Review

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What can lipidomics tell us about the pathogenesis of Alzheimer disease?

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Abstract: Lipids serve many distinct functions in cellular homeostasis such as membrane organization, as a platform for membrane function and protein/protein or protein/lipid interaction, energy storage, as well as secondary messengers in signal transduction. Perturbations in lipid homeostasis may result in abnormal cellular function. Alzheimer's disease (AD) is a neurodegenerative disorder in which the brain represents the primary site of pathology. While there is a plethora of previous work pertaining to AD pathogenesis, the precise mechanism of the disease is still not well-understood. Recent waves of technological advances in the realm of lipidomics have enabled scientists to look at AD pathogenesis from a previously unexplored perspective, and studies have revealed extensive lipid aberrations are implicated in the disease pathology. Herein, we review the critical lipids alternations, which affect amyloid plaque and neurofibrillary tangles formation and accumulation, as well as lipid aberrations related to neuronal and synaptic dysfunction in cells and animal models. We also summarize lipid abnormalities observed in the human cerebrospinal fluid (CSF), as well as other circulating fluids including plasma and serum in association with AD, which could serve as candidate biomarkers to diagnose and monitor the disease.

Keywords: amyloid-β; biomarker; lipids; Tau.

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder clinically characterized by progressive cognitive defects and increasing memory loss (Holtzman, 2001). The hallmarks of AD include extracellular senile plaque (SP) formed by aggregation of amyloid-β (Aβ), intracellular neurofibrillary tangles (NFTs) formed by hyperphosphorylated Tau, as well as loss of neuronal and synaptic integrity (Harman, 1996, 2006). Currently, AD is the sixth-leading cause of death in the United States. About 1% of individuals aged 65 years are affected by AD, and the number increases to 38% for those 85 years or older (Alzheimer's Association, 2013). The incidence of AD increases exponentially with every 5-year increase in age. It is estimated that one in 85 persons worldwide will be living with AD by the year 2050 (Brookmeyer et al., 2007). The debilitating effects of AD, especially in the advanced stages, impose substantial financial burdens on the society and families of the patients, primarily due to the cost associated with caregiving. Thus, there is a pressing need to develop novel therapeutic agents that could help prevent or even treat the disease.

AD can be divided into two categories: early onset AD (EOAD) and late onset AD (LOAD). EOAD accounts for 5–10% of total AD cases. The definition of EOAD is disease occurrence before the age of 65 years (Blennow et al., 2006). Missense mutations that alter a single amino acid in one of the three genes encoding the amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2), can result in EOAD (Campion et al., 1995; Sherrington et al., 1996; Janssen et al., 2003). Moreover, EOAD is inheritable in an autosomal dominant pattern (Campion et al., 1999). For example, the risk to offspring of individuals with EOAD is 50%, if a mutation is found in one of these three genes. In contrast to EOAD, no single gene mutation has been found to be directly responsible for the onset and pathogenesis of LOAD. Nonetheless, it was reported that specific mutations in the gene encoding the lipid transporter protein-apolipoprotein E (ApoE) dramatically

increases the risk of developing LOAD for individuals over 65 years.

The human ApoE protein comprises 299 amino acids and it has three isoforms: namely the ε2, ε3 and ε4. The differences between these three isoforms lie in the amino acid residues at positions 112 and 158: ε2 (Cys112, Cys 158), ε3 (Cys 112, Arg158), ε4 (Arg112, Arg158). For individuals carrying the *APOE ε4* allele, their risk of developing AD is two to three times higher than those who do not carry this allele (Jarvik et al., 1996). Furthermore, ApoE ε4 was observed to exhibit gene dosage effect, in that individuals who carry two copies of this allele have further elevated risk of LOAD, as well as an earlier age of onset. Conversely, ApoE ε2 carriers appear to be somewhat protected from LOAD as evidenced by the higher mean age of LOAD onset (Corder et al., 1993; Poirier et al., 1993).

Lipidomics targets at constructing a comprehensive atlas of lipidome comprising the entire lipid pool within a cell or tissue, and has emerged as an independent discipline at the interface of lipid biology, technology and medicine (Lam and Shui, 2013). An expanding array of technologies, such as liquid chromatography, gas chromatography, mass spectrometry and nuclear magnetic resonance, has been successfully incorporated into the burgeoning field of lipidomcs in order to provide novel, hitherto unknown information pertaining to lipid homeostasis in various aspects of biology and medicine (Lam and Shui, 2013). The brain, which represents the primary site of pathology in AD, is the most lipid-enriched organ in the human body. The basic lipid compositions of the brain are cholesterol, phospholipids and sphingolipids, as well as the lipid derivatives such as 4-hydroxy-2-trans-nonenal (HNE). Furthermore, lipids exert critical structural and physiological roles in maintaining normal brain function. For instance, sulfatide, a major lipid component of myelin sheath, plays important roles in myelinated cell differentiation and gila-axon signaling (Eckhardt, 2008). Aberrant sphingomyelin (SM) and ceramide (Cer) metabolism could induce neuronal endosome/lysosomal dysfunction (Soreghan et al., 2003). Therefore, elucidating changes in brain lipid profiles will undoubtedly confer novel insights pertaining to the pathogenesis of AD and unveil potential markers to facilitate early disease diagnosis. In this review, we will limit the discussion to the contribution of lipidomics in furthering the current understanding of AD pathogenesis based on previous findings in cells and AD animal models (Figure 1), as well as the contribution of lipidomics in uncovering early diagnostic biomarkers for AD.

Lipid changes related to amyloid β-induced AD pathology

Aβ is generated from two successive cleavages of the APP by membrane-bound proteases, β- and γ-secretase (Haass and Selkoe, 2007; Haass et al., 2012). The first cleavage of APP by β-secretase liberates the soluble ectodomain into extracellular space. β-site APP cleavage enzyme 1(BACE1), identified as the enzyme responsible for majority of the β-secretase activity, mediates the first cleavage step. The remaining fragment of APP undergoes subsequent cleavage by γ-secretase to generate a spectrum of Aβ peptides with varying lengths. In this step, γ-secretase not only acts as endoproteolytic enzyme that cuts APP to generate a 48/49 residues form of Aβ, but also function as carboxypeptidase that processively trims longer Aβ intermediates approximately every three residues to form shorter, secreted forms (Wolfe, 2012). The presenilins are the major catalytic component of the γ-secretase complex (De Strooper et al., 2012). Amongst the spectrum of Aβ peptides generated, the variant with 42 amino acids (Aβ42) is substantially more amyloidogenic than its counterparts, and forms the major component of amyloid plaques (Verbeek et al., 1997). Without efficient clearance, Aβ42 deposits generate neurotoxicity and induce the subsequent defects in AD pathogenesis.

APP, BACE1 and components of the γ-secretase complex are all transmembrane proteins. Thus, changes in membrane lipid bilayer composition and organization are expected to exert substantial impact on the trafficking and proteolytic activities of β- and γ-secretases (Hartmann et al., 2007). The changes in β- and γ-secretase activities will consequently affect the production of Aβ42. In the brain, cholesterol (Chol) is mainly located in the myelin sheaths and the cellular membranes of glial cells and neurons. The levels of cholesterol in the brain tissues of AD patients were elevated significantly compared to healthy controls (Cutler et al., 2004; Bandaru et al., 2009). Reducing the membrane cholesterol level has been shown to inhibit the production and secretion of Aβ (Simons et al., 1998). Treatment of cholesterol-enriched vesicles containing BACE1 with the cholesterol extraction compound β-methyl cyclodextrin reduces the binding between BACE1 and the associating membrane. Depletion of membrane cholesterol has also been shown to curb γ-secretase activity in another separate study, which can be restored by replenishing the cholesterol supply (Wahrle et al., 2002). All these data cumulatively indicate that membrane cholesterol modulates the enzymatic activities of β- and γ-secretases. A better association between cholesterol and

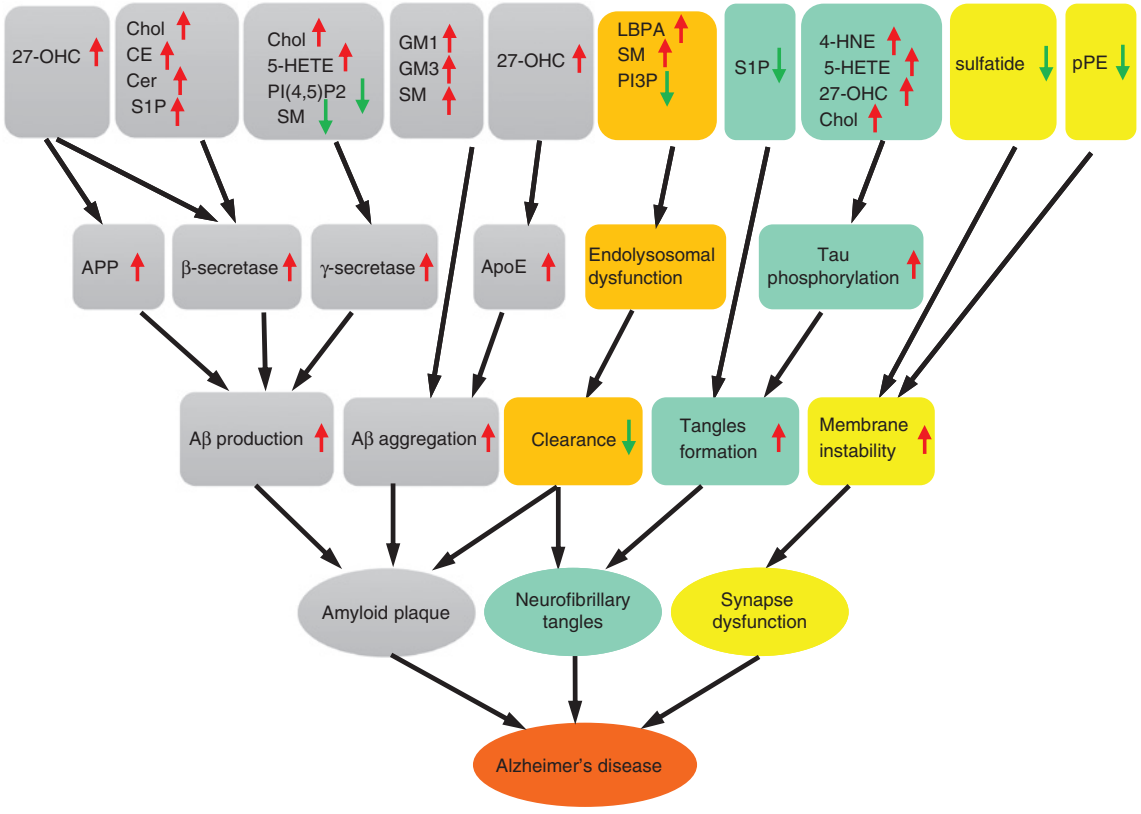

Figure 1: Schematic diagram of lipid changes contributing to AD pathogenesis.
Lipid changes boxed in various colors correspond to changes that are associated with different clinical hallmarks of AD, including amyloid plaque formation (purple), neurofibrillary tangles formation (blue) and synaptic dysfunction (yellow). Perturbations in the endolysosomal pathway could negatively impact the clearance of both amyloid plaques and neurofibrillary tangles and the associated lipid changes are boxed in orange. Red arrow indicates increase; while green arrow indicates decrease.

the activity of β- and γ-secretases was observed in the brain of AD patients. AD brain had significant cholesterol retention and high β- and γ-secretase activities as compared to age matched non-demented controls. β- and γ-secretase activities were highly stimulated by 40 μM of cholesterol in the lysate of AD brain (Xiong et al., 2008). Thus, enhanced cholesterol level may promote the enzyme activities of β- and γ-secretases, thereby accelerating the amyloidogenic processing of APP, resulting in the increased accumulation and deposition of Aβ in AD patients. These *in vitro* observations, however, awaits further validation with respect to their physiological relevance *in vivo*.

In cells, excess free cholesterol is converted into cholesteryl ester (CE) by the enzyme sterol O-acyltransferase 1(ACAT1) under normal physiologic condition. ACAT1 inhibitors such as CP113 or 818, or genetic ablation of ACAT1, have been shown to alleviate Aβ pathology in mouse model (Hutter-Paier et al., 2004; Bryleva et al., 2010). These evidences imply that CE accumulation may also be detrimental to normal brain function by exacerbating Aβ pathology and therefore AD pathogenesis. Indeed, increased CE level has been reported in most AD mouse models (APP, APP/PS1, APP/Tau) except for the PS1 mice (Chan et al., 2012; Tajima et al., 2013). As one of the oxidative products of cholesterol, increased levels of 27-hydroxycholesterol (27-OHC) was shown to increase Aβ level through elevating the protein levels of APP and BACE1 in human neuroblastmal SH-SY5Y cells (Prasanthi et al., 2009). This study indicates that 27-OHC may contribute to AD pathogenesis by the increasing Aβ level, which results from elevating the protein level of APP and BACE1. Furthermore, 27-OHC was also found to induce Aβ aggregation by increasing ApoE levels (Gamba et al., 2012). It has been previously reported that ApoE functions as an Aβ-binding protein in the brain that induces pathological β-pleated sheet conformation changes in Aβ peptides in a manner akin to gangliosides (see below) (Holtzman et al., 2012).

Gangliosides are concentrated in the luminal leaflet of cellular organelles and the outer leaflet of the plasma membrane, and are found to aggregate in raft-like membrane microdomains containing cholesterol (Di Paolo and

Kim, 2011). The distribution of specific gangliosides displays significant regional differences in the brains of AD patients (Kracun et al., 1990, 1991). Incubating Aβ peptides with direct addition of GM1 or raft fractions isolated from GM1-riched cells promotes Aβ oligomerization, while incubation of Aβ peptides with raft fractions extracted from ganglioside-poor cells decreases the rate of Aβ oligomerization (Kim et al., 2006; Chi et al., 2007). Furthermore, ganglioside-binding amino acid sequence has been found in Aβ peptides. Histidine (13) is the key interaction site in Aβ for GM1 inducing conformation change (Williamson et al., 2006). Gangliosides, such as GM1, can bind Aβ and alter the peptide secondary conformation from random coils to the more ordered form of β-pleated sheets (Ariga et al., 2008), which correlates with increased toxicity. GM1-bound-Aβ (GAβ) could act as 'seeds' for Aβ aggregation in neurons and nerve terminal preparation (Di Paolo and Kim, 2011). It has been reported that the assembly and deposition of Aβ in the different regions of brain are all dependent on the local gangliosides pool (Yanagisawa, 2007). GM3 level was observed to increase in both the entorhinal cortex of AD patients and the forebrains of AD mouse models (APP, APP/PS1) (Chan et al., 2012).

Sphingolipids make up approximately one-third of the lipid content in eukaryotic cell membranes, and are highly-enriched in the central nervous system (CNS). Sphingolipids also represent the main components of lipid rafts, the proposed site of function in cellular membranes for membrane-bound secretases including BACE1 and γ-secretase as abovementioned. Exogeneous Cer has previously been shown to increase the half-life of BACE1 and promote the production of Aβ in human neuroglioma cells and Chinese hamster ovary cells (Puglielli et al., 2003). In addition, the level of Cer was observed to increase in different regions of the brain in both AD patients (Han et al., 2002; He et al., 2010; Chan et al., 2012; Filippov et al., 2012) and mouse model of AD (Barrier et al., 2010). In PS1/PS2 deficiency mouse embryonic fibroblast cells, increased level of SM was observed with reduction in the activity of neutral sphingomyelinase (nSMase). Inhibition of nSMase by GW4869, however, results in the accumulation of SM and the reduction of Aβ secretion in SH-SY5Y cells. This result was further supported in COS7 cells by yet another nSMase inhibitor, and also via direct addition of SM (Grimm et al., 2005). These evidences cumulatively indicate that SM accumulation in membrane, which is a result of reduction in nSMase activity, reduces the Aβ production possibly by virtue of its inhibitory effect on γ- secretase activity. While elevated membrane SM levels have been shown to reduce Aβ production, treatment of rat pheochromocytoma (PC) 12 cells with sphingomyelinase inhibitor, GW4869, however, significantly increased the level of SM and Thioflavin S (ThS)-positive Aβ fibrils. The increase in ThS positive Aβ fibrils was abolished in presence of GAβ specific antibody 4396C, which impedes SM and GAβ association (Yuyama and Yanagisawa, 2010), implying that SM represents a key molecule in GAβ generation which subsequently drives amyloid fibrillization in the brain. In corroboration with the hypothesis, treatment of PC12 cells with SM synthase (SMS) inhibitor D609 led to lowered SM levels, and appreciably prevented amyloid generation (Yuyama and Yanagisawa, 2010). Therefore, the interaction between membrane lipid components such as SM with AD-associated proteins is complex, and exerts compound effects on the overall pathogenesis of the disease at by affecting various key steps contributing to disease progression to differing extents or even in an opposite manner.

Sphingosine-1-phosphate (S1P) is a biologically active lipid metabolite generated from sphingosine. The rate-limiting enzymes of S1P generation are sphingosine kinases 1 and 2 (Sphk1 and Sphk2). Attenuating the activity of Sphk1 or overexpression of the S1P-degrading enzyme S1P phosphatase-1 (SGPP1) or S1P lyase (SGPL1) has been shown to reduce BACE1 activity and therefore Aβ production. Conversely, addition of S1P into microsomal fraction significantly increased BACE1 activity, with accompanying increase in Aβ production in both mouse primary cortical neurons and neuroblastoma 2a (N2a) cells (Takasugi et al., 2011).

While a myriad of different studies have indicated that accumulation of specific sphingolipid classes, such as Cer, SM, S1P and gangliosides contribute to AD pathogenesis by promoting Aβ accumulation as discussed above, attenuation of the entire sphingolipid biosynthetic pathway via inhibiting serine palmitoyl transferase activity, however, leads to increased production of Aβ42 (Sawamura et al., 2004). These studies therefore demonstrate that the effects of sphingolipids on Aβ production are probably complex, and compensatory mechanisms may operate when the levels of specific sphingolipid classes or species are elevated or diminished. A systematic and comprehensive lipidomic study encompassing all sub-members of the sphingolipid and glycosphingolipid families in patient postmortem tissues or relevant disease models in relation to AD pathogenesis and Aβ accumulation would be indispensable in identifying the actual sphingolipid metabolite governing the central mechanisms underlying amyloid plaque formation.

A classical inhibitor of phosphatidylinositide-3 kinase (PI3K) pathway has been shown to reduce Aβ levels both *in vitro* and *in vivo*, highlighting the potential role of

phosphoinositides in AD pathogenesis (Petanceska and Gandy, 1999; Haugabook et al., 2001). Phosphatidylinositol 3- phosphate (PI3P) is found predominantly on early endosomal membranes, as well as in the intraluminal vesicles of multivescular endosomes (Simonsen et al., 2001). In Hela cells treated with hydrogen peroxide, PI3P was found to be obliterated from endosomes resulting in the perturbations in endocytosis pathway (Kano et al., 2011). PI3P has been shown to regulate receptor sorting but not transport in endolysosomal pathway (Petiot et al., 2003). Conversely, lysobisphosphatidic acid (LBPA) is specifically enriched in the late endosomes. LBPA together with the protein Alix are proposed to regulate fission and fusion processes in the late endosomes (Falguieres et al., 2009). Therefore, altered levels of PI3P and LBPA may have detrimental effects on the normal physiological functioning of the endolysosomal pathway, which may subsequently affect the clearance of deleterious Aβ plaques, thereby negatively impacting AD pathogenesis. Indeed, LBPA accumulation has been previously observed in the entorhinal cortex of AD patients (Chan et al., 2012). In addition, some reports have been shown that perturbed endolysosomal functions leads to a failure in the degradation of amyloid plaques (Cataldo et al., 2000; Pan et al., 2008; Orr and Oddo, 2013).

Phosphatidylinosiol-4, 5-bisphosphate [PI (4, 5) P_2] is the most abundant and well-studied species of phosphoinositide family. The activity of reconstituted γ-secretase complex in liposomes is exquisitely sensitive to PI (4, 5) P_2. In the presence of PI (4, 5) P_2, the activity of γ- secretase is strongly inhibited. This inhibitory effect on APP cleavage was proposed to reflect competitive binding between PI (4, 5) P_2 and APP fragment for the γ- secretase complex (Osawa et al., 2008; Osenkowski et al., 2008). These studies indicate that PI (4, 5) P_2 negatively regulate Aβ production via inhibiting the activity of γ-secretase complex. Conversely, the enzyme 5-lipoxygenase (5-LO) and its metabolic product, 5-hydroxy-eicosateraenoic acid (5-HETE), was found to significantly increase the activities of γ-secretase complex and result in the elevated Aβ levels in Tg2576 mice and N2a cells (Chu and Pratico, 2011).

Lipid changes in hyperphosphorylated Tau-induced AD pathology

In contrast to APP and various secretases responsible for generating the pathological forms of Aβ, which are transmembrane proteins, Tau is a cytoplasmic protein that interacts with and stabilizes microtubules. Under normal physiological conditions, Tau binds to microtubules and serves to stabilize them. In pathological states, however, Tau proteins become hyperphosphorylated. Hyperphosphorylated Tau detach from microtubules, resulting in the subsequent disintegration of the latter. In addition, the hyperphosphorylated Tau undergo alterations in configuration that promote the formation of insoluble neurofibrillary tangles (NFT). The lipid oxidation product 4-HNE was reported to enhance conformational changes in Tau that promote NFT formation (Perez et al., 2000). The level of 5-LO was also found to be correlated with Tau pathology in AD. In the brain tissues of human subjects with observable Tau pathology, 5-LO level was elevated. Inhibition of 5-LO activity in transgenic tau mice reduces the extent of Tau phosphorylation. Inhibition of 5-LO also improves memory defect, rescues synaptic dysfunction and ameliorates Tau pathology in transgenic tau mice (Giannopoulos et al., 2015). Furthermore, in 3xTg mice (APP/PS1/Tau), the 5-LO inhibitor, Zileuton, has been reported to appreciably improve memory defects, as well as ameliorating amyloid and Tau pathology (Chu et al., 2013). In both studies, it was found that 5-LO promote Tau phosphorylation by modulating the activity of CDK5. In another study, knockout 5-LO or the use of 5-LO inhibitor was also found to prevent Tau phosphorylation in 3xTg mice, with accompanying increase in GSK3β activity (Joshi et al., 2013). It has been reported that CDK5 affects Tau phosphorylation at Ser202 and Ser396/404 sites via the regulation of GSK3 activity (Martin et al., 2013). Cholesterol and its oxidative product, 27-OHC, were also reported to increase the level of Tau phosphorylation in both brain organotyptic slices and the hippocampus of rabbits (Ghribi et al., 2006; Marwarha et al., 2010). In postmortem brain tissues of AD human subjects, S1P level was found to be negatively correlated with increasing Tau pathology, coupled with reduction in sphingosine kinase activity (Couttas et al., 2014).

The initiation of autophagy, which has been reported to facilitate the clearance of insoluble NFTs, is strictly dependent upon PI3P biosynthesis (Vergne and Deretic, 2010). Inhibitors of various processes related to autophagy, such as ammonium chloride, chloroquine, 3-methyladenine, as well as various cathepsin inhibitors, was reported to slow down Tau degradation and enhance the formation of Tau aggregates (Wang et al., 2010). These findings imply that PI3P may regulate the clearance of Tau aggregates via stimulating the autophagy pathway. Treatment of PC cells with sphingomyelinase inhibitor G4869 induce the enlargement of Rab-5-GFP positive early endosomes but not Rab-7 positive late endosomes (Yuyama and Yanagisawa, 2010). This study may indicate that SM accumulation affects the physiological function

of early endosomes, which may subsequently impact upon the clearance of amyloid β and NFTs via within the endolysosomal pathway.

Lipid changes in relation to neuronal and synaptic loss

Most sulfatides in the nervous system are concentrated in the oligodendrocytes and Schwann cells. During oligodendrocyte differentiation, sulfatide is first detected at the stage of immature oligodendrocytes and its level is elevated before the mature cells begin to wrap as myelin around axons, suggesting that sulfatides may fulfill other critical functions apart from serving as a structural component of myelin for the maintenance of axonal integrity (Bansal et al., 1988; Pfeiffer et al., 1993; Marcus et al., 2006). In APP mice, the level of sulfatides was decreased compared to the control mice. The reduction in sulfatides, however, was obliterated following ApoE deletion in APP mice (Cheng et al., 2010), indicating that the reduction in sulfatides in APP mice is mediated by ApoE. The drop in sulfatide levels in the brain tissues of AD patients (Han et al., 2002; Bandaru et al., 2009) and AD mouse models (PS1, PS1/APP, APP) (Cheng et al., 2010; Chan et al., 2012) may be associated with oligodendrocytes death and myelin destruction, leading to a failure in maintaining the functional integrity of neurons in the afflicted individuals or animal models. On another note, plasmalogen ethanolamine (pPE) is a major constituent of human neural membranes. pPE deficiency has been shown to adversely affect neural membrane integrity, resulting in myelin sheath defects and axonal dysfunction (Ginsberg et al., 1998).

Lipidomics and the discovery of biomarkers for AD

Preclinical diagnosis of AD nowadays mainly relies on magnetic resonance imaging (MRI) scan of structural changes in the brain, positron emission tomography (PET) for detection of neutral-related molecular changes, as well as quantitation of the levels of Aβ42 and Tau (absolute amount and phosphorylated amount) in the CSF (Dubois et al., 2007). With the advent of lipidomics, an emerging pool of lipid molecular markers for AD has surfaced in the recent decade. For instance, a lower level of lysophosphatidylcholine (LPC) was observed in the CSF of AD patients compared to control (Mulder et al., 2003), which may be indicative of enhanced PC breakdown in AD pathogenesis. As for sphingolipid changes in the CSF of AD patients, increased Cer (Satoi et al., 2005) and SM (Kosicek et al., 2010, 2012) levels and reduction in sulfatide (Han et al., 2003) have been separately reported by different groups. Interestingly, free cholesterol and CE were reduced in the CSF (Mulder et al., 1998) despite their observed increases in the brains of AD patients (Cutler et al., 2004; Bandaru et al., 2009) and AD mouse models (Chan et al., 2012; Tajima et al., 2013). This may imply a reduced conversion of cholesterol to cholesterol oxidation products and their subsequent transport out of the brain into the circulation via the CSF in AD, and therefore a failure in brain cholesterol homeostasis in afflicted individuals.

Although AD is commonly regarded as a disease of brain, ample evidences have now demonstrated that AD is indeed a systemic disease that affects peripheral tissues other than the CNS, even from the incipient stage of the disease (Khan and Alkon, 2015). Peripheral tissues and fluids, such as the blood, serve as a potential source of non-invasive biomarkers for AD diagnosis. A pressing need exists to identify novel biomarkers to delineate the incipient stage of the disease, which would extend the therapeutic window for treatment. Indeed, appreciable efforts have been dedicated by different research groups to unveil lipid-related biomarkers in the plasma/serum of AD patients (Table 1). For instance, decreased total PC (Han et al., 2011) and specific PC species (Oresic et al., 2011; Mapstone et al., 2014; Whiley et al., 2014), as well as total SM (Oresic et al., 2011) have been previously observed using various techniques including electrospray ionization-mass spectrometry, high performance liquid chromatography-mass spectrometry, ultra-performance liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry. Notably, the species PC 38:4 has been identified as a potential AD marker in two independent studies (Oresic et al., 2011; Mapstone et al., 2014). Conversely, elevated plasma Cer level has also been independently observed by a number of research groups (Mielke et al., 2010; Han et al., 2011). In light of the decreasing PC levels in both serum and plasma, accompanied by decreasing LPC and increasing PC hydrolysis products, glycerophosphocholine and phosphocholine, in the CSF (Walter et al., 2004) of AD patients, it is likely that PC breakdown was accelerated in AD pathogenesis. In addition, reduction in SM (Han et al., 2011) and increased Cer levels (Mielke et al., 2010; Han et al., 2011) have been reported in the serum and plasma of AD patients. Most of the clinical studies aforementioned were cross-sectional

Table 1: Lipid changes in peripheral tissues in AD patients.

Materials		Increase in AD	Decrease in AD	Analytical techniques	References
CSF	Lipid class		LPC	ESI-MS/MS	Mulder et al. (2003)
CSF	Lipid class		Slufatide	ESI-MS	Han et al. (2003)
CSF	Lipid class	SM		Nano-HPLC-MS	Kosicek et al. (2012)
	Individual species	SM 14:0, 16:0, 16:1, 20:0, 22:0, 22:1, 24:0, 24:1, 24:2			
CSF	Lipid class	Cer		DAG kinase method	Satoi et al. (2005)
CSF	Lipid class		Chol/CE	Enzymatic methods	Mulder et al. (1998)
CSF	Lipid class	SM		Nano-HPLC-MS	Kosicek et al. (2010)
	Individual species	SM 16:0, 16:1, 22:0			
CSF		4-HNE		GC-MS	Selley et al. (2002)
Serum	Lipid class	SM/sulfatide		ESI-MS/MS	Mielke et al. (2010)
	Individual species	Cer (C16:0, C18:0, C22:0, C24:0, C24:1)			
Serum	Individual species		PC 16:0/18:2, PI 18:0/20:4, pPC 18:0/18:2, PC 18:0/20:4, SM d18:1/24:0	UPLC-MS	Oresic et al. (2011)
			2-ketobutyric, sitosterol,	GCXGC-TOFMS	
Plasma		Arachidic acid (C20:0), erucic acid (C22:1, n-9), vaccenic acid (C18:1, n-7), mead acid (C20:3, n-9)	Cerotic acid (C26:0), linoleic acid (C18:2, n-6)	GC	Iuliano et al. (2013)
Plasma	Individual species		PC 36:6, PC 40:2, PC 40:6, PC 38:0, PC 38:6, PC 40:1, PC 40:2, PC 40:6e, LPC 18:2, PC 38:4e	UPLC-ESI-QTOF-MS	Mapstone et al. (2014)
Plasma	Individual species		CE 32:0, 34:0, 34:6, 32:4, 33:6	LC-MS/MS	Proitsi et al. (2015)
Plasma	Individual species		PC 16:0/20:5, 16:0/22:6, 18:0/22:6;	LC-MS	Whiley et al. (2014)
Plasma	Lipid class		PC/SM	ESI-MS	Han et al. (2011)
	Individual species	Cer C16:0, C21:0	SM 20:0, 21:0, 22:0, 22:1, 23:0, 23:1, 24:1		
Plasma		4-HNE		GC-MS	Selley et al. (2002)

in nature, and may not reveal biomarkers that could effectively indicate the progression of the disease, in particular, the incipient changes that culminate in disease onset.

Mapstone and colleagues (Mapstone et al., 2014) performed a 5-year follow-up study on three defined groups of subjects, namely the control, converter and amnestic mild cognitive impairment (aMCI)/AD. Converter was sub-classified into two groups: pre-converter and post-converter. Pre-converter represent subjects who were later diagnosed as AD during five-year follow-up but without cognitive impairment at entry. By comparing the lipid profiles of pre-converters and controls, the group has identified a panel of ten lipids that were decreased in pre-converter, represented by PC 36:6, PC 40:2, PC 40:6, PC 38:0, PC 38:6, PC 40:1, PC 40:2, PC40:6e, LPC 18:2, PC 38:4e. Notably, the reduction in this panel of lipids is reported to be able to be able to predict the phenoconversion to aMCI/AD within a 2–3 year timeframe with an accuracy of over 90%. This study has demonstrated precisely how lipidomics, in combination with appropriate clinical design, could serve to sieve out effective diagnostic biomarker panel from a repertoire of lipid candidates. These lipid candidates also serve as potential targets for therapeutic intervention. In another separate longitudinal study, Oresic and colleagues have reported reductions in the levels of PC 16:0/18:2, PI 18:0/20:4, plasmalogen PC 18:0/18:2, PC18:0/20:4 in the serum of AD patients compared to control. Interestingly, the level of 2,4-dihydroxybutanoic acid was increased in progressive MCI, which represents individuals who later progress into AD, compared to healthy control, indicating the potential involvement of hypoxia in early AD pathogenesis (Oresic et al., 2011). Another notable longitudinal study was performed in 100 women over a 9-year timeframe (Mielke et al., 2010). The researchers employed the Hopkins Verbal Learning test and the trail making test to classify patients into varying degree of memory

impairment, and analyzed the sphingolipids changes in the different groups across various time-points. The group found that the serum levels of Cer and SM changes in accordance with the onset of memory impairment. In particular, higher levels of total SM, various Cer species including C16:0, C18:0, C22:0, C24:1, C24:0, and sulfatide were associated with a significant higher risk of impairment in the group with mildest cognitive impairment. In addition, two specific ceramides species (C16:0 and C20:0) predicted impairment on immediate recall and psychomotor speed. This study therefore indicated that elevated SM and ceramide levels may be good preclinical predictors of memory impairment.

Peripheral lipid biomarkers have advantages over CSF-based markers or various forms of brain imaging techniques for AD diagnosis in the sense that these studies are non-invasive, as well as relatively simple and inexpensive to perform. While the plethora of lipidomic studies aforementioned has unveiled a myriad of candidate lipid biomarkers for AD diagnosis and treatment intervention, there is an apparent lack of unifying evidence for the disease across the many different studies. While the complex nature of AD pathogenesis may play a substantial role in the current lack of conclusive mechanisms pertaining to the disease, the use of different analytical techniques that include dissimilar classes of lipids, varying methods of data normalization and processing, as well as the different criteria employed to define the clinical groups in each of the lipidomic studies have also contributed to lack of consistent lipid biomarkers drawn from the various studies. A comprehensive method of lipidomic analysis that includes most classes of lipids relevant to neurobiology on a well-defined clinical cohort is imperative to unify the various lipid candidates identified across the different studies.

Future directions

The fields of genomics and proteomics have witnessed an explosion of information in the recent years that have not been matched by corresponding advancements in the field of lipidomics. Lipidomics, as a burgeoning field, has been circumvented by a lack of suitable technological tools with sufficient sensitivity and specificity to provide information with comparable spatial and temporal resolution to rival that of other omics fields. The high structural complexity and diversity of lipids *per se*, however, also play a substantial role in this bottleneck. As aforementioned, the continual development in analytical methods based chiefly upon the principle of liquid chromatography couple to mass spectrometry would remain as a powerful technique to unravel promising lipid candidate markers in proximal fluids such as the plasma for AD diagnosis. Furthermore, as spatial-specific lipid aberrations in the different regions of brain are known to exert varying effects upon disease pathogenesis *per se*, imaging mass spectrometry may confer the much-needed spatial information for better understanding the neurological mechanisms of AD pathogenesis in this aspect. Lipidomics has already offered us a previously unexplored angle of AD pathogenesis by moving scientific efforts away from a solely protein- and gene-centric view in the past decade. Emerging panels of lipid biomarkers and lipid pathways relevant to the disease have been unveiled by the concerted efforts of various research groups dedicated to the lipid aspect of AD pathogenesis, as summarized in this review (Figure 1). A comprehensive lipidomic analytical method that could provide us a wholesome view of the global changes in the lipid landscape specific to AD pathogenesis is, in our view, indispensable to construct a unifying understanding of lipid perturbations in AD pathogenesis amidst the current lipid conundrum stemming from the myriad of lipidomic studies already available.

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