We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Brain Lipids in the Pathophysiology and Treatment of Alzheimer's Disease

Manuel Torres, Xavier Busquets and Pablo V. Escribá

Additional information is available at the end of the chapter

Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder that causes severe and progressive cognitive impairment. The discovery of specific mutations related to AD supported the amyloid cascade hypothesis, which postulates that the accumulation of the amyloid-β (Aβ) peptide triggers neuronal death and dementia. However, most drugs that aim to prevent Aβ accumulation or tau phosphorylation have consistently failed in clinical trials. This would suggest that the amyloid pathology lies downstream of (an)other cellular event(s) that is/are responsible for AD pathogenesis. In this context, several lipid alterations have been described in the brain and in peripheral fluids of patients with AD, suggesting the involvement of lipids in the etiology of this condition. Indeed, the central nervous system (CNS) has the highest lipid content in the body, next to adipose tissue, and it is thought that normalization of brain membrane lipid levels would revert AD-related pathogenic events. In this sense, novel hydroxylated derivatives of docosahexaenoic acid (DHA) such as natural resolvins or synthetic hydroxy-DHA (HDHA, DHALifort) can modulate membrane lipid composition and show remarkable beneficial effects on AD hallmarks, such as prevention of amyloid production and tau phosphorylation, and cognitive restoration in animal models. Therefore, normalization of the neuronal lipid environment by hydroxyl-DHA and/or other lipids may constitute a promising therapy for AD treatment, memory loss and, possibly, other types of dementia.

Keywords: Alzheimer's disease, neurodegeneration, neuroregeneration, hydroxy-DHA, amyloid, tau, neurite dystrophy, inflammation, brain lipids, cholesterol, sphingolipids, lipid rafts, omega-3 PUFAs, HDHA, DHALifort, resolvins, neuroprotectins, lipid biomarkers

1. Introduction

Alzheimer's disease (AD) is the main neurological cause of dementia, and it affects about 46 million people worldwide, mostly elderly adults. The incidence of AD increases exponentially every 5 years from 65 years of age, and it is estimated that 74.7 and 131.5 million people will be living with AD by 2030 and 2050, respectively (World Alzheimer Report, 2015). Patients with AD undergo progressive memory loss, reduced cognitive capacity and eventually, dementia. The debilitating effects of AD, especially at advanced disease stages, impose a substantial financial burden on AD patient's families, primarily due to the cost associated with medical care. However, the etiology of AD still remains largely unclear and although there has been much effort to elucidate the pathophysiological mechanisms underlying this devastating condition over the last 20 years, the principal cause remains unknown, representing an important unmet clinical need. Therefore, AD is undoubtedly one of today's most challenging global public health problems, and there is a pressing need to develop novel therapeutic agents to prevent and treat this disease.

The neuropathological hallmarks of AD include the formation of extracellular senile plaques due to the aggregation of amyloid-β (Aβ; normally associated with local inflammation and dystrophy/swelling of neurites), the formation of intracellular neurofibrillary tangles of hyperphosphorylated tau protein, as well as a loss of synaptic connections and neuronal degeneration [1]. Clinically, AD can be classified into two categories: familial AD (FAD, also known as early-onset AD) and sporadic AD (SAD, also known as late-onset AD). FAD generally accounts for <1% of the total AD cases, and they correspond to a disease variant with onset prior to 65 years of age [2]. This familial form of AD is inherited in an autosomal dominant pattern, and it is caused by mutations in three genes involved in Aβ generation: the amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2) [3]. In contrast to FAD, no single gene mutation has been found to be directly responsible for the onset and pathogenesis of SAD [4]. For the late-onset cases, the principal risk factors are ageing and the apolipoprotein E (ApoE) allele ε4 (see Section 3.1.).

The identification of clinical mutations in APP and presenilins in association with FAD has contributed to our understanding of AD pathogenesis. APP is a transmembrane protein that undergoes primary enzymatic cleavage by an α- or β-secretase in its extracellular (or intraluminal) domain, as well as secondary cleavage by a γ-secretase within the transmembrane region (**Figure 1**). The metalloproteases ADAM10 and/or ADAM17 appear to be responsible for this α-secretase activity and the aspartyl protease BACE-1 (beta-site APP cleaving enzyme 1) corresponds to the β-secretase activity, whereas γ-secretase is an aspartyl proteolytic complex containing four subunits (PS1 or 2, nicastrin, APH1, and PEN-2) [5]. APP cleavage may be produced by β- and γ-secretases in a pathway known as the amyloidogenic route of APP. First, APP β-cleavage produces soluble APP-β (sAPPβ) and a transmembrane C-terminal fragment known as β-CTF or C99. The latter then undergoes γ-secretase cleavage to generate the APP intracellular domain (AICD) and the Aβ peptide, preferentially the Aβ40 and 42 isoforms. Alternatively, APP may be cleaved by α- and γ-secretases in a pathway known as the non-amyloidogenic route of APP where α-secretase cleaves APP right

in the middle of the Aβ sequence (**Figure 1**) to generate soluble APPα (sAPPα) and a transmembrane C-terminal fragment known as α-CTF or C83. The latter undergoes further γ-cleavage to produce AICD and p3 (also known as Aβ17–40/42). In this context, it has been widely reported that FAD mutations induce alterations in APP processing that increased the cellular production of Aβ and augment the Aβ 42/40 ratio. Since mutations in both APP and presenilins are the major causal factors in FAD etiology, altered APP metabolism was assumed to be the principal cause triggering AD, leading to the formulation of the amyloid cascade hypothesis more than 20 years ago. Finally, it is notable that all these participants in APP metabolism, APP and secretases, are membrane-associated proteins influenced by the composition and structure of cell membrane lipids that in turn modulate APP metabolism [6].
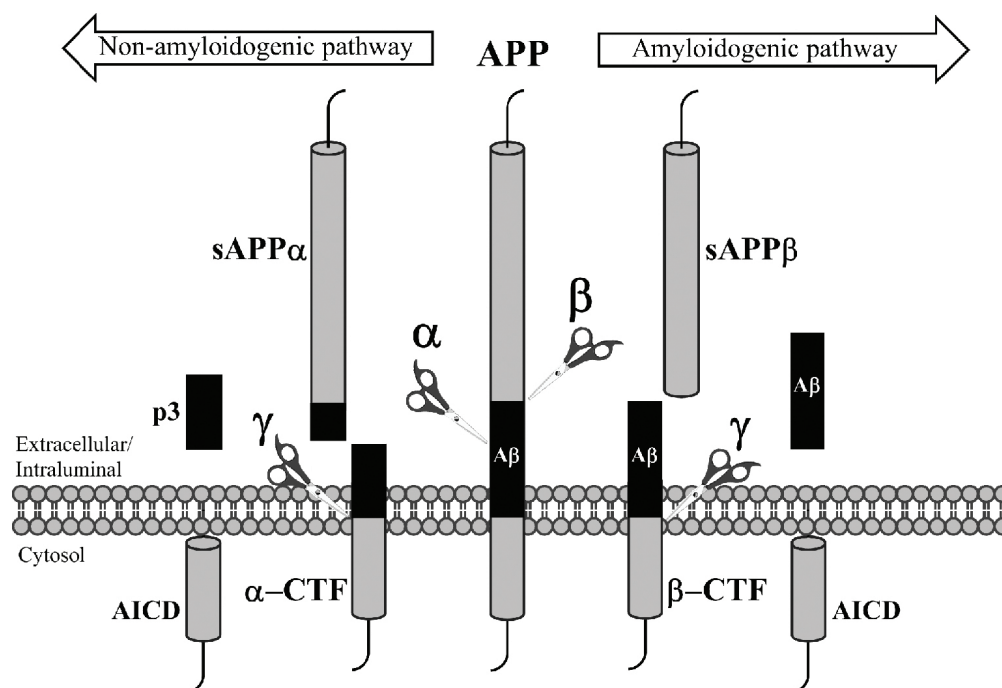
Figure 1. APP processing by secretases. In the non-amyloidogenic pathway, APP is first cleaved by α-secretase at a sequence of amino acids within the Aβ peptide, releasing the sAPPα ectodomain. Further processing of the resulting membrane-associated C-terminal C83 fragment (α-CTF) by γ-secretase leads to the release of the p3 fragment and the APP intracellular domain (AICD). This processing takes place preferentially at the plasma membrane. Conversely, the amyloidogenic pathway is initiated when β-secretase cleaves APP at the amino terminus of the Aβ peptide to release the sAPPβ ectodomain. Further processing of the resulting membrane-associated C-terminal C99 fragment (β-CTF) by γ-secretase releases the Aβ peptide and AICD. This processing normally takes place in acidic cellular compartments like late endosomes. The Aβ peptide produced is normally 40 or 42 amino acids long (Aβ40 or 42) and the Aβ42/40 ratio increases in AD.

2. Historical perspective on the pathophysiology of Alzheimer's disease

For more than 20 years, the accumulation of the Aβ peptide has been considered to be the main cellular/molecular event that triggers AD-related neurodegeneration. Amyloid plaques were first thought to cause AD pathogenesis, and more recently, Aβ-soluble oligomers have gained

more attention as key players in AD etiology [7]. Regardless of the form of amyloid, the amyloid cascade hypothesis postulates that Aβ accumulation in the brain is the major upstream event in AD pathophysiology, whereas other neuropathological features are a result of this primary amyloid pathology, including the formation of neurofibrillary tangles, neuroinflammation, synaptic failure, and eventually neural death [8, 9].

According to the amyloid cascade hypothesis, enhanced amyloidogenic activity of secretases and/or reduced clearance of the Aβ peptide may trigger Aβ accumulation. As a result, the secretases involved in Aβ generation have been extensively targeted by the pharmaceutical industry to develop new compounds to treat AD [10]. In particular, the Aβ42/40 ratio may increase due to FAD mutations and this increase enhances oligomer formation, which may in turn impair synaptic function and provoke neuronal degeneration [7]. At the same time, secreted Aβ42 forms primary extracellular Aβ deposits in the brain parenchyma, first as diffuse plaques and later as insoluble fibrillary plaques. A concomitant local inflammatory response develops around these amyloid deposits (involving microglial and astroglial activation), coupled to synaptic spine loss and neurite dystrophy (neuritic pathology) [11, 12]. Over time, these events result in oxidative stress and altered ion homeostasis. Neurofibrillary tangles appear as a consequence of the altered kinase and phosphatase activities that cause tau protein hyperphosphorylation, and likely its subsequent dysfunction in axonal transport, as well as neurite dystrophy [13, 14]. Finally, the cascade ends with extensive synaptic and neuronal dysfunction, which precedes the well-characterized neuronal death associated with the Aβ and tau pathologies [7]. It is this neuronal degeneration that is responsible for memory loss and dementia in patients with AD.

Amyloid burden in the brain parenchyma is closely associated with tau hyperphosphorylation, axonal dystrophy and inflammatory reaction around amyloid plaques (**Figure 2**). Both, inflammation and axonal dystrophy can promote neuronal degeneration [15, 16]. However, it is still largely unknown which of these events (amyloid, inflammation, or neurite dystrophy) appear first during disease development and how these three events are connected. The amyloid cascade hypothesis postulates that amyloid accumulation, first intracellular and then extracellular, leads to the generation of amyloid plaques. Given the close relationship between Aβ plaque number and size with the surrounding dystrophies and gliosis, these two latter events were proposed to progress in conjunction with Aβ plaque formation. However, evidence is now accumulating against the amyloid cascade hypothesis. On the one hand, therapeutic approaches focused on combating amyloid pathology have generally failed to prevent AD progression in clinical trials (see Section 4, [17, 18]), while on the other hand, transgenic AD animal models, mostly created by incorporating human mutated APP and/or PS1 into the animal genome, do not recapitulate all the neuropathological features of AD, and not even the large scale neuronal death that occurs during this pathology [19]. Moreover, the alterations to membrane lipids in neurons of patients with AD suggest that the changes to lipid bilayer could be the first event in the amyloid cascade and related pathways [6]. Indeed, the normalization of membrane lipids is associated with cognitive restoration (see Section 5.2). Accordingly, the amyloid pathology may not actually be the first initial event driving the events that provoke neuronal degeneration.
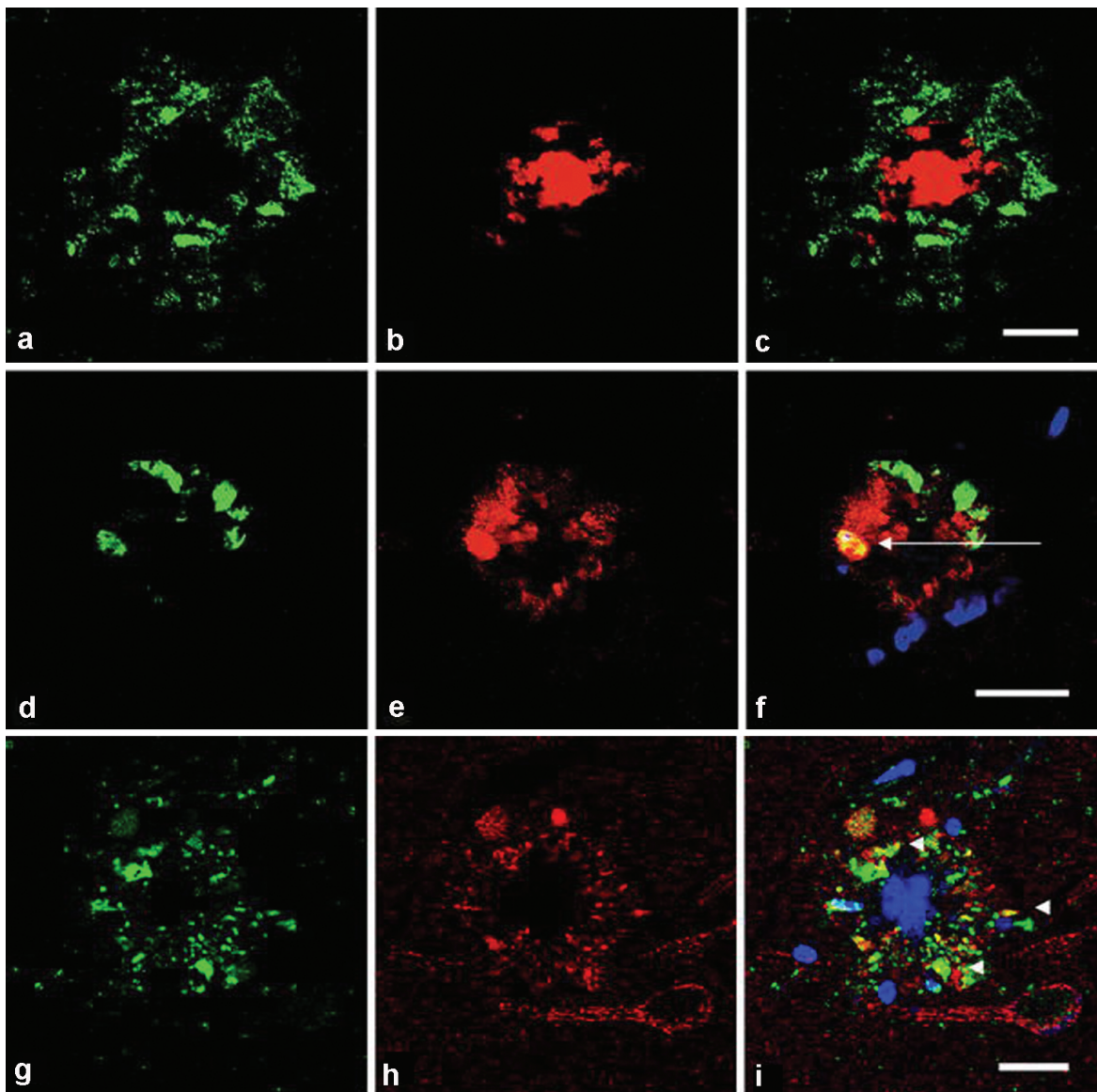
Figure 2. Dystrophic neurites surrounding β-amyloid plaques in AD patient's brain. (a–c) Double-labeling immuno-fuorescence and confocal microscopy to mitochondrial porin (a; green) and β-amyloid plaques (b; red). Porin immu-nostaining revealed mitochondrial enrichment in dystrophic neurites surrounding amyloid plaques (c). (d–f) Double-labeling immunofluorescence and confocal microscopy to mitochondrial porin (d; red), and phosphorylated tau (pThr181) (b; green) show co-segregation of porin and hyperphosphorylated tau in dystrophic neurites (long arrow in f). (g–i) Double-labeling immunofuorescence and confocal microscopy to lysosomal associated protein 1 (LAMP-1) (a; green) and mitochondrial porin (b; red). LAMP-1 and porin co-localize in a subset of cellular processes (c; arrowheads) suggesting engulfment of mitochondria into matured autophagic vesicles and participation of lysosomes in its degra-dation in distrophic neurites. β-Amyloid is stained in blue. Bar 10 μm (a–c and d–f), and 20 μm (g–i). Adapted from [12] with permission of Springer.

It also appears that axon swelling or dystrophy can precede extracellular amyloid deposition in certain animal models, in which autophagic vesicles with all the necessary enzymatic machinery to produce the Aβ peptide are evident [20–23]. In this sense, dystrophic axons have been proposed to be an intracellular source of secreted Aβ that would seed extracellular amyloid plaques. Protein deposits containing APP fragments can be seen in the brain

parenchyma of aged wild-type mice, originating from axonal varicosities, further supporting this hypothesis. These data suggest that axonal dystrophy occurs first, leading thereafter to extracellular amyloid deposition in the early stages of the disease. In fact, it has been proposed that neurite dystrophy could reflect a conserved neuroprotective strategy to overcome the age-related accumulation of misfolded proteins, which in turn may represent a molecular mechanism of Aβ plaque deposition that potentially underlies the shift from normal to pathological aging [24, 25]. Nevertheless, Aβ alone may promote axonal atrophy through its interactions with the p75 neurotrophin receptor (p75NTR) in axon membranes [26]. Together, the evidence suggests that dystrophy and extracellular Aβ deposition are involved in a positive feedback loop whereby axon dystrophy is a source of extracellular Aβ, and the latter promotes axonal atrophy.

In terms of neuroinflammation, it is widely accepted that Aβ deposition alone might be sufficient to induce an inflammatory reaction that subsequently contributes to neuronal death and cognitive decline in AD [15]. However, this fact does not necessarily imply that Aβ plaque formation precedes microglial activation in AD. During normal aging, microglial activation aims to clear the misfolded proteins contained in fragmented neurites and aggregated into senile plaques. Interestingly, during AD-related pathological ageing, microglia cells recruited around plaques phagocytose Aβ and this could constitute part of the microglial mechanism to clear misfolded proteins, also during normal ageing [25]. Thus, in a scenario characterized by age-related chronic inflammation, microglia would be highly responsive to further activation which would drive their differentiation toward a classic phenotype characterized by pro-inflammatory cytokine secretion, in turn impairing axon trafficking, promoting Aβ accumulation and cell death [25, 27]. However, this putative role for AD-associated neuroinflammation is not supported by evidence showing that the inflammatory response is not neurotoxic and, indeed, it is even neuroprotective in a transgenic mouse model of AD [28]. In fact, from early in the amyloid pathology, alternative neuroprotective microglia are activated around amyloid plaques supporting neuronal survival, and this alternative phenotype is also present during animal ageing. By contrast, the classic microglial phenotype that is characterized by cytotoxic cytokine secretion only appears at advanced ages, associated with the presence of soluble Aβ oligomers and neuronal loss [27, 28]. Thus, these evidences show that alternative neuroprotective microglia may be present at advanced ages and coexist with classic microglial activation. In summary, although it is widely accepted that neuroinflammation promotes neuronal degeneration, it remains unclear how brain inflammation participates in the shift from normal to pathological ageing.

Hence, determining whether amyloid pathology is the first event in the pathway to AD-associated neuronal degeneration and dementia appears to be a particularly relevant issue, especially after the repeated fiascos in clinical trials of drugs targeting Aβ and related molecular entities. There is a close relationship among Aβ, inflammatory and neurite pathologies in AD because they all appear at early stages of the disease and all three are involved in neuronal death. In the present chapter, we will review how these neuropathological hallmarks are related to AD-associated membrane lipid alterations, as there can now be no shadow of doubt that brain lipids and the pathways they are involved in influence the pathophysiology of AD.

3. Brain lipid alterations in Alzheimer's disease

3.1. Cholesterol and sphingolipid homeostasis in AD

The amyloid cascade hypothesis was postulated because FAD mutations cause Alzheimer's disease, and they induce abnormal APP processing that leads to the well-characterized amyloid pathology [9]. Since the pathological hallmarks are exactly the same for both FAD and SAD, the same cascade of neuropathological events is thought to occur in both these disease variants. However, in addition to the influence of FAD clinical mutations on APP metabolism, these mutations may also have additional effects on other signaling cascades. In fact, presenilins (PSs) are the catalytic center of the γ-secretase complex, which cleaves more than 60 type I membrane proteins (one type of single transmembrane spanning region in integral proteins) [29, 30]. More than 160 clinical mutations have been described for PS1 and most of those that were studied induce loss of function of γ-secretase activity [31, 32]. These mutations may exert additional effects on cellular signaling as a consequence of the altered processing of certain membrane proteins that could influence lipid cellular homeostasis. Interestingly, γ-secretase loss of function induced by the ablation of PSs or by transgenic expression of PS1 mutants provoked a severe imbalance in the cholesterol content of the plasma membrane and intracellular membranes [33, 34]. In this sense, PS ablation increased the overall levels of cholesterol and sphingomyelin (SM) in cells, whereas the local concentration of cholesterol at the plasma membrane was dramatically reduced, resulting in the intracellular accumulation of cholesterol and cholesterol-rich membrane domains, such as lipid rafts [33, 34]. These observations demonstrate the impact of γ-secretase loss of function on the cell membrane lipid composition.

In the human brain, cholesterol is mainly transported in lipoprotein particles that predominantly contain ApoE. Interestingly, ApoE has been identified as a risk factor for SAD suggesting that altered cholesterol transport might also be related to the pathogenesis of late-onset AD [35]. The human ApoE protein is comprised of 299 amino acids and it has three isoforms, namely ApoE2, ApoE3, and ApoE4. The differences between these three isoforms lie in the amino acid residues at positions 112 and 158: ApoE2 (Cys112, Cys158), ApoE3 (Cys112, Arg158), and ApoE4 (Arg112, Arg158). In particular, subjects carrying the ApoE4 allele have a 3- to 4-fold higher risk of developing AD than those who do not carry this allele. Furthermore, ApoE4 was observed to exhibit a gene dose–effect, such that individuals who carry two copies of this allele have an even higher risk of suffering AD and an earlier age of onset. The effects of the ApoE4 isoform on AD risk are maximal between the ages of 60 and 70 years old, ApoE4 allele being present in more than 50% of all AD cases. Conversely, ApoE2 carriers appear to be somewhat protected from AD compared with ApoE3 carriers [36]. In this context, the ApoE4 isoform is less efficient in promoting cholesterol flux in neurons and astrocytes, and it also compromises cell uptake of cholesterol-containing lipoproteins compared with the other ApoE isoforms [37]. Furthermore, individuals carrying the ApoE4 allele accumulate less ApoE lipoprotein in the brain than non-ApoE4 carriers [38]. Hence, the expression of ApoE4 in SAD cases appears to alter cholesterol homeostasis in neurons in a similar way as that induced by γ-secretase loss-of-function in PS1-deficient cells and transgenic models of AD harboring

clinical PS1 mutations [33, 34]. In such AD models, the loss of γ-secretase activity leads to impaired uptake of lipoproteins from the extracellular media due to the poor internalization of ApoE receptors like the LDLR (low-density lipoprotein receptor) [34]. In AD patients with the ApoE4 allele, cholesterol uptake would be impaired due to the lower affinity of ApoE4 to bind neuronal lipoprotein receptors, and to the lower concentration of circulating ApoE than in individuals carrying the ApoE2 or ApoE3 alleles [38, 39]. In any case, poorer membrane incorporation of neuronal cholesterol leads to increased *de novo* cholesterol synthesis and an altered neuronal distribution. Thus, altered cholesterol homeostasis is a key aspect of AD pathogenesis and alterations to cholesterol may represent a meeting point in the pathogenesis of FAD and SAD, driving the same neuropathological events in both disease variants, such as increased amyloidogenic APP processing.

The central nervous system (CNS) contains around 25% of the cholesterol in the body and evidence is accumulating that cholesterol homeostasis is indeed associated with AD pathogenesis. High cholesterol and high-density lipoprotein (HDL) in blood plasma are correlated with Aβ load in the brains of patients with AD [40, 41] and that increased cholesterol levels are associated with the incidence of AD [42, 43]. Furthermore, high or low cholesterol levels have often been related to enhanced or diminished Aβ production, respectively, in cell and animal models of AD, although these results are a little controversial [42, 44, 45]. What is more, lipidomic studies have shown that levels of cholesterol, certain cholesterol esters, and certain SM species are upregulated in the brain of patients with AD. This correlation is particularly strong in the case of patients with AD harboring the ApoE4 allele, although some contradictory results have also been reported in this respect [46–49]. Finally, altered cholesterol distribution and transport have been causally linked to neurodegenerative diseases in addition to AD, such as Huntington's and Niemann–Pick Type C diseases [44].

Cholesterol is an essential structural component of cell membranes and one of the major components of the functional membrane microdomains known as lipid rafts, together with sphingolipids such as SM and gangliosides. These microdomains are highly ordered membrane structures that serve as platforms for cell signaling, ligand-receptor binding, protein sorting, and other activities in the cell. Interestingly, amyloidogenic APP processing and Aβ aggregation have been proposed to take place in lipid rafts [50]. In fact, the activities of both BACE-1 and γ-secretase are enhanced in this type of membrane microdomains [51, 52]. In this context, compelling evidence supports the involvement of cholesterol and sphingolipids in the amyloidogenic processing of APP. On the one hand, membrane enrichment of these lipids could alter the biophysical properties of the lipid bilayer, affecting secretase activity in a manner that leads to the production of the longer pathogenic Aβ peptides instead of the shorter p3 peptide [53] (see **Figure 1**). On the other hand, cholesterol and SM storage disorders impair intracellular trafficking of APP, resulting in the accumulation of APP, APP-CTFs, and Aβ in autophagic vesicles of the endolysosomal pathway [54, 55]. Accordingly, impaired distribution of cholesterol and SM is accompanied by the downregulation of proteins involved in endosomal redistribution and fusion to the plasma membrane (SNAREs and RABs) in PS1-deficient cells [33]. These evidences suggest that dysfunctional vesicular trafficking between the plasma membrane and intracellular compartments may be caused by membrane lipid alterations that

lead to the neuritic pathology and altered APP processing in FAD transgenic models [33, 56]. Additional studies have also linked shingolipid lysosomal accumulation to autophagic dysfunction and dystrophic neurite formation in AD [55, 57]. Such results indicate that cellular accumulation of sphingolipids could induce key cytopathological changes characteristic of AD, such as alterations to the autophagic/lysosomal system, increased generation of Aβ and accumulation of APP-CTFs in autophagic vesicles at dystrophic neurites, as occurs in an age-dependent manner in transgenic mouse models of AD [58]. Interestingly, a cholesterol-enriched diet in healthy mice also leads to insulin-like growth factor 1 (IGF1) impairment and insulin-mediated pro-survival signaling, which in turn promotes tau hyperphosphorylation in neurons [59]. Together, this evidence suggests that altered cholesterol/sphingolipid homeostasis may promote the neurite pathology, tau hyperphosphorylation, and amyloido-genic APP processing in AD.

Nevertheless, it cannot be ruled out that AD-related membrane lipid alterations can also potentiate the neurotoxicity of the Aβ oligomers in AD patient's brains. In fact, lipid rafts may serve as a platform for the cellular interactions with soluble Aβ oligomers, in turn promoting tau hyperphosphorylation and inhibiting synaptic plasticity by hindering LTP (long-term potentiation) in the brain [60, 61]. Moreover, raft-associated lipids such as cholesterol, SM, and the GM1 ganglioside revert the fibrillar Aβ into soluble oligomers, such that altered cellular lipid homeostasis may actually potentiate the severity of the amyloid pathology in AD [62].

3.2. Polyunsaturated fatty acids in AD

Polyunsaturated fatty acids (PUFAs) are those fatty acids that contain more than one double bond in their backbone. They are abundant in cell membranes, and they are mainly incorpo-rated into membrane phospholipids. The carbon next to the carboxyl group is known as the α carbon, the next one is the β carbon, and so forth, until the final carbon called the ω carbon. Thus, ω-3 fatty acids have the first double bond between the third and fourth C atoms from the ω carbon. For instance, 22:6 ω-3 or 22:6 n-3 (docosahexaenoic acid, DHA) indicates a 22-carbon chain with six double bonds and with the first double bond between the third and fourth carbons from the CH_3 end. The physiological properties of unsaturated fatty acids largely depend on the position of the first unsaturation relative to the end position. The essential fatty acids α-linolenic acid (ALA, 18:3 ω-3) and linoleic acid (LA, 18:2 ω-6) must be incorporated through the diet, and they are the starting point for the synthesis of longer and more unsaturated PUFAs such as arachidonic acid (ARA, 20:4 ω-6), eicosapentaenoic acid (EPA, 20:5 ω-3), and DHA (22:6 ω-3). However, conversion of ALA to longer PUFAs in humans is very inefficient and therefore, these long PUFAs are normally incorporated through the diet, particularly through fish intake [63].

The membranes of the cells in the brain are rich in ω-3 PUFAs such as DHA and EPA. Since AD is a cognitive disorder and DHA is involved in normal cognitive development, the DHA levels in the AD brain have been analyzed extensively. As a result, it is widely accepted that in the human brain AD courses with diminished DHA levels, although a number of discrep-ancies in this respect have also been observed [64]. These discrepancies may reflect the brain region studied as the neurodegeneration associated with AD does not affect all brain areas

homogeneously. In the hippocampus, one of the regions primarily affected in AD, decreased DHA levels are associated with reduced levels of PE (phosphatidylethanolamine) or PE plasmalogens [65–69], supporting a relationship between lower DHA levels and cognitive decline in AD. Moreover, there is significant experimental evidence in animal models that hippocampal DHA deficiency or enrichment is associated with reduced or increased learning memory abilities, respectively [70]. At the cellular level, exposure to ω-3 PUFAs enhances synaptic plasticity by increasing LTP and synaptic protein expression, in turn leading to increased dendritic spine density and hippocampal neurogenesis. In addition, ω-3 PUFAs have antioxidant, anti-inflammatory, and anti-apoptotic effects, thereby promoting neuronal survival during normal ageing and in AD. On the other hand, PUFA deficits are related to enhanced amyloidogenic APP processing and cell susceptibility to Aβ neurotoxicity, particularly as ω-3 PUFA deficiency downregulates neuroprotective signaling (e.g., ERK signaling). Therefore, PUFA deficits may enhance neuron degeneration and cognitive impairment in AD [71].

It still remains largely unclear how ω-3 PUFAs exert their cellular functions and consequently, what signaling cascades are impaired in the brain due to their deficiency. Such ω-3 PUFAs maintain the structural functionality of neural cell membranes. Indeed, in consonance with the reduced levels of DHA in the human AD brain, lipid rafts obtained from AD brain cortex also exhibited significantly less DHA than age-matched controls [72]. Interestingly, the biophysical and structural properties of PE and DHA in membranes are opposed to those of cholesterol and SM. Thus, these abnormalities in lipid raft composition may provoke strong modifications to the membrane structure of neurons such as alteration of membrane viscosity, rigidity and thickness, lateral lipid packing, lipid order, and other parameters, which could in turn be relevant to secretase activity and the production of Aβ [73]. Accordingly, decreased PUFA levels in lipid rafts would be coupled to enriched cholesterol and sphingolipids, thereby promoting the detrimental effects on neurons including the neurite dystrophy, tau hyperphosphorylation, and amyloidogenic APP processing that drives neuronal degeneration (see Section 3.1.).

Alternatively, DHA may be released from phospholipids due to the activity of PLA2 (phospholipase A2), acting as a signaling molecule, and DHA can be hydroxylated to produce several secondary bioactive lipids such as resolvins (RVs) and protectins. DHA hydroxylation is mediated through lipoxygenase-15 (LOX-15) or acetylated cyclooxygenase-2 (COX-2) [63]. Compounds derived from DHA are classified as D-series RVs or protectins, while those formed from EPA are designated as E-series RVs. DHA can be hydroxylated on carbon 17 by 15-LOX or acetylated COX-2, leading to stereoselective formation of 17S- or 17R-hydroxy-DHA (17-HDHA), respectively. These derivatives may be further hydroxylated to give rise to trihydroxy derivatives such as the D1, D2, D3, and D4 17-(S/R)-RVs (D-series RVs), and the dihydroxy 17-(R)- and 17(S)-protectin, the latter also known as neuroprotectin D1 (NPD1). EPA can be stereoselectively hydroxylated to 18-(S/R)-hydroxy-EPA (18-HEPA) by cytochrome P450 or acetylated COX-2, which is further processed to form E1, E2 and E3 18-(S/R)-RVs (E-series RVs: **Figure 3**). Both, 17-HDHA and 18-HEPA serve as markers for RVs and protectins, and remarkably, their presence in blood is directly related to the intake of ω-3 PUFAs in animal

models [74]. In addition, these PUFA derivatives are thought to exert their biological function by mechanisms that go beyond the simple regulation of lipid membrane composition and structure. In fact, non-esterified DHA, RVs and protectins may bind to different fatty acid (FA) receptors such as the retinoid X receptor (RXR), G protein-coupled receptors (GPCRs), peroxisome proliferator-activated receptors (PPARs), and fatty-acid binding proteins (FABPs). Although the exact signaling cascade mediated by many of these proteins has not been identified, the mechanism of action of DHA or HDHA derivatives like NPD1 has been proposed to involve PPARγ activation. Indeed, NPD1 is known to promote PPARγ activation more intensely than DHA and as such, the neuroprotective effects of DHA may be mediated by NPD1 and/or other DHA-derived hydroxylated bioactive derivatives in the brain [75, 76].
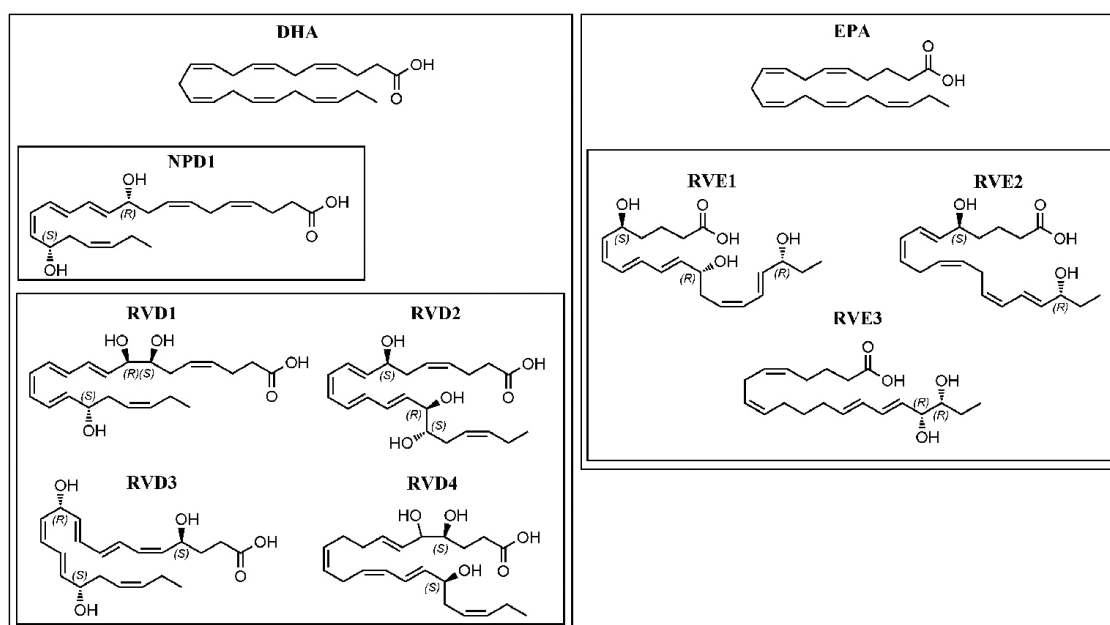
Figure 3. Chemical structure of specialized pro-resolving mediators derived from DHA and EPA ω-3 fatty acids. DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) may be released from phospholipids through PLA2 (phospholipase A2) activity and converted into bioactive hydroxylated fatty acids with potent anti-inflammatory properties, known as resolvins and protectins. This conversion may be mediated by several enzymes, including lipoxygenase-15 (LOX-15), acetylated cyclooxigenase-2 (COX-2), and cytochrome P450. Compounds derived from DHA are classified as D-series resolvins (RVs: left panel), while those formed from EPA are designated as E-series resolvins (right panel). Although resolvins normally includes trihydroxy fatty acids, DHA can be also transformed into dihydroxylated compounds denominated as protectins. Within this group, neuroprotectin D1 (NPD1: see left panel) is the best studied DHA-derived hydroxylated compound in terms of AD therapy, and it displays anti-inflammatory, anti-apoptotic, and anti-amyloidogenic properties.

The balance between ω-6 to ω-3 intake has a strong impact on brain health. In Western diets, this ratio is about 10–20:1, while in other cultures and also historically, this ratio has been as low as 1–2:1. Total fat intake as well as the ω-6 to ω-3 ratio in Western diets has increased significantly since the Industrial Revolution, indicating that Western diets are deficient in ω-3 PUFAs [77]. Epidemiological studies, including correlational studies and migration studies, suggest a protective effect against AD of ω-3 PUFAs and fish oil (an important source of ω-3 PUFAs), such that the role of nutrition in preventing AD arouses increasing hope, particularly

with reference to ω-3 PUFA dietary intake. One recent meta-analysis reviewed a total of six cohort studies performed in the USA and Europe to address how dietary intake of long-chain ω-3 PUFAs or fish correlates with the incidence of dementia and AD [78]. This meta-analysis found a significant lower risk of AD associated with high fish intake. Such an association was most pronounced when the follow-up period was at least five years and fish intake was 500 g or more per week, such that fish consumption is inversely correlated with AD incidence in a temporal and quantitative manner. A dose–response meta-analysis also showed that for every 100 g per week dietary fish intake the risk of AD falls 11%. This neuroprotective effect of fish intake was mainly attributed to its high long-chain ω-3 PUFA content, particularly DHA [79]. Interestingly, the same meta-analysis also revealed that dietary intake of ω-3 PUFAs alone (not linked to fish consumption) did not lower the risk of dementia or AD. Moreover, an earlier randomized trial reached the same conclusions in patients with mild-to-moderate AD who were administered DHA [80]. Nevertheless, most of the individual studies evaluating the relationships between ω-3 PUFA intake and AD risk suggest there is a potential protective effect of these long ω-3 PUFAs on the incidence of AD, although no significant statistical differences were reached in the pooled analysis.

The discrepancies between fish and ω-3 PUFA consumption in relation to AD incidence may be explained by different factors in terms of the dietary composition or socioeconomic status of the individual. In this context, dietary intake of long-chain ω-3 PUFAs may also be accompanied by the intake of other saturated fats, which would attenuate the neuroprotective effect of ω-3 PUFAs. Alternatively, fish is also a good source of vitamins, essential amino acids and other nutrients, which could in turn be responsible for the beneficial effect attributed to fish in AD prevention. The fact that DHA is converted into bioactive derivatives that mediate its beneficial effects in CNS cannot be overlooked. In this context, the neuroprotective effect of fish intake could be also attributed to PUFA derivatives present in fish, such as hydroxylated forms of PUFAs or PUFA forms easily transformable into bioactive derivatives similar to NPD1 [81]. In fact, fish oil consumption has recently been related to increased levels of total DHA and NPD1-like derivatives in the mouse brain, without any modification of free (unesterified) DHA levels [82]. Hence, fish oil intake promotes elevated levels of NPD1 without affecting basal levels of free DHA in the brain. These data bring to light a central role for ω-3 PUFA hydroxylated bioactive derivatives in the prevention and treatment of AD (see Section 5.2.).

3.3. Specific lipid alterations as potential biomarkers in AD

Modern lipidomic analysis allows a comprehensive atlas to be built up of all the lipid alterations existing in the AD brain. Current laboratory techniques, such as ultra/high pressure liquid chromatography (U/HPLC) and gas chromatography (GC) coupled to mass spectrometry (MS) allow the vast majority of lipids in cells and animal tissues to be studied. Since the brain is the most lipid-enriched organ in the human body, after adipose tissue, alterations in lipid composition might be involved in many neurological disorders, including AD [44]. An in-depth lipidomic analysis performed in the postmortem brain of patients with AD showed heterogeneous changes in lipid metabolism in AD-affected patients [47]. As expected, the cerebellum lipid profile was largely unaffected whereas significant lipid changes were

observed in the prefrontal and entorhinal cortex of AD brains when compared with age-matched controls. These changes demonstrate that lipid alterations are restricted to AD-affected brain regions (principally the cortex and hippocampus) and that they are not present in unaffected regions like the cerebellum. Interestingly, the prefrontal cortex displays more severe lipid alterations, with a decrease in PE, LPC (lyso-phosphatidylcholine), and sulfatides, together with elevated levels of ceramides (including glucosyl- and galactosyl-ceramides, Cer) and DAG (diacylglycerol). By contrast, in the entorhinal cortex, significant increases are only evident in LBPA (lysobiphosphatidic acid), SM, ganglioside GM3, and cholesterol esters (ChoE). In addition, polyunsaturated PE 40:6, 38:6, and 38:4 species were markedly downregulated in the prefrontal cortex, whereas there was a general decrease in long-chain fatty acids (≥40C) and a corresponding increase in short-chain fatty acids (≤34C) that is compatible with the lower levels of PE carrying DHA in the brain of patients with AD. Unexpectedly, the entorhinal cortex displays more species of the polyunsaturated lipid pools in PC (phosphatidylcholine) and PE. The different lipid alterations between these two brain regions may reflect different aspects or stages of AD pathophysiology, since the entorhinal cortex is known to be affected earlier and more severely than neocortical areas [83].

AD progresses from a pre-symptomatic stage to mild cognitive impairment (MCI), mild AD and to severe AD with a gradual deterioration in cognitive abilities. Unfortunately, the clinical manifestation of the disease is preceded by a long prodromal phase, during which neuropathological lesions arise, including neuron death. For this reason, clinical diagnosis of AD is unreliable, particularly at early disease stages. Hence, there is a strong need to find peripheral biomarkers to reliably diagnose AD early, thereby enabling early treatment and better therapeutic efficacy. Most approaches to fluid-based biomarker discovery have focused on Aβ42, total tau and phosphorylated tau in cerebrospinal fluid (CSF). Although these are useful to distinguish symptomatic patients from normal controls or other dementias, these CSF biomarkers lack predictive value in preclinical patients, and they are only useful to confirm the clinical diagnosis [84]. Thus, given the brain lipid alterations in AD, lipidomic analysis of lipid derivatives in biological fluids may represent a reliable way to identify non-invasive biomarkers for early AD diagnosis [85].

Of the lipid changes reported in the CSF, plasma, and serum of patients with AD, many do not necessarily correlate with those described previously in the CNS [6]. For instance, free cholesterol and ChoE were reported to be downregulated in the CSF although they are increased in the brain of patients with AD [86] (see Section 3.1.). However, six different long-chain ChoE species in plasma allowed patients with AD to be accurately discriminated from healthy controls (ChoE 32:0, 34:0, 34:6, 32:4, 33:6, and 40:4). These metabolites accumulated more strongly in healthy controls than in MCI, and in MCI than in AD, such that they were proposed as potential biomarkers for early AD diagnosis [87]. Total PC levels and specific PC species have also been proposed as reliable biomarkers, with diminished PC levels in the CSF of patients with AD accompanied by lowered LPC and increased PC hydrolytic products such as glycerophosphocholine and phosphocholine, suggesting that PC breakdown might be enhanced in AD pathogenesis [88]. Notably, a set of 10 PC metabolites was specifically depleted in the plasma of healthy individuals who later suffered phenoconversion towards MCI/AD.

These subjects were diagnosed as AD during a 5-year follow-up even though they displayed no cognitive impairment at entry. The PC species identified were diacyl PC 36:6, 38:0, 38:6, 40:1, 40:2, 40:6, PC acyl-alkyl 40:6, and LPC 18:2, as well as the acylcarnitines (ACs) propionyl AC (C3), and C16:1-OH [89]. It is noteworthy that control subjects (not previously diagnosed with AD) did not display any of these modifications, while already diagnosed patients with AD also showed decreased levels of these PC species. Moreover, downregulation of this panel of lipids predicted phenoconversion from healthy to MCI/AD within a 2–3 year time frame with 90% accuracy [89]. These data were supported by independent studies showing decreased levels of PC 38:4, 38:6, and 40:6 in the plasma or serum of AD subjects [86, 90]. In addition, a variety of peripheral lipid changes were also reported that might potentially be useful for early AD diagnosis, such as lower levels of SM and increased levels of Cers in the plasma or serum of patients with AD. In particular, there were significantly fewer SM species containing long chains (e.g., 22 and 24 carbon atom acyl chains) in AD subjects [86, 91]. In parallel, increased Cer levels were reported in the plasma of patients with AD [91, 92]. SM can be metabolized into Cers, second messengers that regulate cellular differentiation, proliferation and apoptosis. Upregulated levels of Cers were concomitant with significant reductions in SM in the plasma of patients with AD. A correlation between the decrease in SM and the increase in Cers was particularly robust in the ratios of SM and Cer species with identical fatty acyl chains. Cer alterations were particularly evident in mild-to-moderate stages of AD [91]. Moreover, it is noteworthy that upregulated Cer levels were significantly correlated with the onset of memory impairment, supporting the role of Cers as potential AD biomarkers [92].

In conclusion, a wide range of peripheral fluid changes have been described that could be used as biomarkers for early AD diagnosis. However, many of the clinical studies involved are cross-sectional in nature and some of them do not reveal reliable biomarkers to test disease progression. Nevertheless, longitudinal studies with several years of follow-up do identify promising biomarkers for early AD diagnosis that reliably predict cognitive impairment and the onset of AD.

4. Prevention and treatment of Alzheimer's disease

The main risk factors for dementia are age and genetics (see more information about AD risk factors at http://www.alz.org/alzheimers_disease_causes_risk_factors.asp), although other risk factors may also influence the onset of dementia. For instance, since the brain is nourished by a rich network of blood vessels, cardiovascular alterations are considered a risk factor for neurological disorders. In fact, vascular dementia is linked to morphological changes to blood vessels which are in turn present in other types of dementia like AD. Indeed, a healthy cardiovascular system is frequently linked to brain protection [93]. In this context, the control of blood cholesterol levels, blood pressure, and body weight is recommended to maintain good brain health. In fact, high-fat diets and sedentary lifestyles are becoming major concerns in terms of their contribution to the high incidence of dementia in Western society, whereas regular physical exercise and heart-healthy diets are also good habits to lower the risk of dementia [35].

Only two types of drugs are currently available to treat Alzheimer's disease: acetylcholinesterase inhibitors (often shortened to just "cholinesterase inhibitors") and NMDA receptor antagonists. Cholinesterase inhibitors (donepezil, rivastigmine, and galantamine) bind to and reversibly inactivate cholinesterases, inhibiting acetylcholine hydrolysis. Such inhibition results in increased acetylcholine concentrations at cholinergic synapses and indeed, AD involves a substantial loss of cholinergic neurons in the neocortex and hippocampus, which in turn contributes to the AD symptomatology and to memory impairment in particular. Therefore, increased levels of acetylcholine are thought to protect against the death of cholinergic neurons, alleviating AD symptoms [94]. Memantine is a low-affinity voltage-dependent antagonist of glutamatergic NMDA receptors. By binding to the NMDA receptor, memantine inhibits the sustained influx of Ca^{2+} ions from the extracellular milieu, thereby preventing neuronal death by excitotoxicity. Such a pathogenic mechanism can be mediated by the Aβ oligomers that bind to NMDA receptor as agonists, favoring Ca^{2+} influx and neuronal excitotoxicity [95]. Interestingly, memantine preserves physiological receptor activity, such that released glutamate can still mediate receptor activation leading to neuronal depolarization in postsynaptic neurons [96]. However, neither cholinesterase inhibitors nor NMDA antagonists have disease-modifying effects in AD and they are generally viewed as palliative treatments with marginal to minimal clinical efficacy, either alone or in combination. Therefore, only a small percentage of patients with AD respond to these treatments and these responders normally undergo a short period of cognitive stabilization after which they again suffer from the cognitive decline associated to largescale neuronal degeneration [97, 98]. This scenario highlights the unmet clinical need for the treatment of AD and related conditions.

Developing disease-modifying drugs (DMDs) capable of preventing neuron degeneration and thereby counteracting AD progression is one of the most pressing challenges of modern pharmacology. Since the pathological process of AD begins many years before its clinical diagnosis, the optimal time for a disease-modifying therapy may be during the prodromal stage of AD. Therefore, clinical diagnosis of AD must be achieved when patients show no relevant clinical signs. Indeed, the development of DMDs will require the concomitant incorporation of reliable biomarkers to identify early stages of AD (see Section 3.3). Hitherto, no DMDs are available for AD and although several have been tested up to phase 3, none has yet achieved marketing approval. The recurrent failures in clinical trials raise a number of questions about our understanding of AD pathophysiology. In this sense, the amyloid cascade hypothesis has not only influenced the study of AD pathophysiology over the past 2 decades but also, the choice of drug targets (see Section 2). Therefore, most clinical trials have set out to prevent Aβ accumulation, either by inhibiting its production/aggregation or enhancing its clearance, as well as reducing tau phosphorylation [99, 100]. However, it remains unclear if these two hallmarks of AD are a cause or consequence of the disease. In fact, they could lie downstream of previous molecular/cellular alterations, as a result of the disease pathology (damage response proteins) and/or as products of an endogenous protective response to disease-induced damage. Nonetheless, over the past 20 years the main focus of biomedical research and the associated drug discovery programs for AD have targeted brain amyloid or tau hyperphosphorylation, and the associated formation of neurofibrillary tangles [18].

Mutations in the BACE-1 gene have not been related to AD but elevated levels of this enzyme have consistently been found in both the brain and CSF of patients with AD [101–103]. Since β-secretase activity is pathologically elevated in AD, BACE1 inhibition has been addressed as a potential therapeutic approach to combat AD. In fact, both genetic deletion of BACE-1 and administration of a BACE-1 inhibitor rescued cognitive deficits and lowered brain Aβ production in AD mouse models. Interestingly, although BACE-1 has other substrates, its inhibition was apparently free of side effects in AD mice [104, 105]. The latest generation of small molecule BACE-1 inhibitors has achieved satisfactory brain penetration and a robust reduction in cerebral Aβ in preclinical animal models. Furthermore, administration of most of these inhibitors in humans also reduced Aβ and sAPPβ levels, whereas sAPPα (the α-secretase cleavage product) was enhanced in the CSF. This observation is consistent with BACE-1 inhibition since β- and α-secretase compete for APP processing (see **Figure 1**). Many of these BACE-1 inhibitors are still in phase-1 clinical trials where safety and tolerability are tested but some of them are currently in phase 2/3, although no clinical efficacy data are as yet available (**Table 1**). Interestingly, one such drug (LY2886721 from Eli Lilly Company) was discontinued in a phase-2 trial because a number of subjects developed hepatic toxicity, although they were not associated with the mechanism of action of BACE1 [106].

Drug	Synonyms	Company	Mechanism of action	Result of study	Clinical trial ID*	Observations
LY2886721	–	Eli Lilly & Co.	β-Secretase inhibitor	Discontinued in phase 2	NCT01561430	Altered liver biochemistry
AZD3293	LY3314814	Astra Zeneca/ Eli Lilly & Co.	β-Secretase inhibitor	Ongoing in phase 2/3	NCT02245737	–
Verubecestat	MK-8931 MK-8931-009	Merck	β-Secretase inhibitor	Ongoing in phase 2/3	NCT01739348 NCT01953601	–
E2609	–	Eisai/Biogen Idec	β-secretase inhibitor	Ongoing in phase 2	NCT02322021	–
Semagacestat	LY450139	Eli Lilly & Co.	γ-secretase inhibitor	Discontinued in phase 3	NCT01035138 NCT00762411 NCT00594568	Lack of clinical improvement Increased risk of skin cancer and infections.
Avagacestat	BMS-708163	Bristol-Myers Squibb	Notch-sparing γ-secretase inhibitor	Discontinued in phase 3	NCT00890890	Lack of clinical improvement Increased rate of skin cancers
Begacestat	GSI-953	Pfizer	Notch-sparing γ-secretase inhibitor	Phase-1 trial completed	NCT00547560	–

Drug	Synonyms	Company	Mechanism of action	Result of study	Clinical trial ID*	Observations
Tarenflurbil	R-flurbiprofe MPC-7869	Myriad Genetics & Laboratories	γ-Secretase modulator	Discontinued in phase 3	NCT00105547 NCT00380276 NCT00322036	Lack of clinical improvement Low potency and poor brain penetration
Tramiprosate	NC-531 Homotaurine 3APS	Neurochem, Inc	Aβ aggregation inhibitor	Discontinued in phase 3	NCT00314912 NCT00088673 NCT00217763	Lack of clinical improvement
Scyllo-inositol	AZD-103 ELND005	Elan Corporation, Speranza Therapeutics, Transition Therapeutics, Inc.	Aβ aggregation inhibitor	Discontinued in phase 2	NCT00568776 NCT00934050	Lack of clinical improvement
Rosiglitazone	Avandia	GlaxoSmithKline	Anti-diabetic drug Aβ clearance enhancer	Discontinued in phase 3	NCT00428090 NCT00550420	Lack of clinical improvement
AN-1792	AIP 001	Janssen Pfizer	Aβ-targeted active immunotherapy	Discontinued in phase 2	NCT00021723	Brain inflammation Aseptic meningoencephalitis
Bapineuzumab	AAB-001	Janssen Pfizer	Aβ-targeted passive immunotherapy	Discontinued in phase 3	NCT00676143 NCT00667810 NCT00998764 NCT00996918	Lack of clinical improvement
Solanezumab	LY2062430	Eli Lilly & Co.	Aβ-targeted passive immunotherapy	Ongoing in phase 3	NCT00905372 NCT00904683 NCT01127633 NCT01900665	–
Gantenerumab	RO4909832 RG1450	Chugai Pharmaceutical Co. Ltd. Hoffmann-La Roche	Aβ-targeted passive immunotherapy	Ongoing in phase 3	NCT01224106 NCT02051608	–
Aducanumab	BIIB037	Biogen	Aβ-targeted passive immunotherapy	Ongoing in phase 3	NCT02477800 NCT02484547	–
Ponezumab	PF-04360365	Pfizer	Aβ-targeted passive immunotherapy	Discontinued in phase 2	NCT00722046 NCT00945672	Lack of clinical improvement

Drug	Synonyms	Company	Mechanism of action	Result of study	Clinical trial ID*	Observations
Valproate	Depakote, Depakene	Abbott Laboratories	Tau phosphorylation inhibitor	Discontinued in phase 3	NCT00071721	Lack of clinical improvement Brain volume loss
Lithium **	Lithium carbonate	Public institutions	Tau phosphorylation inhibitor	Ongoing in phase 2	ISRCTN72046462 (see at isrctn.com) NCT01055392 NCT02129348 NCT00088387	Discrepant results reported Apparently effective in early AD (amnestic MCI) but not in mild-to-moderate AD
Epothilone D	BMS-241027	Bristol-Myers Squibb	Microtubule stabilizer	Discontinued in phase 1	NCT01492374	No reasons reported regarding discontinuation in phase 1
TPI 287	–	Cortice Biosciences	Microtubule stabilizer	Ongoing in phase 1	NCT01966666	
Methylthioninium (MT)	Methylene Blue Rember TM TRx-0014	TauRx Therapeutics Ltd	Tau aggregation inhibitor	Discontinued in phase 2	NCT00684944 NCT00515333	Discrepant results reported Blinding of phase-2 trial has been questioned
LMT-X	Methylene Blue TRx-0237	TauRx Therapeutics Ltd	Tau aggregation inhibitor	Phase 3 completed	NCT01689233 NCT01689246 NCT01626378	No results available as yet
ACI-35	–	AC Immune SA Janssen	Tau-targeted active immunotherapy	Phase 1 completed	ISRCTN13033912 (see at isrctn.com)	–
AADvac1	Axon peptide 108 conjugated to KLH	Axon Neuroscience SE	Tau-targeted active immunotherapy	Ongoing phase 1	NCT02031198	–
RG7345	RO6926496	Roche	Tau-targeted passive immunotherapy	Discontinued in phase 1	NCT02281786	No reasons reported regarding discontinuation in phase 1

Some data in this table are available at http://www.alzforum.org/therapeutics/.
*Clinical trial IDs were obtained from Clinicaltrials.gov unless specified.
**Information regarding the clinical use of lithium was obtained from [121, 122] and Clinicaltrials.gov.

Table 1. Developed disease-modifying drugs for AD treatment in clinical trials.

Clinical mutations in PS1 are supposed to induce a loss of γ-secretase function that in turn prevents Aβ generation and increases the Aβ 42/40 ratio (an increase in the longer vs. shorter Aβ isoforms) [31]. Such loss of function is then translated into increased neuronal Aβ production, which is further potentiated with the ageing in AD mice harboring FAD mutations [23, 58]. This pathological mechanism is associated with accumulation of autophagic vesicles in axonal dystrophies surrounding amyloid plaques, which are principally formed by long hydrophobic isoforms of Aβ like Aβ42. Therefore, γ-secretase inhibition or modulation has also been studied as a plausible therapeutic approach against AD, although non-specific effects hinder the development of γ-secretase inhibitors (GSI) as DMDs given that γ-secretase also cleaves several type-I transmembrane proteins such as the Notch receptor, N-cadherin, ErbB4, and p75NTR (see Section 2).

Semagacestat was the first GSI to undergo clinical trials, and it reduced Aβ concentrations in the mouse CNS and human plasma [107, 108]. Two large phase-3 trials with semagacestat were prematurely interrupted due to serious adverse events, including hematological alterations, and an increased risk of skin cancer and infections that were attributed to inhibition of the Notch signaling pathway. Furthermore, a worsening of cognition was observed in AD-treated patients [109]. Notch-sparing GSIs (second generation inhibitors) and modulators (agents that shift γ-secretase cleavage from longer to shorter Aβ species without affecting Notch cleavage) were then designed for clinical development. Avagacestat and begacestat were first conceived as notch-sparing GSIs that supposedly display greater selectivity for APP than for Notch cleavage [10], although this was recently reported not to be the case [31]. Therefore, these drugs are also likely to fail and indeed, the poor clinical efficacy of Avagacestat was coupled to an increased rate of skin cancers, again suggesting side effects attributable to Notch signaling inhibition (see **Table 1**). Finally, some non-steroidal anti-inflammatory drugs (NSAIDs) modulate γ-secretase (GSMs), decreasing the abundance of Aβ42 while increasing that of Aβ38. Tarenflurbil (the R-enantiomer of flurbiprofen) was tested in a phase-3 trial but it did not slow cognitive decline in patients, while it did increase the frequency of dizziness, anemia, and infection. This failure of tarenflurbil was attributed to its low potency and poor brain penetration [10, 99].

Aggregation of Aβ monomers into higher molecular weight oligomers is thought to be a key neurotoxic event leading to neurodegeneration in the amyloid pathology [7]. For this reason, some DMDs also target this conversion to fight AD. Tramiprosate and scyllo-inositol are two compounds that prevent the transition from Aβ monomers to oligomers, thus favoring Aβ clearance from the brain by insulin-degrading enzyme (IDE) and neprilysin [110]. In addition, scyllo-inositol can also directly bind to Aβ oligomers, promoting their dissociation. Both these drugs have been involved in phase-2 clinical trials and both reduced Aβ42 levels in the CSF of treated patients. In a larger phase-3 study, tramiprosate failed to induce clinical improvement, and thus, further clinical evaluation is still necessary. Scyllo-inositol, also failed to produce significant clinical improvement in a phase-2 trial. Rosiglitazone is an anti-diabetic drug that improves spatial learning and memory abilities, and it mildly decreases Aβ42 brain levels by activating PPARγ and upregulating IDE in AD mice [111]. This drug was involved

in phase-2 and phase-3 clinical trials, although the inconclusive results in phase 2 were followed by a lack of clinical efficacy in a larger phase-3 study [99, 112].

Another therapeutic approach to promote Aβ clearance was based on immunization toward Aβ. Active immunization by vaccination stimulates the immune response to promote antibody formation against pathogenic forms of Aβ, such as Aβ42. Active Aβ immunotherapy has been studied since 1999 when the generation of Aβ antibodies was shown to produce clearance of cerebral Aβ by phagocytic microglia in animal models [113]. Unfortunately, this revolutionary approach soon suffered its first setback in a phase-2 trial to test active immunization using full length human Aβ42 peptide, with some patients developing brain inflammation with aseptic meningoencephalitis and provoking the termination of the clinical study [99]. Passive immunotherapy is an alternative strategy and recent approaches were based on shorter Aβ immunogens, such as the humanized monoclonal antibody to Aβ1–5, bapineuzumab, which binds to both soluble and fibrillar forms of Aβ. Despite the evidence of adverse effects in phase-1 trials, bapineuzumab advanced to phases 2 and 3 where it failed to demonstrate clinical efficacy in patients with AD. Another antibody against Aβ is Solanezumab, a humanized monoclonal antibody against Aβ16–24 that preferentially binds to soluble Aβ. In phase-2 trials, solanezumab was found to be safe while increasing plasma and CSF levels of Aβ40 and Aβ42, an indication of decreased plaque load in the brain. However, solanezumab had no effect on behavioral outcomes. Despite the lack of efficacy in phase 2, the antibody advanced to phase-3 trials in patients with mild-to-moderate AD where the primary endpoints, both cognitive and functional, were not achieved [18]. Many other humanized antibodies have been developed, directed at different regions of the Aβ peptide, some entering phase-3 trials (Gantenerumab and Aducanumab) and others having been discontinued (Ponezumab; **Table 1**).

According to the amyloid cascade hypothesis, Aβ accumulation precedes and drives tau hyperphosphorylation via the activation of different kinases, including cyclin dependent kinase 5 (CDK5) and glycogen synthase kinase 3β (GSK3β) [14, 114]. Tau hyperphosphorylation is thought to destabilizes neuronal microtubules, impairing axonal transport and leading to neurite pathology, finally resulting in deficient synaptic function and neuronal death [115, 116] (see Section 2). In this context, DMDs were developed to inhibit tau phosphorylation, as well as compounds that prevent tau aggregation. GSK3β is the main enzyme involved in tau hyperphosphorylation, and lithium and valproate are both drugs that inhibit GSK3β and reduce tau phosphorylation in animal models [117]. Unexpectedly, valproate impaired the cognitive and functional status, and it was also associated with a reduced brain volume in patients with AD receiving the drug in clinical trials [118]. Lithium is neuroprotective in animal models of AD, not only via the inhibition of GSK-3β but also through the remodeling of Aβ plaques, leading to a decrease in the number of dystrophic axons, reduced neuronal degeneration and improved cognitive scores in AD mice [119, 120]. However, no conclusions have been reached regarding the clinical efficacy of lithium for AD treatment. Some clinical trials failed to demonstrate a protective effect of lithium on cognitive performance, although a more recent clinical study showed that lithium reduced cognitive decline patients with early AD (amnesic MCI) [121, 122]. Tau hyperphosphorylation compromises its ability to bind to microtubules in AD, provoking microtubule instability. In this sense, epothilone D and TPI

287, synthetic paclitaxel-derived microtubule-stabilizing drugs with good BBB permeability, were assessed in phase-1 trials of safety and tolerability. Unfortunately, epothilone D was recently discontinued (see **Table 1**). Tau hyperphosphorylation also provokes tau aggregation which is also considered a key neurotoxic event in AD [123]. LMT-X is a new version of methylene blue, a compound that was tested and discontinued in a phase-2 trial to treat AD. LMT-X is an inhibitor of tau aggregation that specifically disrupts tau-tau interactions in the microtubule binding region. In a phase-2 trial, this new drug slowed down the cognitive decline in a subgroup of patients, and it is now being tested in phase-3 trials, although information about clinical efficacy is not yet available [124, 125]. Finally, two tau-derived peptide vaccines that stimulate active immunization entered phase I studies: AADvac1 and ACI-35. AADvac1 is a synthetic peptide corresponding to a naturally occurring, truncated and misfolded form of tau. ACI-35 is a liposomal vaccine containing a synthetic peptide corresponding to human protein tau sequence 393–408 (numbering according to the tau 2N4R isoform), with phosphorylated S396 and S404 residues. Vaccination with these peptides improves neurobehavioral deficits in AD rodents while ACI-35 is characterized by a rapid and robust polyclonal antibody response specific to phosphorylated tau in WT and AD mice [125]. In addition, passive immunization has also been investigated using a humanized monoclonal antibody targeting pS422 phospho-tau. In AD mice, chronic administration of this antibody reduced hyperphosphorylated tau accumulation [126], although clinical studies with this antibody were recently discontinued in phase 1 (see **Table 1**).

The aforementioned therapeutic approaches summarize the attempts to develop DMDs based on the amyloid cascade hypothesis, principally focused on Aβ and hyperphosphorylated tau protein. With several anti-amyloid drugs now having failed in late stage clinical trials, many critical voices in the scientific community have questioned the validity of the amyloid hypothesis to explain the pathophysiology of AD and as platform on which to develop DMDs for AD therapy. Moreover, the incidence of serious side effects observed in human trials is another drawback to the clinical development of these types of drugs, particularly when many of these adverse effects are associated with the mechanism of action of the compounds tested. However, the amyloid hypothesis cannot be disregarded due the lack of reliable biomarkers to detect efficacy at early stages, and because many of the compounds in clinical trials cross the BBB poorly or cause side effects that forced trials to be discontinued before efficacy could be evaluated [18, 127].

5. The role of brain lipids in preventing and treating Alzheimer's disease

5.1. Therapeutic approaches based on inhibitors of cholesterol biosynthesis

Over the last 2 decades, the relationship between cholesterol levels and the risk of developing AD has become more evident, in turn encouraging the use of statins to treat or prevent AD (see Section 3.1.). Statins are a group of drugs used to treat hypercholesterolemia as they inhibit HMG-CoA reductase, the principal enzyme involved in cholesterol synthesis. In animal models of AD, simvastatin administration to guinea pigs decreased brain and CSF Aβ levels,

an effect that is reversed by discontinuing the treatment [128]. By contrast, simvastatin failed to modify brain levels of Aβ in other studies but it improved the cognitive capacity of transgenic AD mice [129]. Thus, it appears that simvastatin can possibly prevent cognitive decline in AD mice without affecting amyloidogenic APP processing, in turn suggesting that the amyloid pathology may be a consequence more than the primary causal agent of AD, possibly due to changes in membrane lipids. In another study, lovastatin and pravastatin reduced the amount of Aβ in the brains of AD mice, while simultaneously increasing the levels of sAPPα [130]. Therefore, the results of preclinical research into these drugs are encouraging, although the outcome of human studies has been inconsistent, in part due to the differences in study design and data analysis [131].

While several observational studies in human subjects support the hypothesis that statins may prevent AD development, other studies argue against such effects [132]. Nevertheless, some clinical trials are investigating the use of statins in AD, such as simvastatin or atorvastatin. The first trial to analyze the effect of simvastatin on cognitive scores and APP processing was completed in 2003. This clinical study was performed over 12 weeks on patients with AD, and it reported changes in APP metabolites in the CSF: sAPPα and sAPPβ levels were significantly reduced but not those of Aβ or tau. Remarkably, a significant cognitive improvement in response to simvastatin treatment was found in patients with AD [133]. Unexpectedly, subsequent results based on a 12 month treatment failed to show such cognitive improvements in the same patients, even though cholesterol metabolism was altered in the brain [134]. Unfortunately, a later larger trial performed on 406 mild-to-moderate AD patients also failed to identify clinical benefits of simvastatin (the multicenter CLASP trial). This CLASP trial (clinicaltrials.gov ID: #NCT00053599) evaluated the safety and efficacy of an 18 month treatment with simvastatin to prevent AD progression. Once again, simvastatin treatment lowered lipid levels but it did not slow the progressive AD-related decline in cognitive performance [135]. Despite the apparent lack of clinical improvement on cognition in patients with AD, the University of Wisconsin (Madison, USA) evaluated simvastatin in cognitively normal people at risk of developing FAD. This study (ESPRIT study: clinicaltrials.gov ID: #NCT00486044), compared the changes in CSF Aβ and cognitive scores following simvastatin or placebo administration, as well as markers of cholesterol metabolism and inflammation. Again, no specific effect of simvastatin was observed on CSF Aβ or tau levels but a improvement in terms of cognitive performance was reported [136]. As a result, a follow-up study attempted to evaluate similar outcome measures after a longer course of simvastatin (the SHARP study; clinicaltrials.gov ID: #NCT00939822). Additional clinical trials with a more precise methodological design are also being developed to define the clinical efficacy of simvastatin. For instance, the SIMaMCI study (clinicaltrials.gov ID: #NCT00842920) on 445 subjects assesses the time until participants suffer phenoconversion to dementia, with conversion being defined as an increase in the Clinical Dementia Rating (CDR) score above 0.5. The trial also focuses on the change in cognitive scores from a healthy state to MCI and dementia.

Other clinical studies have assessed atorvastatin, lovastatin, and pravastatin in AD. The only clinical trial showing cognitive improvement associated with atorvastatin administration was

a phase-2 pilot study comparing a 1-year course of atorvastatin to a placebo in patients with mild-to-moderate-AD who were also taking a cholinesterase inhibitor and vitamin E (clinicaltrials.gov: #NCT00024531). This study reported trends towards benefits on cognition and function [137, 138], leading to a larger phase-3 randomized trial involving 640 patients to confirm the potential clinical benefits of atorvastatin in patients with mild-to-moderate AD also treated with donepezil (the LEADe study; clinicaltrial.gov ID: #NCT00151502). Unfortunately, no clinical benefit was observed after 18 months of treatment [139, 140], and this was considered the definitive trial on atorvastatin regarding symptomatic AD treatment. It is worth noting that APP metabolites were not assessed in these studies and that decreased circulating cholesterol, as well as improved neurovascular response and cerebral blood flow were found in atorvastatin-treated patients with AD (clinicaltrials.gov: #NCT00751907) [141]. Lovastatin has been less frequently studied in randomized AD trials, and it was shown to be efficient in reducing serum Aβ levels in patients AD, although no cognitive evaluations were performed (clinicaltrial.gov: #NCT00046358) [142]. In the case of pravastatin, APP processing was not analyzed and the cognitive evaluation of treated patients revealed no significant improvement relative to the placebo group (clinicaltrial.gov: # NCT00303277) [143].

The substantial variability in outcome from these human studies makes it difficult to ascertain whether statins might have a beneficial role in preventing or treating AD. One possible reason to explain such inconsistency relates to the ability of statins to cross the BBB and enter the brain. In this respect, the chemical structure of statins can vary greatly, which justifies why some of them cross the BBB better than others. Accordingly, simvastatin and lovastatin appear to cross the BBB via passive diffusion, whereas pravastatin depends on an active transport system. Although this could justify the lack of clinical effect of pravastatin in clinical trials, it is also true that pravastatin reduced Aβ load in AD mice, suggesting that pravastatin does reach the brain and exert its pharmacological effects [130, 144]. In this sense, clinical studies have investigated different statins with substantial variation in BBB permeability, making it difficult to reconcile the conflicting findings in the literature.

Another confounding factor would be the AD patient's ApoE genotype which may affect the effectiveness of statins in AD prevention and treatment. In fact, individuals with the ApoE4 allele may experience less benefit from statin treatment in terms of cholesterol levels than others with the E2 or E3 alleles [145]. Therefore, although some trials in humans have taken the ApoE genotype into account, not all do. In addition, statins have a number of pleitropic effects on physiology and metabolism besides lowering cholesterol levels. For instance, statins can alter the expression of genes related to cell growth, signaling, trafficking, and apoptosis, which in turn can potentially affect the results of trials. In this sense, inhibition of HMG-CoA reductase activity can lead to decreased isoprenylation of proteins which in turn may cause a variety of downstream effects [146]. Thus, low isoprenoid levels may inhibit the secretory APP pathway leading to intracellular accumulation of APP metabolites that bias their analysis in the CSF or plasma [147].

In summary, cholesterol-lowering drugs such as statins have potential therapeutic effects for the treatment of AD. Based on preclinical studies in animal models and clinical trials in humans, statins represent a valuable group of compounds with promising therapeutic effects

in AD. However, individual statins show different outcomes in terms of APP metabolism and cognitive improvement. In part, these disparities may be explained by the variability in BBB permeability and the different biochemical effects of these drugs observed to date.

5.2. Therapeutic approaches based on PUFAs

Neuroprotective effects of long-chain ω-3 PUFAs (see Section 3.2.) encouraged a number of clinical trials to assess the effects of ω-3 fatty acid administration to patients with AD over a defined time period, particularly focusing on the cognitive benefits of DHA and EPA. Interestingly, decreases in plasma DHA are associated with cognitive decline in healthy elderly adults and DHA administration to these patients improved the physiological memory loss and cognitive decline that frequently appears in the elderly [148] (clinicaltrials.gov ID: #NCT0027813). However, DHA administration to patients with AD did not significantly improve cognitive scores [80] (clinicaltrials.gov ID: #NCT00440050). Another randomized study involving administration of a commercially available fish oil as source of DHA and EPA only improved cognition in a small subgroup of patients with very mild cognitive dysfunction, with no clear beneficial effects in most patients [149] (clinicaltrials.gov ID: #NCT00211159). Finally, the most recent trial was carried out on a small group of patients with mild-to-moderate AD who were administered fish oil containing DHA and EPA. In this pilot study, significant recovery of cognitive capacity was evident in the patients treated with fish oil (with or without lipoic acid supplementation) [150] (clinicaltrials.gov ID: #NCT00090402). Together, these studies indicate that DHA supplementation may represent a plausible therapeutic approach for the treatment of the physiological age-related cognitive decline, although it is unclear what type of ω-3 PUFAs could be used to treat AD. Some of these discrepancies in the different randomized studies may reflect the source of the ω-3 PUFAs administered to the patients. As yet there is no consensus with regards the defined sources of ω-3 PUFAs or a standard ratio or dose of DHA and EPA: Quinn et al. [80] evaluated 2 g/day DHA, Freund-Levi et al. [149] evaluated the effect of fish oil administration with a DHA and EPA content of 1.7 and 0.6 g/day, respectively (EPAX 1500 TG; Pronova Biocare, Norway), and Shinto et al. [150] evaluated a fish oil daily dose containing 675 mg DHA and 975 mg EPA, the latter trial being the only efficacious treatment against AD in humans and having a different DHA:EPA ratio with respect to the former.

It is likely that differences in the source of ω-3 PUFAs together with variable DHA:EPA ratios might explain the variation in the results observed when treating AD patients with long-chain ω-3 PUFAs. Moreover, the presence of mercury in some fish oil supplements may provoke some neurological problems that could counteract the beneficial effects of DHA and related compounds. In this context, ω-3 PUFAs also exert their physiological function through the production of hydroxylated bioactive derivatives, such as NPD1 (see Section 3.2.). In fact, it has been demonstrated that NPD1 levels are dramatically reduced in the AD brain, even more so than DHA [68]. These data suggest that abnormally low levels of DHA in AD would be accompanied by impaired conversion of this fatty acid into NPD1 and other RVs. In fact, reduced levels of 15-LOX, the key enzyme involved in the generation of the D-series RVs and protectins, were observed in the brain of patients with AD, in turn demonstrating that lipid

second messenger generation from DHA is impaired in AD [68]. Assuming that the conversion of DHA into hydroxylated derivatives is needed to mediate DHA-related physiological activity, such 15-LOX modifications could at least partially explain why DHA administration did not improve cognition in patients with AD. In this context, it is noteworthy that some cognitive improvement was observed when fish oil alone was used as the source of ω-3 PUFAs, suggesting that these oils might contain other PUFAs that impart neuroprotection independently of DHA and EPA (hydroxylated PUFAs such as RVs or other PUFA derivatives) [81]. This hypothesis is supported by the high efficacy of HDHA(see below DHALifort) on cognitive score and by the aforementioned epidemiological meta-analysis showing an inverse correlation between AD incidence and fish oil intake but not with DHA/EPA (ω-3 PUFA) intake (see Section 3.2) [78].

DHA-derived NPD1 produces many beneficial effects in animal and cell models of AD [75]. On the one hand, NPD1 suppresses Aβ42 peptide shedding by downregulating BACE-1 activity while enhancing α-secretase activity, thereby upregulating sAPPα levels and shifting the cleavage of APP from the amyloidogenic to the non-amyloidogenic pathway. Thus, NPD1 stimulated secretion of sAPPα strengthens neurotrophic signaling and prevents Aβ oligomer neurotoxicity, which may in turn be accompanied by a number of beneficial effects, such as the prevention of neuronal and axonal injury, improved neuronal plasticity, and enhanced learning memory [151–153]. In addition, like other RVs, NPD1 also displays anti-inflammatory properties. Indeed, NPD1 administration decreases Aβ42-triggered expression of the pro-inflammatory COX-2 and of B-94 (a TNF-α-inducible pro-inflammatory factor), and it prevents apoptosis in cultured cells by upregulating the expression of anti-apoptotic members of the Bcl-2 protein family.

The neuroprotective properties of NPD1 have encouraged the development of new pharmacological approaches based on hydroxylated derivatives of ω-3 PUFAs to treat AD. Regardless of the use of natural RVs and protectins to treat inflammatory and neurodegenerative diseases [154], synthetic ω-3-PUFA bioactive hydroxyl derivatives have also been used to treat such disorders. This kind of therapeutic approach, aimed at modulating brain lipids to treat neurological diseases, is framed within so-called membrane lipid therapy (MLT) [155–157]. In this context, a novel hydroxylated derivative named HDHA (2-hydroxy-docosahexanoic acid) has been proposed as a promising therapeutic approach to treat AD. HDHA (DHALifort; PharmaConcept, Hungary) administration influences the brain lipid composition, increasing the PE species carrying long-chain PUFAs, which are significantly reduced in patients with AD (see Section 3.3.). Upon normalization of the membrane lipid composition by HDHA treatment, the membrane structure recovers the presence of liquid-disordered prone membrane structures [158] (**Figure 4**). These lipid changes are paralleled with a reduction in Aβ accumulation and tau hyperphosphorylation, and recovery of cognitive scores in a transgenic mouse model of AD (5xFAD mice) [159, 160] (see **Figure 4**).

HDHA also enhances the survival of neuron-like cells exposed to different insults, such as oligomeric Aβ and NMDA-mediated neurotoxicity (*in vitro*), and it promotes hippocampal neuronal cell proliferation in 5XFAD mice *in vivo* [159, 160], suggesting that HDHA induced neuroregeneration both *in vivo* and *in vitro*, which in part may explain its efficacy against

neurodegeneration and memory loss. As part of its mechanism of action, HDHA dampens the binding affinity of oligomeric and fibrillar Aβ to lipid-raft membrane domains. Moreover, it enhances the unfolded protein response (UPR) and autophagy in neuron-like cells, which in turn may promote neuronal survival [160, 161]. In this sense, although the molecular role of autophagy in AD is complex and still largely unknown, it is thought that activation of salvage autophagy would avoid the intracellular accumulation of Aβ and its precursors by reducing the neuritic pathology (see **Figure 2**) [162, 163]. Therefore, the pleitropic effects of HDHA have proven beneficial to treat AD, suggesting that its molecular target is an upstream entity such as the membrane lipid bilayer. Thus, the normalization of the PE, DHA, cholesterol, and SM content mediated by HDHA would restore membrane lipid structure, which in turn would regulate amyloidogenic secretase activity tau phosphorylation and neuronal degeneration.
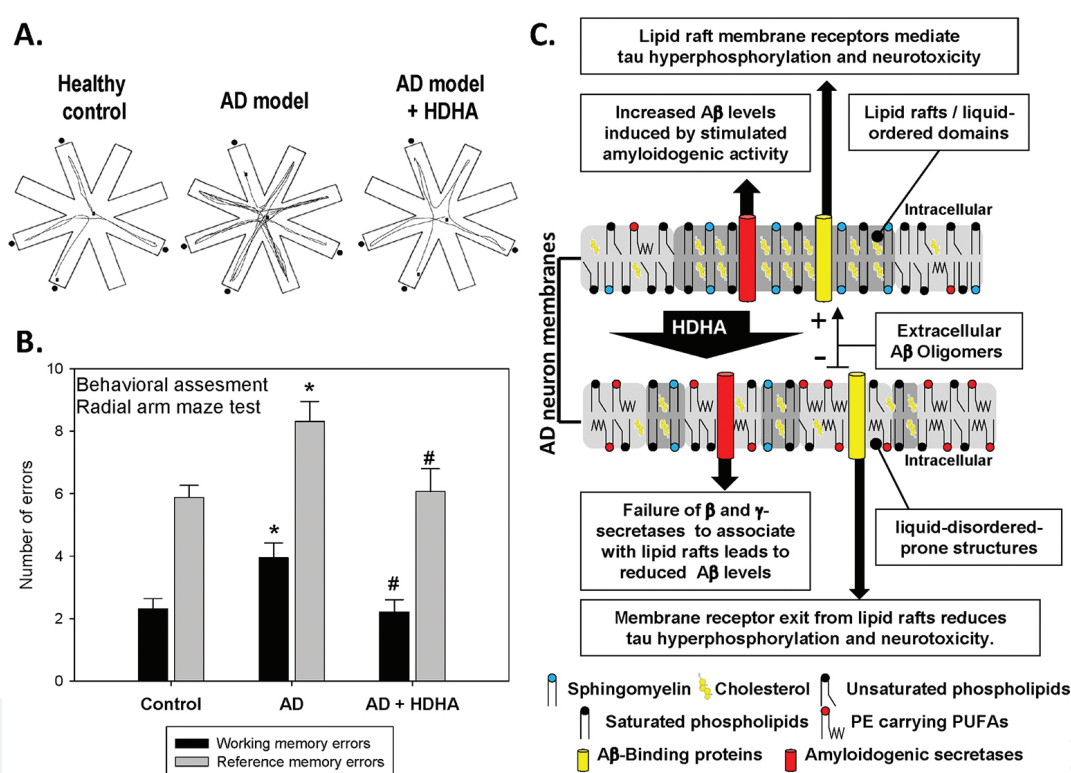
Figure 4. Proof of concept for the use of HDHA in AD mice and the proposed molecular mechanism of action. (A) Diagrams showing representative outlines of control and AD mice (5xFAD mice) that received HDHA or the vehicle alone, in the Radial Arm Maze test (RAM). A black point at the end of one arm represents where the mice find a food pellet. (B) Quantitative analysis of test performance is addressed by quantifying working (reentry of an arm already visited) and reference (entry into an unbaited arm) memory errors. Both parameters increased significantly in AD mice while HDHA treatment prevented such behavioral impairment until cognitive scores were almost totally reverted to those of the controls. Bars represent the mean ± SEM. One-way ANOVA followed by Bonferroni's post hoc test: *$p < 0.05$, difference relative to healthy controls; #: $p < 0.05$ difference relative to the untreated AD group. C) Postulated mechanism of action for HDHA. HDHA enriches brain membranes in PE carrying DHA and other long PUFAs. These lipid changes may influence the structure of the cell membrane by promoting the appearance of liquid-disordered prone structures and potentially preventing AD-related cell signaling by: (i) downregulating APP amyloidogenic processing and Aβ-induced tau protein hyperphosphorylation; and (ii) decreasing neuron vulnerability to extracellular toxic agents such as oligomeric Aβ. Together, this evidence supports a neuroprotective role of HDHA that may be associated with the improved cognitive capabilities observed in AD mice. Adapted from [159, 160].

Interestingly, the cellular heat shock response (HSP) depends on the plasma membrane composition, such that increased membrane fluidity is related to enhanced expression of heat-shock proteins (HSP) [164]. In this context, these proteins (particularly Hsp70, Hsp60, and Hsp27) are involved in the mechanism of action of lithium in compacting Aβ plaques, lowering the density of dystrophic neurites and preventing neuronal degeneration in a mouse model of AD [119]. Therefore, lipid derivatives like HDHA that enhance membrane fluidity might also reduce the neurite pathology and prevent neuronal loss in AD via a mechanism involving Hsp expression. Regardless of amyloid production and the neuritic pathology, inflammation is also a key player in AD. In this sense, another synthetic hydroxyl derivative of ARA, 2-HARA (2-hydroxy-arachidonic acid) is a COX-1 and COX-2 inhibitor [165]. The inhibitory effect over COX-1 has been related to alternative microglia activation, as well as reduced Aβ production and tau hyperphosphorylation in a transgenic model of AD [166]. Thus, 2-HARA may be a promising therapeutic approach to mitigate the inflammatory component of AD, driving microglia activation towards an alternative neuroprotective phenotype, and reducing AD-related amyloid and tau pathologies. To summarize, MLT is a therapeutic concept targeting membrane lipids that could be used to treat neurological disorders such as AD. In this context, recent findings about ω-3 PUFA RV-like mediators, such as HDHA and 2-HARA, offer a wide range of possibilities to design new bioactive compounds to treat neurodegenerative diseases.

6. Concluding remarks

After adipose tissue, the human brain is the organ with the largest amount of lipids in the body. There is compelling evidence that lipid homeostasis is altered in AD, suggesting that the plasma membrane lipid composition and structure plays a critical role in the pathophysiology of AD and hence in its therapy. Therefore, lipid alterations might be responsible for other downstream neuropathological hallmarks of AD, including amyloid and neurite pathologies, as well as inflammation and neuron loss, which eventually causes the cognitive deterioration evident in patients with AD. Accordingly, a number of clinical trials have been set up to investigate how the regulation of cholesterol and PUFA hydroxyl derivatives such as HDHA may constitute promising therapeutic approaches to treat this devastating condition.

7. Review criteria

The PubMed database (NCBI, National Library of Medicine, USA) was searched for relevant, both original and review, articles using the keywords mentioned at the beginning of the present chapter either by separate or with multiple combinations. The papers were selected accordingly to their adhesion to the main subject of the present review and the expert authors' knowledge of the field. In addition, interesting and useful information has been achieved from http://www.alzforum.org/ and http://clinicaltrials.gov/, as well as from books at the Library of the University of the Balearic Islands (Palma de Mallorca, Spain).

Acknowledgements

Conflict of interest

Author details

Manuel Torres[1,2*], Xavier Busquets[1] and Pablo V. Escribá[1*]

*Address all correspondence to: manuel.torres.phd@gmail.com and Pablo.escriba@uib.es

1 Laboratory of Molecular Cell Biomedicine, University of the Balearic Islands, Palma de Mallorca, Spain

2 Lipopharma Therapeutics S.L., Palma de Mallorca, Spain

References

[1]   Castellani RJ, Rolston RK, Smith MA. Alzheimer disease. Dis Mon. 2010;56(9):484–546.

[2]   Annaert W, Cupers P, Saftig P, De Strooper B. Presenilin function in APP processing. Ann N Y Acad Sci. 2000;920:158–64.

[3]   Price DL, Sisodia SS. Mutant genes in familial Alzheimer's disease and transgenic models. Annu Rev Neurosci. 1998;21:479–505.

[4]   Harman D. Alzheimer's disease pathogenesis: role of aging. Ann N Y Acad Sci. 2006;1067:454–60.

[5]   De Strooper B. Proteases and proteolysis in Alzheimer disease: a multifactorial view on the disease process. Physiol Rev. 2010;90(2):465–94.

[6]   Xiang Y, Lam SM, Shui G. What can lipidomics tell us about the pathogenesis of Alzheimer disease? Biol Chem. 2015;396(12):1281–91.

[7]   Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol. 2007;8(2):101–12.

[8]   Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992;256(5054):184–5.

[9]   Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002;297(5580):353–6.

[10] De Strooper B, Vassar R, Golde T. The secretases: enzymes with therapeutic potential in Alzheimer disease. Nat Rev Neurol. 2010;6(2):99–107.

[11] Duyckaerts C, Delatour B, Potier M-C. Classification and basic pathology of Alzheimer disease. Acta Neuropathol. 2009;118(1):5–36.

[12] Pérez-Gracia E, Torrejón-Escribano B, Ferrer I. Dystrophic neurites of senile plaques in Alzheimer's disease are deficient in cytochrome c oxidase. Acta Neuropathol. 2008;116(3):261–8.

[13] Jin M, Shepardson N, Yang T, Chen G, Walsh D, Selkoe DJ. Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphory-lation and neuritic degeneration. Proc Natl Acad Sci U S A. 2011;108(14):5819–24.

[14] Spires-Jones TL, Stoothoff WH, de Calignon A, Jones PB, Hyman BT. Tau pathophysi-ology in neurodegeneration: a tangled issue. Trends Neurosci. 2009;32(3):150–9.

[15] Heneka MT, O'Banion MK. Inflammatory processes in Alzheimer's disease. J Neuro-immunol. 2007;184(1–2):69–91.

[16] Nixon RA. Autophagy, amyloidogenesis and Alzheimer disease. J Cell Sci. 2007;120(Pt 23):4081–91.

[17] Krstic D, Knuesel I. The airbag problem-a potential culprit for bench-to-bedside translational efforts: relevance for Alzheimer's disease. Acta Neuropathol Commun. 2013;1:62.

[18] Mullane K, Williams M. Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond? Biochem Pharmacol. 2013;85(3):289–305.

[19] Wong PC, Cai H, Borchelt DR, Price DL. Genetically engineered mouse models of neurodegenerative diseases. Nat Neurosci. 2002;5(7):633–9.

[20] Yu WH, Cuervo AM, Kumar A, Peterhoff CM, Schmidt SD, Lee J-H, et al. Macroautophagy – a novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease. J Cell Biol. 2005;171(1):87–98.

[21] Zhang X-M, Cai Y, Xiong K, Cai H, Luo X-G, Feng J-C, et al. Beta-secretase-1 elevation in transgenic mouse models of Alzheimer's disease is associated with synaptic/axonal pathology and amyloidogenesis: implications for neuritic plaque development. Eur J Neurosci. 2009;30(12):2271–83.

[22] Doehner J, Genoud C, Imhof C, Krstic D, Knuesel I. Extrusion of misfolded and aggregated proteins--a protective strategy of aging neurons? Eur J Neurosci. 2012;35(12):1938–50.

[23] Sanchez-Varo R, Trujillo-Estrada L, Sanchez-Mejias E, Torres M, Baglietto-Vargas D, Moreno-Gonzalez I, et al. Abnormal accumulation of autophagic vesicles correlates with axonal and synaptic pathology in young Alzheimer's mice hippocampus. Acta Neuropathol. 2012;123(1):53–70.

[24] Mattson MP, Magnus T. Ageing and neuronal vulnerability. Nat Rev Neurosci. 2006;7(4):278–94.

[25] Krstic D, Knuesel I. Deciphering the mechanism underlying late-onset Alzheimer disease. Nat Rev Neurol. 2013;9(1):25–34.

[26] Knowles JK, Rajadas J, Nguyen T-VV, Yang T, LeMieux MC, Vander Griend L, et al. The p75 neurotrophin receptor promotes amyloid-beta(1–42)-induced neuritic dystrophy *in vitro* and *in vivo*. J Neurosci. 2009;29(34):10627–37.

[27] Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 2015;14(4):388–405.

[28] Jimenez S, Baglietto-Vargas D, Caballero C, Moreno-Gonzalez I, Torres M, Sanchez-Varo R, et al. Inflammatory response in the hippocampus of PS1M146L/APP751SL mouse model of Alzheimer's disease: age-dependent switch in the microglial phenotype from alternative to classic. J Neurosci. 2008;28(45):11650–61.

[29] Wakabayashi T, De Strooper B. Presenilins: members of the gamma-secretase quartets, but part-time soloists too. Physiology (Bethesda). 2008;23:194–204.

[30] De Strooper B, Annaert W. Novel research horizons for presenilins and γ-secretases in cell biology and disease. Annu Rev Cell Dev Biol. 2010;26:235–60.

[31] Chávez-Gutiérrez L, Bammens L, Benilova I, Vandersteen A, Benurwar M, Borgers M, et al. The mechanism of γ-Secretase dysfunction in familial Alzheimer disease. EMBO J. 2012;31(10):2261–74.

[32] Bentahir M, Nyabi O, Verhamme J, Tolia A, Horré K, Wiltfang J, et al. Presenilin clinical mutations can affect gamma-secretase activity by different mechanisms. J Neurochem. 2006;96(3):732–42.

[33] Thimiri Govinda Raj DB, Ghesquière B, Tharkeshwar AK, Coen K, Derua R, Vanderschaeghe D, et al. A novel strategy for the comprehensive analysis of the biomolecular composition of isolated plasma membranes. Mol Syst Biol. 2011;7:541.

[34] Tamboli IY, Prager K, Thal DR, Thelen KM, Dewachter I, Pietrzik CU, et al. Loss of gamma-secretase function impairs endocytosis of lipoprotein particles and membrane cholesterol homeostasis. J Neurosci. 2008;28(46):12097–106.

[35] Martins IJ, Hone E, Foster JK, Sünram-Lea SI, Gnjec A, Fuller SJ, et al. Apolipoprotein E, cholesterol metabolism, diabetes, and the convergence of risk factors for Alzheimer's disease and cardiovascular disease. Mol Psychiatry. 2006;11(8):721–36.

[36] Bu G. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. Nat Rev Neurosci. 2009;10(5):333–44.

[37] Martins IJ, Berger T, Sharman MJ, Verdile G, Fuller SJ, Martins RN. Cholesterol metabolism and transport in the pathogenesis of Alzheimer's disease. J Neurochem. 2009;111(6):1275–308.

[38] Poirier J. Apolipoprotein E, cholesterol transport and synthesis in sporadic Alzheimer's disease. Neurobiol Aging. 2005;26(3):355–61.

[39] Rapp A, Gmeiner B, Hüttinger M. Implication of apoE isoforms in cholesterol metabolism by primary rat hippocampal neurons and astrocytes. Biochimie. 2006;88(5):473–83.

[40] Launer LJ, White LR, Petrovitch H, Ross GW, Curb JD. Cholesterol and neuropathologic markers of AD: a population-based autopsy study. Neurology. 2001;57(8):1447–52.

[41] Pappolla MA, Bryant-Thomas TK, Herbert D, Pacheco J, Fabra Garcia M, Manjon M, et al. Mild hypercholesterolemia is an early risk factor for the development of Alzheimer amyloid pathology. Neurology. 2003;61(2):199–205.

[42] Puglielli L, Tanzi RE, Kovacs DM. Alzheimer's disease: the cholesterol connection. Nat Neurosci. 2003;6(4):345–51.

[43] Whitmer RA, Sidney S, Selby J, Johnston SC, Yaffe K. Midlife cardiovascular risk factors and risk of dementia in late life. Neurology. 2005;64(2):277–81.

[44] Vetrivel KS, Thinakaran G. Membrane rafts in Alzheimer's disease beta-amyloid production. Biochim Biophys Acta. 2010;1801(8):860–7.

[45] Ledesma MD, Dotti CG. Amyloid excess in Alzheimer's disease: what is cholesterol to be blamed for? FEBS Lett. 2006;580(23):5525–32.

[46] Bandaru VVR, Troncoso J, Wheeler D, Pletnikova O, Wang J, Conant K, et al. ApoE4 disrupts sterol and sphingolipid metabolism in Alzheimer's but not normal brain. Neurobiol Aging. 2009;30(4):591–9.

[47] Chan RB, Oliveira TG, Cortes EP, Honig LS, Duff KE, Small SA, et al. Comparative lipidomic analysis of mouse and human brain with Alzheimer disease. J Biol Chem. 2012;287(4):2678–88.

[48] Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, et al. Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. Proc Natl Acad Sci U S A. 2004;101(7):2070–5.

[49] Ledesma MD, Abad-Rodriguez J, Galvan C, Biondi E, Navarro P, Delacourte A, et al. Raft disorganization leads to reduced plasmin activity in Alzheimer's disease brains. EMBO Rep. 2003;4(12):1190–6.

[50] Lai AY, McLaurin J. Mechanisms of amyloid-Beta peptide uptake by neurons: the role of lipid rafts and lipid raft-associated proteins. Int J Alzheimers Dis. 2010;2011:548380.

[51] Kalvodova L, Kahya N, Schwille P, Ehehalt R, Verkade P, Drechsel D, et al. Lipids as modulators of proteolytic activity of BACE: involvement of cholesterol, glycosphingolipids, and anionic phospholipids *in vitro*. J Biol Chem. 2005;280(44):36815–23.

[52] Holmes O, Paturi S, Ye W, Wolfe MS, Selkoe DJ. Effects of membrane lipids on the activity and processivity of purified γ-secretase. Biochemistry. 2012;51(17):3565–75.

[53] Askarova S, Yang X, Lee JCM. Impacts of membrane biophysics in Alzheimer's disease: from amyloid precursor protein processing to Aβ peptide-induced membrane changes. Int J Alzheimer's Dis. 2011;2011:134971.

[54] Runz H, Rietdorf J, Tomic I, de Bernard M, Beyreuther K, Pepperkok R, et al. Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. J Neurosci. 2002;22(5):1679–89.

[55] Tamboli IY, Hampel H, Tien NT, Tolksdorf K, Breiden B, Mathews PM, et al. Sphingolipid storage affects autophagic metabolism of the amyloid precursor protein and promotes Abeta generation. J Neurosci. 2011;31(5):1837–49.

[56] Lazarov O, Morfini GA, Pigino G, Gadadhar A, Chen X, Robinson J, et al. Impairments in fast axonal transport and motor neuron deficits in transgenic mice expressing familial Alzheimer's disease-linked mutant presenilin 1. J Neurosci. 2007;27(26):7011–20.

[57] Boland B, Kumar A, Lee S, Platt FM, Wegiel J, Yu WH, et al. Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. J Neurosci. 2008;28(27):6926–37.

[58] Torres M, Jiménez S, Sánchez-Varo R, Navarro V, Trujillo-Estrada L, Sánchez-Mejias E, et al. Defective lysosomal proteolysis and axonal transport are early pathogenic events that worsen with age leading to increased APP metabolism and synaptic Abeta in transgenic APP/PS1 hippocampus. Mol Neurodegener. 2012;7:59. Epub 2012/11/24.

[59] Bhat NR, Thirumangalakudi L. Increased tau phosphorylation and impaired brain insulin/IGF signaling in mice fed a high fat/high cholesterol diet. J Alzheimers Dis. 2013;36(4):781–9.

[60] Rushworth JV, Griffiths HH, Watt NT, Hooper NM. Prion protein-mediated toxicity of amyloid-β oligomers requires lipid rafts and the transmembrane LRP1. J Biol Chem. 2013;288(13):8935–51.

[61] Kuperstein I, Broersen K, Benilova I, Rozenski J, Jonckheere W, Debulpaep M, et al. Neurotoxicity of Alzheimer's disease Aβ peptides is induced by small changes in the Aβ42 to Aβ40 ratio. EMBO J. 2010;29(19):3408–20.

[62] Martins IC, Kuperstein I, Wilkinson H, Maes E, Vanbrabant M, Jonckheere W, et al. Lipids revert inert Abeta amyloid fibrils to neurotoxic protofibrils that affect learning in mice. EMBO J. 2008;27(1):224–33.

[63] Ibarguren M, López DJ, Escribá PV. The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health. Biochim Biophys Acta. 2014;1838(6):1518–28.

[64] Plourde M, Fortier M, Vandal M, Tremblay-Mercier J, Freemantle E, Bégin M, et al. Unresolved issues in the link between docosahexaenoic acid and Alzheimer's disease. Prostaglandins Leukot Essent Fatty Acids. 2007;77(5–6):301–8.

[65] Söderberg M, Edlund C, Kristensson K, Dallner G. Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. Lipids. 1991;26(6):421–5.

[66] Prasad MR, Lovell MA, Yatin M, Dhillon H, Markesbery WR. Regional membrane phospholipid alterations in Alzheimer's disease. Neurochem Res. 1998;23(1):81–8.

[67] Han X, Holtzman DM, McKeel DW. Plasmalogen deficiency in early Alzheimer's disease subjects and in animal models: molecular characterization using electrospray ionization mass spectrometry. J Neurochem. 2001;77(4):1168–80.

[68] Lukiw WJ, Cui J-G, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, et al. A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. J Clin Invest. 2005;115(10):2774–83.

[69] Astarita G, Jung K-M, Berchtold NC, Nguyen VQ, Gillen DL, Head E, et al. Deficient liver biosynthesis of docosahexaenoic acid correlates with cognitive impairment in Alzheimer's disease. PLoS One. 2010;5(9):e12538.

[70] Belkouch M, Hachem M, Elgot A, Van AL, Picq M, Guichardant M, et al. The pleiotropic effects of omega-3 docosahexaenoic acid on the hallmarks of Alzheimer's disease. J Nutr Biochem. 2016;38:1–11.

[71] Su H-M. Mechanisms of n-3 fatty acid-mediated development and maintenance of learning memory performance. J Nutr Biochem. 2010;21(5):364–73.

[72] Martín V, Fabelo N, Santpere G, Puig B, Marín R, Ferrer I, et al. Lipid alterations in lipid rafts from Alzheimer's disease human brain cortex. J Alzheimers Dis. 2010;19(2):489–502.

[73] Shaikh SR. Biophysical and biochemical mechanisms by which dietary N-3 polyunsaturated fatty acids from fish oil disrupt membrane lipid rafts. J Nutr Biochem. 2012;23(2):101–5.

[74] Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014;510(7503):92–101.

[75] Stark DT, Bazan NG. Neuroprotectin D1 induces neuronal survival and downregulation of amyloidogenic processing in Alzheimer's disease cellular models. Mol Neurobiol. 2011;43(2):131–8.

[76] Zhao Y, Calon F, Julien C, Winkler JW, Petasis NA, Lukiw WJ, et al. Docosahexaenoic acid-derived neuroprotectin D1 induces neuronal survival via secretase- and PPARγ-mediated mechanisms in Alzheimer's disease models. PLoS One. 2011;6(1):e15816.

[77] Simopoulos AP. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. Biomed Pharmacother. 2006;60(9):502–7.

[78] Wu S, Ding Y, Wu F, Li R, Hou J, Mao P. Omega-3 fatty acids intake and risks of dementia and Alzheimer's disease: a meta-analysis. Neurosci Biobehav Rev. 2015;48:1–9.

[79] Barberger-Gateau P, Raffaitin C, Letenneur L, Berr C, Tzourio C, Dartigues JF, et al. Dietary patterns and risk of dementia: the Three-City cohort study. Neurology. 2007;69(20):1921–30.

[80] Quinn JF, Raman R, Thomas RG, Yurko-Mauro K, Nelson EB, Van Dyck C, et al. Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. JAMA. 2010;304(17):1903–11.

[81] Hong S, Tjonahen E, Morgan EL, Lu Y, Serhan CN, Rowley AF. Rainbow trout (Oncorhynchus mykiss) brain cells biosynthesize novel docosahexaenoic acid-derived resolvins and protectins—mediator lipidomic analysis. Prostaglandins Other Lipid Mediat. 2005;78(1–4):107–16.

[82] Afshordel S, Hagl S, Werner D, Röhner N, Kögel D, Bazan NG, et al. Omega-3 polyunsaturated fatty acids improve mitochondrial dysfunction in brain aging—impact of Bcl-2 and NPD-1 like metabolites. Prostaglandins Leukot Essent Fatty Acids. 2015;92:23–31.

[83] Gómez-Isla T, Price JL, McKeel DW, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. J Neurosci. 1996;16(14):4491–500.

[84] Ritter A, Cummings J. Fluid biomarkers in clinical trials of Alzheimer's disease therapeutics. Front Neurol. 2015;6:186.

[85] Khan TK, Alkon DL. Peripheral biomarkers of Alzheimer's disease. J Alzheimers Dis. 2015;44(3):729–44.

[86] Orešič M, Hyötyläinen T, Herukka S-K, Sysi-Aho M, Mattila I, Seppänan-Laakso T, et al. Metabolome in progression to Alzheimer's disease. Transl Psychiatry. 2011;1:e57.

[87] Proitsi P, Kim M, Whiley L, Pritchard M, Leung R, Soininen H, et al. Plasma lipidomics analysis finds long chain cholesteryl esters to be associated with Alzheimer's disease. Transl Psychiatry. 2015;5:e494.

[88] Walter A, Korth U, Hilgert M, Hartmann J, Weichel O, Hilgert M, et al. Glycerophosphocholine is elevated in cerebrospinal fluid of Alzheimer patients. Neurobiol Aging. 2004;25(10):1299–303.

[89] Mapstone M, Cheema AK, Fiandaca MS, Zhong X, Mhyre TR, MacArthur LH, et al. Plasma phospholipids identify antecedent memory impairment in older adults. Nat Med. 2014;20(4):415–8.

[90] Olazarán J, Gil-de-Gómez L, Rodríguez-Martín A, Valentí-Soler M, Frades-Payo B, Marín-Muñoz J, et al. A blood-based, 7-metabolite signature for the early diagnosis of Alzheimer's disease. J Alzheimers Dis. 2015;45(4):1157–73.

[91] Han X, Rozen S, Boyle SH, Hellegers C, Cheng H, Burke JR, et al. Metabolomics in early Alzheimer's disease: identification of altered plasma sphingolipidome using shotgun lipidomics. PLoS One. 2011;6(7):e21643.

[92] Mielke MM, Bandaru VVR, Haughey NJ, Rabins PV, Lyketsos CG, Carlson MC. Serum sphingomyelins and ceramides are early predictors of memory impairment. Neurobiol Aging. 2010;31(1):17–24.

[93] Zambón D, Quintana M, Mata P, Alonso R, Benavent J, Cruz-Sánchez F, et al. Higher incidence of mild cognitive impairment in familial hypercholesterolemia. Am J Med. 2010;123(3):267–74.

[94] Kása P, Rakonczay Z, Gulya K. The cholinergic system in Alzheimer's disease. Prog Neurobiol. 1997;52(6):511–35.

[95] Texidó L, Martín-Satué M, Alberdi E, Solsona C, Matute C. Amyloid β peptide oligomers directly activate NMDA receptors. Cell Calcium. 2011;49(3):184–90.

[96] Rogawski MA, Wenk GL. The neuropharmacological basis for the use of memantine in the treatment of Alzheimer's disease. CNS Drug Rev. 2003;9(3):275–308.

[97] Raina P, Santaguida P, Ismaila A, Patterson C, Cowan D, Levine M, et al. Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline. Ann Intern Med. 2008;148(5):379–97.

[98] Kaduszkiewicz H, Zimmermann T, Beck-Bornholdt H-P, van den Bussche H. Cholinesterase inhibitors for patients with Alzheimer's disease: systematic review of randomised clinical trials. BMJ. 2005;331(7512):321–7.

[99] Salomone S, Caraci F, Leggio GM, Fedotova J, Drago F. New pharmacological strategies for treatment of Alzheimer's disease: focus on disease modifying drugs. Br J Clin Pharmacol. 2012;73(4):504–17.

[100] Caraci F, Bosco P, Leggio GM, Malaguarnera M, Drago F, Bucolo C, et al. Clinical pharmacology of novel anti-Alzheimer disease modifying medications. Curr Top Med Chem. 2013;13(15):1853–63.

[101] Zetterberg H, Andreasson U, Hansson O, Wu G, Sankaranarayanan S, Andersson ME, et al. Elevated cerebrospinal fluid BACE1 activity in incipient Alzheimer disease. Arch Neurol. 2008;65(8):1102–7.

[102] Hébert SS, Horré K, Nicolaï L, Papadopoulou AS, Mandemakers W, Silahtaroglu AN, et al. Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. Proc Natl Acad Sci U S A. 2008;105(17):6415–20.

[103] O'Connor T, Sadleir KR, Maus E, Velliquette RA, Zhao J, Cole SL, et al. Phosphorylation of the translation initiation factor eIF2alpha increases BACE1 levels and promotes amyloidogenesis. Neuron. 2008;60(6):988–1009.

[104] Chang W-P, Huang X, Downs D, Cirrito JR, Koelsch G, Holtzman DM, et al. Beta-secretase inhibitor GRL-8234 rescues age-related cognitive decline in APP transgenic mice. FASEB J. 2011;25(2):775–84.

[105] Ohno M, Sametsky EA, Younkin LH, Oakley H, Younkin SG, Citron M, et al. BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease. Neuron. 2004;41(1):27–33.

[106] Vassar R. BACE1 inhibitor drugs in clinical trials for Alzheimer's disease. Alzheimers Res Ther. 2014;6(9):89.

[107] Henley DB, May PC, Dean RA, Siemers ER. Development of semagacestat (LY450139), a functional gamma-secretase inhibitor, for the treatment of Alzheimer's disease. Expert Opin Pharmacother. 2009;10(10):1657–64.

[108] Bateman RJ, Siemers ER, Mawuenyega KG, Wen G, Browning KR, Sigurdson WC, et al. A gamma-secretase inhibitor decreases amyloid-beta production in the central nervous system. Ann Neurol. 2009;66(1):48–54.

[109] Doody RS, Raman R, Farlow M, Iwatsubo T, Vellas B, Joffe S, et al. A phase 3 trial of semagacestat for treatment of Alzheimer's disease. N Engl J Med. 2013;369(4):341–50.

[110] Numata K, Kaplan DL. Mechanisms of enzymatic degradation of amyloid Beta microfibrils generating nanofilaments and nanospheres related to cytotoxicity. Biochemistry. 2010;49(15):3254–60.

[111] Pedersen WA, McMillan PJ, Kulstad JJ, Leverenz JB, Craft S, Haynatzki GR. Rosiglita-zone attenuates learning and memory deficits in Tg2576 Alzheimer mice. Exp Neurol. 2006;199(2):265–73.

[112] Gold M, Alderton C, Zvartau-Hind M, Egginton S, Saunders AM, Irizarry M, et al. Rosiglitazone monotherapy in mild-to-moderate Alzheimer's disease: results from a randomized, double-blind, placebo-controlled phase III study. Dement Geriatr Cogn Disord. 2010;30(2):131–46.

[113] Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature. 1999;400(6740):173–7.

[114] Hanger DP, Anderton BH, Noble W. Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. Trends Mol Med. 2009;15(3):112–9.

[115] Kanaan NM, Pigino GF, Brady ST, Lazarov O, Binder LI, Morfini GA. Axonal degeneration in Alzheimer's disease: when signaling abnormalities meet the axonal transport system. Exp Neurol. 2013;246:44–53.

[116] Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, et al. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J Neuropathol Exp Neurol. 2005;64(2):113–22.

[117] Tariot PN, Aisen PS. Can lithium or valproate untie tangles in Alzheimer's disease? J Clin Psychiatry. 2009;70(6):919–21.

[118] Fleisher AS, Truran D, Mai JT, Langbaum JBS, Aisen PS, Cummings JL, et al. Chronic divalproex sodium use and brain atrophy in Alzheimer disease. Neurology. 2011;77(13):1263–71.

[119] Trujillo-Estrada L, Jimenez S, De Castro V, Torres M, Baglietto-Vargas D, Moreno-Gonzalez I, et al. *In vivo* modification of Abeta plaque toxicity as a novel neuroprotective lithium-mediated therapy for Alzheimer's disease pathology. Acta Neuropathol Commun. 2013;1(1):73.

[120] Mudher A, Shepherd D, Newman TA, Mildren P, Jukes JP, Squire A, et al. GSK-3beta inhibition reverses axonal transport defects and behavioural phenotypes in Drosophila. Mol Psychiatry. 2004;9(5):522–30.

[121] Forlenza OV, Diniz BS, Radanovic M, Santos FS, Talib LL, Gattaz WF. Disease-modifying properties of long-term lithium treatment for amnestic mild cognitive impairment: randomised controlled trial. Br J Psychiatry. 2011;198(5):351–6.

[122] Hampel H, Ewers M, Bürger K, Annas P, Mörtberg A, Bogstedt A, et al. Lithium trial in Alzheimer's disease: a randomized, single-blind, placebo-controlled, multicenter 10-week study. J Clin Psychiatry. 2009;70(6):922–31.

[123] Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Sarmiento J, Troncoso J, Jackson GR, et al. Identification of oligomers at early stages of tau aggregation in Alzheimer's disease. FASEB J. 2012;26(5):1946–59.

[124] Pritchard SM, Dolan PJ, Vitkus A, Johnson GVW. The toxicity of tau in Alzheimer disease: turnover, targets and potential therapeutics. J Cell Mol Med. 2011;15(8):1621–35.

[125] Grüninger F. Invited review: drug development for tauopathies. Neuropathol Appl Neurobiol. 2015;41(1):81–96.

[126] Collin L, Bohrmann B, Göpfert U, Oroszlan-Szovik K, Ozmen L, Grüninger F. Neuronal uptake of tau/pS422 antibody and reduced progression of tau pathology in a mouse model of Alzheimer's disease. Brain. 2014;137(Pt 10):2834–46.

[127] De Strooper B, Chávez Gutiérrez L. Learning by failing: ideas and concepts to tackle γ-secretases in Alzheimer's disease and beyond. Annu Rev Pharmacol Toxicol. 2015;55:419–37.

[128] Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, Keller P, et al. Simvastatin strongly reduces levels of Alzheimer's disease beta -amyloid peptides Abeta 42 and Abeta 40 *in vitro* and *in vivo*. Proc Natl Acad Sci U S A. 2001;98(10):5856–61.

[129] Li L, Cao D, Kim H, Lester R, Fukuchi K-I. Simvastatin enhances learning and memory independent of amyloid load in mice. Ann Neurol. 2006;60(6):729–39.

[130] Chauhan NB, Siegel GJ, Feinstein DL. Effects of lovastatin and pravastatin on amyloid processing and inflammatory response in TgCRND8 brain. Neurochem Res. 2004;29(10):1897–911.

[131] Shepardson NE, Shankar GM, Selkoe DJ. Cholesterol level and statin use in Alzheimer disease: I. Review of epidemiological and preclinical studies. Arch Neurol. 2011;68(10):1239–44.

[132] Shepardson NE, Shankar GM, Selkoe DJ. Cholesterol level and statin use in Alzheimer disease: II. Review of human trials and recommendations. Arch Neurol. 2011;68(11):1385–92.

[133] Sjögren M, Gustafsson K, Syversen S, Olsson A, Edman A, Davidsson P, et al. Treatment with simvastatin in patients with Alzheimer's disease lowers both alpha- and beta-cleaved amyloid precursor protein. Dement Geriatr Cogn Disord. 2003;16(1):25–30.

[134] Hoglund K, Thelen KM, Syversen S, Sjogren M, von Bergmann K, Wallin A, et al. The effect of simvastatin treatment on the amyloid precursor protein and brain cholesterol

metabolism in patients with Alzheimer's disease. Dement Geriatr Cogn Disord. 2005;19(5–6):256–65.

[135]   Sano M, Bell KL, Galasko D, Galvin JE, Thomas RG, van Dyck CH, et al. A randomized, double-blind, placebo-controlled trial of simvastatin to treat Alzheimer disease. Neurology. 2011;77(6):556–63.

[136]   Carlsson CM, Gleason CE, Hess TM, Moreland KA, Blazel HM, Koscik RL, et al. Effects of simvastatin on cerebrospinal fluid biomarkers and cognition in middle-aged adults at risk for Alzheimer's disease. J Alzheimers Dis. 2008;13(2):187–97.

[137]   Sparks DL, Sabbagh MN, Connor DJ, Lopez J, Launer LJ, Browne P, et al. Atorvastatin for the treatment of mild to moderate Alzheimer disease: preliminary results. Arch Neurol. 2005;62(5):753–7.

[138]   Sparks DL, Sabbagh MN, Connor DJ, Lopez J, Launer LJ, Petanceska S, et al. Atorvastatin therapy lowers circulating cholesterol but not free radical activity in advance of identifiable clinical benefit in the treatment of mild-to-moderate AD. Curr Alzheimer Res. 2005;2(3):343–53.

[139]   Feldman HH, Doody RS, Kivipelto M, Sparks DL, Waters DD, Jones RW, et al. Randomized controlled trial of atorvastatin in mild to moderate Alzheimer disease: LEADe. Neurology. 2010;74(12):956–64.

[140]   Jones RW, Kivipelto M, Feldman H, Sparks L, Doody R, Waters DD, et al. The Atorvastatin/Donepezil in Alzheimer's Disease Study (LEADe): design and baseline characteristics. Alzheimers Dement. 2008;4(2):145–53.

[141]   Carlsson CM, Xu G, Wen Z, Barnet JH, Blazel HM, Chappell RJ, et al. Effects of atorvastatin on cerebral blood flow in middle-aged adults at risk for Alzheimer's disease: a pilot study. Curr Alzheimer Res. 2012;9(8):990–7.

[142]   Friedhoff LT, Cullen EI, Geoghagen NS, Buxbaum JD. Treatment with controlled-release lovastatin decreases serum concentrations of human beta-amyloid (A beta) peptide. Int J Neuropsychopharmacol. 2001;4(2):127–30.

[143]   Shepherd J, Blauw GJ, Murphy MB, Bollen ELEM, Buckley BM, Cobbe SM, et al. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. Lancet. 2002;360(9346):1623–30.

[144]   Tsuji A, Saheki A, Tamai I, Terasaki T. Transport mechanism of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitors at the blood-brain barrier. J Pharmacol Exp Ther. 1993;267(3):1085–90.

[145]   Carmena R, Roederer G, Mailloux H, Lussier-Cacan S, Davignon J. The response to lovastatin treatment in patients with heterozygous familial hypercholesterolemia is modulated by apolipoprotein E polymorphism. Metabolism. 1993;42(7):895–901.

[146] Johnson-Anuna LN, Eckert GP, Keller JH, Igbavboa U, Franke C, Fechner T, et al. Chronic administration of statins alters multiple gene expression patterns in mouse cerebral cortex. J Pharmacol Exp Ther. 2005;312(2):786–93.

[147] Cole SL, Grudzien A, Manhart IO, Kelly BL, Oakley H, Vassar R. Statins cause intracellular accumulation of amyloid precursor protein, beta-secretase-cleaved fragments, and amyloid beta-peptide via an isoprenoid-dependent mechanism. J Biol Chem. 2005;280(19):18755–70.

[148] Yurko-Mauro K, McCarthy D, Rom D, Nelson EB, Ryan AS, Blackwell A, et al. Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. Alzheimers Dement. 2010;6(6):456–64.

[149] Freund-Levi Y, Eriksdotter-Jönhagen M, Cederholm T, Basun H, Faxén-Irving G, Garlind A, et al. Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. Arch Neurol. 2006;63(10):1402–8.

[150] Shinto L, Quinn J, Montine T, Dodge HH, Woodward W, Baldauf-Wagner S, et al. A randomized placebo-controlled pilot trial of omega-3 fatty acids and alpha lipoic acid in Alzheimer's disease. J Alzheimers Dis. 2014;38(1):111–20.

[151] Jimenez S, Torres M, Vizuete M, Sanchez-Varo R, Sanchez-Mejias E, Trujillo-Estrada L, et al. Age-dependent accumulation of soluble amyloid beta (Abeta) oligomers reverses the neuroprotective effect of soluble amyloid precursor protein-alpha (sAPP(alpha)) by modulating phosphatidylinositol 3-kinase (PI3K)/Akt-GSK-3beta pathway in Alzheimer mouse model. J Biol Chem. 2011;286(21):18414–25.

[152] Fragkouli A, Papatheodoropoulos C, Georgopoulos S, Stamatakis A, Stylianopoulou F, Tsilibary EC, et al. Enhanced neuronal plasticity and elevated endogenous sAPPα levels in mice over-expressing MMP9. J Neurochem. 2012;121(2):239–51.

[153] Thornton E, Vink R, Blumbergs PC, Van Den Heuvel C. Soluble amyloid precursor protein alpha reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. Brain Res. 2006;1094(1):38–46.

[154] Lorente-Cebrián S, Costa AGV, Navas-Carretero S, Zabala M, Laiglesia LM, Martínez JA, et al. An update on the role of omega-3 fatty acids on inflammatory and degenerative diseases. J Physiol Biochem. 2015;71(2):341–9.

[155] Escribá PV. Membrane-lipid therapy: a new approach in molecular medicine. Trends Mol Med. 2006;12(1):34–43.

[156] Escribá PV, Busquets X, Inokuchi J-I, Balogh G, Török Z, Horváth I, et al. Membrane lipid therapy: modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res. 2015;59:38–53.

[157] Nicolson GL, Ash ME. Lipid replacement therapy: a natural medicine approach to replacing damaged lipids in cellular membranes and organelles and restoring function. Biochim Biophys Acta. 2014;1838(6):1657–79.

[158] Ibarguren M, López DJ, Encinar JA, González-Ros JM, Busquets X, Escribá PV. Partitioning of liquid-ordered/liquid-disordered membrane microdomains induced by the fluidifying effect of 2-hydroxylated fatty acid derivatives. Biochim Biophys Acta. 2013;1828(11):2553–63.

[159] Fiol-Deroque MA, Gutierrez-Lanza R, Terés S, Torres M, Barceló P, Rial RV, et al. Cognitive recovery and restoration of cell proliferation in the dentate gyrus in the 5XFAD transgenic mice model of Alzheimer's disease following 2-hydroxy-DHA treatment. Biogerontology. 2013;14(6):763–75.

[160] Torres M, Price SL, Fiol-Deroque MA, Marcilla-Etxenike A, Ahyayauch H, Barceló-Coblijn G, et al. Membrane lipid modifications and therapeutic effects mediated by hydroxydocosahexaenoic acid on Alzheimer's disease. Biochim Biophys Acta. 2014;1838(6):1680–92.

[161] Torres M, Marcilla-Etxenike A, Fiol-deRoque MA, Escribá PV, Busquets X. The unfolded protein response in the therapeutic effect of hydroxy-DHA against Alzheimer's disease. Apoptosis. 2015;20(5):712–24.

[162] Yang D-S, Stavrides P, Mohan PS, Kaushik S, Kumar A, Ohno M, et al. Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits. Brain. 2011;134(Pt 1):258–77.

[163] Schaeffer V, Lavenir I, Ozcelik S, Tolnay M, Winkler DT, Goedert M. Stimulation of autophagy reduces neurodegeneration in a mouse model of human tauopathy. Brain. 2012;135(Pt 7):2169–77.

[164] Török Z, Crul T, Maresca B, Schütz GJ, Viana F, Dindia L, et al. Plasma membranes as heat stress sensors: from lipid-controlled molecular switches to therapeutic applications. Biochim Biophys Acta. 2014;1838(6):1594–618.

[165] Lopez DH, Fiol-Deroque MA, Noguera-Salvà MA, Terés S, Campana F, Piotto S, et al. 2-hydroxy arachidonic Acid: a new non-steroidal anti-inflammatory drug. PLoS One. 2013;8(8):e72052.

[166] Choi S-H, Aid S, Caracciolo L, Minami SS, Niikura T, Matsuoka Y, et al. Cyclooxygenase-1 inhibition reduces amyloid pathology and improves memory deficits in a mouse model of Alzheimer's disease. J Neurochem. 2013;124(1):59–68.