysis were shared online, more than 500 lipid standards were synthesized and made available for other researchers.

3. The Lipid Bank, Japan (www.lipidbank.jp) [51]. It is the official database of the Japanese Conference on the Biochemistry of Lipids (JCBL) worked actively during 1987 and 2007, and was funded by various agencies including a Grant-in-Aid for Scientific Research (Grant-in-Aid for Publication of Scientific Research Results) from the Japan Society for the Promotion of Science.

FUTURE PERSPECTIVES

Classical lipid panel involving triglyceride, cholesterol, high density lipoprotein and low density lipoprotein assays may become out of date in some future and may partially or completely be replaced by new biomarkers discovered from MS based lipidomic analysis in defining lipid signatures of pathological states from blood lipidome [52]. A growing data on lipid species for various tissues in several pathological states will be ready for bioinformatic analysis supported by progressive advancement in computing technology. Biomarker and drug discovery is expected to speed up by analysis of high throughput data from large clinical trials.

Table 2. Some representative clinical studies related with Lipidomics

Diseases	Sample size	Method	References
coronary artery disease	Plasma samples from 3-different cohorts Corogene study (n=160), Special Program University Medicine-Inflammation in Acute Coronary Syndromes (SPUM-ACS) cohort (n=1637) Bergen Coronary Angiography Cohort (BECAC) (n=1580),	UHPLC system equipped with QTRAP mass spectrometer	Laaksonen et al (2016) [53]
calcific coronary artery disease	Serum samples no calcification (n=26), mild calcification (n=27), severe calcification (n=17)	UHPLC-system, Q-TOF mass spectrometer	Djekic et al (2016) [54]
diabetes mellitus (DM) and myocardial infarction (MI)	Serum samples from Malmö Diet and Cancer Cardiovascular Cohort Controls (n=316), DM (n=171), MI (n=99)	Shotgun MS analysis (LC-MS/MS) coupled to a robotic nanoflow ion source	Kjellqvist et al (2016) [55]
cardiovascular risk in diabetes mellitus	Plasma samples from the ADVANCE trial (Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation) case-cohort (n=3779)	Targeted lipidomic analysis, liquid chromatography electrospray ionization-tandem mass spectrometry	Alshehry et al (2016) [56]
gestational diabetes	Serum samples Prospective study of 817 pregnant women discovery cohort (n=200), validation cohort (n=617)	Direct infusion high resolution MS for discovery cohort, LC–MS for validation	Lu et al (2016) [57]
preeclampsia	Placental tissue healthy pregnancies (n=68), preeclampsia (n=23), intrauterine growth restriction pregnancies (n=10)	chip based nano-electrospray ionization source coupled to a hybrid linear ion trap-triple quadrupole mass spectrometer	Brown et al (2016) [58]
bipolar disorder	Erythrocyte membrane controls (n=28), high risk group (n= 30), ultra-high risk group (n=36), bipolar I disorder patients (n=35)	gas chromatography	McNamara et al (2016) [59]
prostate cancer	Urine samples controls (n=13), prostate cancer patients (n=15)	shotgun analysis, hybrid triple quadrupole/linear ion trap mass spectrometer equipped with a robotic nanoflow ion source	Skotland et al (2017) [60]
oncocytic thyroid tumors	Thyroid tissue specimens First set (oncocytic thyroid tumors, nononcocytic thyroid tumors, and normal thyroid tissues; 10 samples each). Second set (5 oncocytic thyroid tumors, 10 nononcocytic thyroid tumors).	Desorption electrospray ionization mass spectrometry	Zhang et al (2016) [61]
ovarian cancer	Plasma samples control (n=11), benign gynecological tumor (n=27), ovarian cancer (n=27)	nontargeted lipidomics approach, ultraperformance liquid chromatography-electrospray ionization- quadrupole time-of-flight-mass spectrometry (UPLC-ESI-QTOF-MS)	Zhang et al (2016) [62]

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