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Evaluation of BACE1 silencing as a therapy against Alzheimer disease

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**Evaluation of Bace₁ silencing as a
therapy against Alzheimer disease:**
Implications for lipid metabolism and
inflammatory response

Javier Gustavo Villamil Ortiz

Evaluation of Bace₁ silencing as a therapy against Alzheimer disease: Implications for lipid metabolism and inflammatory response

PhD thesis

Javier Gustavo Villamil Ortiz

Evaluation of Bace₁ silencing as a therapy against Alzheimer disease:

Implications for lipid metabolism and inflammatory response.

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CHAPTER 1

General introduction

Alzheimer Disease

Alzheimer's disease (AD) is the most common type of dementia, it is estimated that more than 35 million people worldwide suffer from it. As the average age of the world population is increasing, it is estimated that the prevalence of AD will double in the next 20 years (dos Santos Picanco et al. 2018)(Duthey 2013). AD is characterized by an irreversible and progressive deterioration of brain regions, with a typical etiology of pathological events; the first alterations occur in the temporal lobe and the dentate gyrus of the hippocampus, which has a direct correlation with short-memory problems. Later the parietal lobe is affected, altering spatial visualization processes and resulting in the loss of knowledge of daily habits and uses. Finally, the frontal lobe is affected, which leads to behavioral alterations (Iacono et al. 2014). Several molecular lesions have been reported in AD, being the accumulation of misfolded proteins the most extensively described feature in the AD brain, as well as oxidative stress, inflammatory damage, energy failure, and synaptic dysfunction which finally inducing cell death pathway activation (Heppner, Ransohoff, and Becher 2015b).

Two forms of Alzheimer's disease are known, familial and the sporadic forms, and they have diverse genetic components; familial AD has been associated with mutations in the amyloid precursor protein (APP) gene, and in genes for presenilin 1 (PSEN 1) and presenilin 2 (PSEN 2), which are the most commonly mutated genes in familial AD (Landman et al. 2006). A range of genes has been associated with the sporadic AD, including the E4 allele of the apolipoprotein gene (APOE), which increases the risk for AD 3-fold in heterozygotes and 15-fold in homozygotes. In addition, it is known that APOE is associated with the processing and accumulation of A β peptide (J.-B. Zhu et al. 2017). The ApoE gene is located on chromosome 19 (4 exons); has 3 common isoforms: APOE2 (8%), APOE3 (78%) and APOE4 (14%); APOE is a lipid transport molecule; APOE2 and APOE3 bind to (HDL) and ApoE4 (LDLR) (Stocker et al. 2018). Alterations in APOE4 lead to an increase in cholesterol levels. In the brain, APOE is mainly expressed by astrocytes. It is suggested that APOE facilitates amyloid beta (A β) aggregation by converting an α helix in A β into a β sheet (Rebeck 2017). APOE also has an important role in the redistribution of lipids during regeneration of neurons after brain after excitotoxicity events (Rebeck 2017). There is evidence that the stability of synapses is related to APOE activity. Consequently, it was shown that the APOE E4 allele induces a reduction of synaptic enzymes such as choline acetyltransferase and glutaminase which contribute to the increased risk of neurodegeneration (Uddin et al. 2018; Lai et al. 2006).

The main pathological characteristics of AD are plaques consisting of A β peptide, dystrophic neurites in the neocortex, neurofibrillary tangles (NFTs) of tau protein in structures of the medial temporal lobe, neuronal loss in white matter, inflammation, among others (dos Santos Picanco et al. 2018). Alzheimer dementia is related to the density of NFTs in the neocortex and hippocampus, and it is due to the accumulation of soluble tau, while plaques

are less clearly related to the severity of dementia (Kametani and Hasegawa 2018). Another important characteristic of AD is the response of glial cells, where the size and number of processes of astrocytes are increased in the white and gray matter. Microglia activity is also increased in gray matter containing A β plaques and NFTs, with increasing processes size and expression of the major histocompatibility class 2 complex (MHC-II), and increased expression levels of lysosomal enzymes and complement receptors (Heppner, Ransohoff, and Becher 2015b). Furthermore, microglia activation has been associated with inflammasome complex formation, which includes the production of Apoptosis-associated speck-like protein (ASC specks). ASC specks protein are released by microglia and have a high binding affinity to A β fragments, which facilitates the formation of A β oligomers and aggregates, acting as inflammation-driven cross-seed for A β pathology (Venegas et al. 2017; Bamberger et al. 2003).

Neuropathological hallmarks

AD has been characterized by the accumulation of specific proteins in neurons or in the brain parenchyma. Two main histopathological markers such as A β aggregates (amyloidopathy), and NFT (tauopathy). Furthermore accumulations of intracellular lipid granules surround A β plaques which were included as an AD histopathological marker as well (Sato and Morishita 2015). Amyloidopathy has been described widely in AD cases, it is characterized by the aggregation of A β , resulting from the aberrant cleavage of the amyloid precursor protein (APP) by the enzyme β -secretase 1 (BACE1) and γ -secretase enzyme complex, which

Figure 1. Alzheimer Disease is a multifactorial disorder, at the gross anatomical level, AD brains have atrophy associated with neuronal apoptosis, resulting in loss of brain volume. At the molecular level, loss of synapses, protein aggregation, of A β fragments and tau hyperphosphorylated is observed. In association with glia cell reaction, since, microgliosis and astroglisis have been reported. Graphics source (CREATIVE COMMONS LICENCE and Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License n.d.).

generates the formation of extracellular and intracellular prone A β fragments (Hardy 2002). An imbalance between production and degradation, and accumulation of β -amyloid peptide causes an excess of A β -plaques which has been pointed as a main initiating factor and neuropathological marker in AD. This process is called “amyloid hypothesis,” describing that A β -42 peptides accumulation is toxic to brain cells and experimental evidence suggest that it accumulation precedes and induce tau aggregation (Bloom 2014b) (Figure 1).

Amyloidopathy in Alzheimer Disease

The amyloid precursor protein (APP), 695 to 770 amino acids in size, is a type I membrane glycoprotein, particularly expressed in neurons where it is anterogradely transported in vesicles to different cellular compartments including synapses (Kamal et al. 2001). It is mainly involved in cell signaling, growth and differentiation (Nalivaeva and Turner 2013), and the structural and functional alteration of this protein has been related to carcinogenesis, type II diabetes and neurodegeneration as AD (Venkataramani et al. 2010; Takayama et al. 2009).

There are two divergent pathways in the natural metabolic processing of APP, known as the non-amyloidogenic and amyloidogenic pathways, respectively (Figure 1) (Barage and Sonawane 2015). The first pathway processes around 90% of APP, via the cleavage by the zinc-binding metalloproteinase α -secretase, whose activity is mainly regulated by protein kinase C (PKC), this enzyme cleaves APP in the peptide domain known as A β , and the resulting fragment is the precursor protein of α soluble amyloid protein precursor (sAPP α) (LeBlanc, Koutroumanis, and Goodyer 1998; Mattson, Guo, and Geiger 1999). This fragment is taken up by the membrane enzyme complex γ -secretase, which consists of five subunits: 1) presenilin 1 (PS1), 2) presenilin 2 (PS2), 3) APH1 (anterior pharynx-defective 1), 4) enhancer presenilin 2 (PEN2) and 5) nicastrin, and these proteins together perform the intramembrane proteolysis of the sAPP α fragment (Selkoe and Kopan 2003; Wolfe 2010). The activation of this pathway has not been associated with any pathology; on the contrary, its activation is considered as neuroprotective (Lichtenthaler 2012; Obregon et al. 2012).

The amyloidogenic pathway starts with the cleavage of the APP by the integral membrane protein type 1 known as BACE1 (Ben Halima et al. 2016), which cleaves at aspartate (Asp) amino acid residues. In neurons, APP cleavage occurs specifically at Asp624 (Barao et al. 2016), releasing a fragment called soluble amyloid β precursor protein (sAPP- β) which induces differentiation to neurons during embryonic development (Freude et al. 2011) and the truncated fragment of APP known as C99 or Carboxy-terminal β fragment (β -CTF) (Zhang et al. 2012). The latter can be processed at different sites by the γ -secretase complex to generate the intracellular domain of APP (AICD: transcriptional regulator mainly of proteins of the membrane) and 2 A β fragments of 40 and 42 amino acids in size, respectively (Koelsch 2017) (Figure 2).

There is extensive scientific evidence that A β fragments, and predominantly A β 1-42, have a hydrophobic structure which favors aggregation and extraneuronal accumulation in insoluble plaques, deposited in the parenchyma and cerebral vasculature. This phenomenon disturbs the cell homeostasis, affecting neuronal synapses and memory processes, characteristic events in AD (Bignante et al. 2013; Lefort, Pozueta, and Shelanski 2012). This process occurs mainly in lipid rafts where the association and cleavage APP by BACE1 has been described, and consequently high levels of cholesterol the (main component of lipid rafts) increases the proteolytic activity of BACE1 resulting in increased generation of A β fragments. These findings suggest that lipids are implicated in the production of A β and that the understanding of this role will allow a more comprehensive approach to the complex neuropathological processes that occur in AD, as well as facilitating the generation of novel therapeutic strategies aimed at recovering neuronal homeostasis (Ma et al. 2007; Evin and Hince 2013).

Figure 2. Processing of Amyloid Precursor Protein (APP). The non-amyloidogenic pathway start with α -secretase activity which results in the cleavage of APP to produce the soluble fragment sAPP α . The terminal fragment of APP (α -CTF) is cleavage by γ -secretase to produce non amyloidogenic peptide 3 (p3) and the intracellular APP fragment (AICD). In the amyloidogenic pathway APP is cleaved by β -secretase at short distance to the transmembrane domain, which release a soluble fragment sAPP β . However, the β -CTF is cleavage by γ -secretase to generate the amyloidogenic peptide fragments of different lengths such as A β 40 or 42 amino acids. Independently of the pathway, CTF is released into the cytosol and it migrate to the nucleus to activate gene transcription. Taken and modified from “Alzheimer’s disease: the lipid connection” (T Hartmann, Kuchenbecker, and Grimm 2007).

Tauopathy in AD.

Tauopathy is characterized by the aggregation of neurofibrillary tangles (NFT) of hyperphosphorylated tau protein. Under physiological conditions, tau protein induces stability during the polymerization and depolymerization of microtubules and vesicle transport, however, it is also demonstrated that tau is the major antigenic component of neurofibrillary tangles.

The property of tau to bind to microtubules is established by post-translational modifications, mainly by phosphorylation of serine/threonine residues, which modulate the affinity of tau for microtubules. The ability of tau protein to modulate the dynamics of microtubules, give tau an important role in the maintenance of axonal transport, allowing the mobilization of signaling molecules, trophic factors and other essential cellular components such as mitochondria (Mandelkow et al. 2003; Shahpasand et al. 2012) and vesicles, which are determinants for neuronal function and viability (Ballatore, Lee, and Trojanowski 2007). However, under pathological conditions, the hyperphosphorylation of tau causes loss of association with microtubules promoting toxic aggregates, NFTs, that are one of the most important neuropathological markers of the severity of AD (Congdon and Sigurdsson 2018b).

Formation of NFTs begins with tau detachment from microtubules; this detachment is induced by the increase of phosphorylation rate and/or decreasing of dephosphorylation in the cytosol (Jouanne, Rault, and Voisin-Chiret 2017). Subsequently, small deposits of non-fibrillar tau are formed, called pre-tangles, which undergo conformational changes resulting in the formation of paired helical filaments (PHFs) to finally form the neurofibrillary tangles. Additionally, it has been established that the malfunction and alterations in the activity of tau phosphatases are direct causes of neurofibrillary tangle formation (Bloom 2014b; Morris et al. 2011).

The phosphorylation of tau in different sites has different impacts on biologic and pathogenic functions. Quantitative in vitro studies demonstrate that tau phosphorylation at Ser262, Thr231, and Ser235, inhibit microtubule binding between 10 and 35%; Studies of binding kinetics between hyperphosphorylated tau and normal tau show that Ser199 / Ser202 / Thr205, Thr212, Thr231 / Ser235, Ser262 / Ser356, and Ser422, are critical phosphorylation sites, which convert tau into an inhibitory molecule, which recruit normal tau and microtubule-associated proteins such as MAP1 and MAP2, producing microtubular disassembly (Stoothoff and Johnson 2005). On the other hand, phosphorylation's in Thr231, Ser396, and Ser422 promote auto-aggregation of tau forming fibrillar tau; tau mutations in Ser396 and Ser404, are fibrillogenic; phosphorylation in regions rich in proline, inhibit microtubule assembly activity and promote tau aggregation; tau phosphorylation in the C-terminal tail increases its activity and strongly promotes its aggregation; phosphorylation's at the C-terminus and in nearby regions to microtubule binding sites, decreases its activity and destabilizes microtubules. In fact, the NFT and A β aggregation levels are tightly correlated with low scores during cognitive tests on human AD patients and AD mice models. Despite this correlation has been described, the precise mechanism that relates these two proteinopathies still remains poorly understood for AD (Bloom 2014a; Congdon and Sigurdsson 2018a).

Interestingly recent studies have been shown the lipid accumulation as third hallmark, being a potential and important link to understand the etiopathology of AD, since it is relevant during the aggregation process of A β and NFT.

The link between lipids and Alzheimer's

Lipids in AD have been the least studied factor by the scientific community, despite several studies have shown in AD brains that the levels of lipid aggregation were increased compared to healthy CNS, suggesting an aberrant modification of lipid metabolism. Furthermore, biochemical alterations have been reported in the composition of lipids in post-mortem brains diagnosed with AD (Hirsch-Reinshagen, Burgess, and Wellington 2009). The nervous system has a high lipid content and the neuronal lipid composition regulates the traffic and activity of membrane-anchored proteins that can control cellular A β levels, such as the BACE1 enzyme and the γ -secretase complex, which are regulated by cholesterol, sphingomyelin (SM) and phospholipids (Kalvodova et al. 2005; Walter 2012). Furthermore the extreme aggregation of A β also causes toxic effects by altering the membrane lipid composition, affecting the activity of phospholipases A, D and C (Desbène et al. 2012; Sanchez-Mejia and Mucke 2010; Jang et al. 2013). Additionally, has been established that the main genetic risk factor for sporadic AD (ApoE4) is related with alterations in lipid metabolism in the CNS. This is supported by the observation that the level of phosphoinositol biphosphate (PIP2) was reduced in postmortem human brain tissue of ApoE4 carriers, in the brain of ApoE4 knock-in (KI) mice, and in primary neurons expressing ApoE4 alleles. In contrast, in neurons with high ApoE3 levels, phospholipid levels were increased. (Schreiber et al. 2017; Bu 2009; Rebeck 2017; L. Zhu et al. 2015).

The influence of lipids on proteinopathies is proposed through the processing of APP and production of A β , as the proteolytic enzymes involved in this process are integral membrane proteins, implying that the lipid environment of these enzymes modulates their catalytic activity as well as their translocation to the plasma membrane. Therefore the metabolism of cholesterol, as well as other membrane lipids, aroused interest to understand the complex development of AD (Ma et al. 2007). In addition, different lines of evidence related lipid species such as ceramide, cholesterol and triglyceride levels with A β aggregation (Panchal et al. 2014; Tall 2008; Listenberger et al. 2003). However, the relationship between lipid metabolism and alterations therein, and its potential contribution to AD is still poorly understood.

Cholesterol, metabolism and Alzheimer's disease.

Cholesterol is a multifunctional molecule. The brain is the organ with the highest cholesterol content, containing approximately 25% of the total human body cholesterol, which is located in only 2% of the total weight of the human body. It is the main component of the plasma membrane of neurons, glia cells as well as myelin, and plays an essential role in the maintenance of the structure, plasticity and neuronal functionality (Pfrieger 2003; Salter and Beggs 2014). The metabolism of cholesterol is a key factor in the successful maintenance of neuronal functions and that its alteration triggers neurodegenerative pathologies such as AD (Amira Zarrouk et al. 2018).

Most of the cerebral cholesterol is synthesized de novo since the blood-brain barrier (BBB) prevents the free passage of cholesterol from the blood plasma to the brain, BBB allows the limited passage of chemically modified cholesterol molecules such as 27-hydroxycholesterol and the 24-hydroxycholesterol. However, the cholesterol exchange rate is very low, estimated at 1% per day (H.-L. Wang et al. 2016b). It has been shown that the ability of mature neurons to synthesize cholesterol is low, which makes glial cells the main source of this molecule in the brain (Giudetti et al. 2016). Therefore, the metabolism of cerebral cholesterol is complex and must be finely regulated by various proteins that control processes such as synthesis, storage, removal and transport. Failures of this complex metabolism plays a crucial role in major human diseases (Lin et al. 2015).

Hydroxy-Methyl-Glutaryl CoA-Reductase (HMG-CoA-R), is a key enzyme in the cholesterol synthesis pathway since it catalyzes the production rate of 3-hydroxy-methyl-glutaryl CoA to mevalonate which is the precursor molecule for cholesterol (Sharpe and Brown 2013). In fact one pharmacological strategy against alterations in cholesterol metabolism focuses on HMG-CoA-R inhibitors such as statins which decrease hypercholesterolemia and reducing the risk of developing amyloidopathy as well (Ostrowski et al. 2007), this is supported by epidemiological studies that have demonstrated a correlation between high cholesterol levels and AD (Kivipelto et al. 2001; Tobias Hartmann, Kuchenbecker, and Grimm 2007). At the molecular level, it has been demonstrated that statins such as simvastatin and lovastatin significantly decrease the levels of A β 40 and 42 peptides in primary cultures of hippocampal and cortical neurons, and this effect is repeated in cerebrospinal fluid and brain homogenates of murine models treated with high doses of simvastatin (Fassbender et al. 2001; Saeedi Saravi et al. 2017). Other pharmacological inhibitors of the cholesterol pathway, such as BM15.766, inhibit the activity of the enzyme 7 dehydrocholesterol reductase that acts in one of the final steps of cholesterol biosynthesis, reducing cholesterol and A β levels (Reid et al. 2007; Fonseca et al. 2010; Alonso, Mata, and Mata 2005).

On the other hand, the pharmacological inhibition of the enzyme Acetyl-Coenzyme A acetyltransferase 1 (ACAT1) reduces the levels cholesterol esters and indirectly A β aggregation, since this protein regulates the excess of free cholesterol by storing cholesterol esterified in structures known as lipid drops, which demonstrates that hypercholesterolemia and ACAT1 activity increase A β levels in neuronal cultures (Chang et al. 2006; Huttunen et al. 2010). Consequently, the genetic deletion of ACAT1 increases the 24s-hydroxycholesterol levels allowing cholesterol excesses to cross the blood-brain barrier and be carried to the peripheral blood flow avoiding its accumulation in the brain, which correlate with A β reduction and improves the cognitive capacity in AD murine models (S. R. Murphy et al. 2013; Bryleva et al. 2010). These data suggest that the balance between free cholesterol and cholesterol esters play a key role in the development of amyloidopathy.

Role of lipid rafts in AD

Lipid rafts are microdomains of cell membranes, composed mainly of stable associations of cholesterol, sphingolipids and specific membrane proteins, their composition makes it a dense membrane structure, allowing specific interaction between lipid-lipid, protein-lipid and protein-protein interaction (Lingwood and Simons 2010). Multiple functions for lipid rafts have been identified, among which its role in signaling, angiogenesis, cellular structure, regulation of enzymatic activity and cholesterol homeostasis are highlighted (Marin et al. 2017).

On the other hand, whether cholesterol levels or sphingolipids of membranes enriched in phosphatidylcholine are increased, the over-activation of γ -secretase is observed, indicating that the lipid environment in the membrane directly regulates the proteolytic activity of enzymes such as BACE1 and indirectly the production of aberrant peptides. However, the association between BACE1 and cellular membrane can occur inside or outside the lipid rafts, however, aberrant sections of APP occur mainly in the proteolysis performed within these microdomains (Choi et al. 2017). Probably due to the preferential formation of co-clusters between BACE1, γ -secretase and APP in cholesterol-enriched environments as in lipid rafts, rapid endocytosis of these clusters is observed, favoring the conformation of aggregates of intracellular A β (Bharadwaj et al. 2018; Marquer et al. 2011). These findings demonstrate that the composition of membrane lipid structures induce pathological processes and that the reestablishment of the levels and physiological conditions of cholesterol, sphingolipids, phospholipids and others are an interesting therapeutic target to reduce the formation of amyloidogenic aggregates and stop the progress of AD as these data suggest that an imbalance in lipid metabolism favors the proteolytic activity over APP by BACE1 and γ -secretase, reinforcing the importance of lipids in the pathogenesis of AD.

Sphingolipids and Alzheimer's disease

Sphingolipids represent one of the main lipids in cell membranes linked to cell signaling, its main fraction is sphingomyelin (SM) with 85% of all sphingolipids and is characterized by the presence of phosphocholine and ceramide (Cer). In the metabolism of lipids, Cer is the most important branch point in the synthesis of sphingolipids since from this lipid different metabolites are derived as glycosphingolipids (GLs) better known as gangliosides (GM) (Noel, Ingrand, and Barrier 2017; Dinkins, Wang, and Bieberich 2017).

High levels of SM reduce the levels of A β by inhibiting the activity of γ -secretase, this was verified in experiments where the activity of the enzyme sphingomyelinase (SMase) was inhibited, this enzyme converts the SM to Cer, and this inhibition results in a significant increase SM levels, decreased levels of Cer and amyloid peptides (Figure 3) (M. M. Mielke et

Figure 3. Cholesterol induces the activity of BACE1 and γ -secretase within lipid rafts. The rafts are small and highly dispersed at the cell surface, because they contain only a few proteins, such as APP, γ -secretase, and BACE1. In addition, the cholesterol at lipid rafts give stability to BACE1 activity, which is necessary to cleave APP. Graphic source (CREATIVE COMMONS LICENCE and Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License n.d.) A close correlation has been established between high cholesterol levels and the activity of BACE1 and γ -secretase enzymes, which promotes amyloidogenesis (Figure 3)(Díaz et al. 2014). In vitro studies show that a reduction in membrane cholesterol levels through the use of components such as β -methyl cyclodextrin (β MCD), causes a decrease in the activity of β and γ secretases, reduction of A β peptide levels, and there is evidence of a loss of association between BACE1 and membranes enriched with cholesterol (Rituper et al. 2013).

al. 2011; He et al. 2010). Interestingly, there has been an increase in the activity of SMase in the brains of patients diagnosed with a familial AD with PSEN1 mutations (Fonteh et al. 2015). On the other hand, decreasing Cer levels can have a beneficial effect, since a significant increase in Cer has been reported during the early stages of AD, as this lipid favors the processing of the APP protein by BACE1, giving stability during BACE1 activity, creating platforms enriched by Cer (Panchal et al. 2014). However, the complete inhibition of sphingolipids biosynthesis by down-regulation of the enzyme palmitoyltransferase (precursor of the metabolic pathway of sphingolipids) induce increasing of A β 1-42 synthesis. Interestingly, this phenomenon can be reversed since the addition of exogenous sphingosine restores the ratio between the synthesis of amyloid peptides in neuronal cultures, which suggests that sphingolipids can regulate the proteolytic activity of BACE1 and γ -secretase (Grimm et al. 2011).

Other sphingolipids associated with AD are the gangliosides or glycosphingolipids. Several studies indicate that the total ganglioside pattern is significantly altered in AD brains, which

highlights the importance of glycosphingolipids (Fukami et al. 2017b). For example, the increase in monoganglioside 1 (GM1) and the increase in the enzymatic activity of sialidase (glycolipid regulator) in AD (Fukami et al. 2017a). In histopathological analyses performed on senile plaques, it was observed that both cholesterol and GM1 bind to A β , this result suggests that the GM1/A β complex could function as a starting point in the aggregation of the peptide, since A β levels increase up to 10 times in the presence of GM1, with respect to control groups (Yanagisawa 2005), since GM1 enhances the catalytic activity of γ -secretase while decreasing the activity of α -secretase. Consequently, the inhibition of enzymes that process these gangliosides decreases the levels of A β , in vitro and in vivo models. (Yuyama and Yanagisawa 2010). However, more studies are needed to clarify the cellular role of GM1 in both physiological and pathological conditions.

On the other side, A β peptide interact with the sphingolipids through a sequence of binding to sphingolipids. This characteristic confers affinity for sites where the concentration of sphingolipids is high, as lipid rafts which enhance the amyloidogenesis, both extra and intracellular (Yahi and Fantini 2014; Mahfoud et al. 2002). These data together confirm the relationship between SMase, Cer, gangliosides, enzymes, and AD, however, further research is still needed to fully understand the mechanisms of lipid regulation and its influence on AD.

Phospholipids and Alzheimer's disease

Different studies in the brains of patients diagnosed with AD showed alterations in the metabolism and composition of phospholipids, among which the phosphorylated derivatives of phosphatidylinositol (phosphoinositide) stand out, as well as alterations in the production and activity of the enzymes phospholipase A2 (PLA2), C (PLC) and D (PLD), phosphoinositide kinase and various phosphatases (A Zarrouk et al. 2017; Amira Zarrouk et al. 2018). In both in vitro and in vivo models have been showed that the use of phospholipid inhibitors such as phosphatidylinositol-3 kinase (PI3K) causes a significant decrease in the levels of A β in the brain (Jimenez et al. 2011). In addition, phospholipids closely related to signaling, regulation of ion channels, membrane structure, endocytosis and exocytosis such as Phosphatidylinositol-4, 5-bisphosphate (PtdIns (4, 5) P2) negatively regulate the production of the A β peptide since it is substrate of the γ -secretase enzyme that effectively competes with APP, therefore in the presence of PtdIns (4, 5) P2 reduces the production of A β 42 in cell cultures. The metabolism of PtdIns (4,5) P2 is regulated by PLC, therefore blocking this phospholipase indirectly reduces the non-amyloidogenic activity of α -secretase and promote the amyloidogenic pathway, in the absence of PtdIns (4, 5) P2 (Uranga et al. 2017b). However, other researchers found that the inhibitory effect of PLC is anti-amyloidogenic, since it decreases the levels of A β 42 and prevents the excessive intake of calcium into the neuron through the NMDAR receptors preventing the loss of intracellular calcium homeostasis and decreasing the loss of synapses, both events observed in the AD (Uranga et al. 2017a). There is still an

extensive debate that defines the pros and cons of using PLC inhibitors as a therapy against AD. However, it seems that phospholipases are a key point in the understanding of different neurodegenerative diseases.

On the other hand, phospholipase D has also been related to the generation of amyloid peptides. From the DNA sequence coding for PLD, 3 isoenzymes PLD1, PLD2 and the most recent one associated to mitochondria called mitoPLD1 have been identified, however the studies have focused mainly on PLD1 and PLD2 which share motives that are essential in phospholipid catalysis. These enzymes have a phosphate binding motif for appropriate targeting, they also contain a phosphatidylinositol-bisphosphate binding motif (PIP2) which is critical for catalytic activity (Frohman 2015; Oliveira et al. 2010). However, PLD1 has a motif, which is a negative regulatory element of this catalytic activity. PLD1 and 2 hydrolyze phosphatidylcholine (PC) to generate phosphatidic acid (PA) and choline (Oliveira et al. 2010). PA is a bioactive molecule that allows the formation and fusion of membranes in the secretory, lysosomal and autophagy pathways. The action of PA phosphatases originates diverse molecules such as diacylglycerol (DAG) and lysophosphatidic acid (LPA) which are found mainly in the membranes of transport vesicles (Liu et al. 2013).

Overexpression of PDL1 in hippocampal neurons increased the transport of APP towards the plasma membrane, and the growth of neurites and these events do not occur in neurons with AD, on the contrary, under physiological conditions PDL1 negatively regulates the catalytic activity of PS1 (γ -secretase) and its transport to the cell surface, preventing the formation of A β 42. On the other hand, the derivatives of this protein allow the formation of intracellular transport and degradation vesicles favoring the maintenance of neuronal homeostasis (Cai et al. 2006). These studies support the action of different phospholipids (phosphoinositols, PA and DAG) in AD specifically in the formation of amyloid aggregates and the subsequent intracellular ion imbalance among other molecular events that lead to the loss of synapses and finally to neuronal death, which turns these molecules into potential therapeutic targets to be explored against this disease (Tanabe, Nakajima, and Ito 2014).

Arachidonic Acid, PLA, Docosahexaenoic Acid (DHA) and Alzheimer's

Arachidonic acid (AA) is one of the main omega-6 fatty acids present in food of animal origin. In addition, the body synthesizes AA by transforming linoleic acid present in cereals, nuts or vegetable oils. AA has been considered as a lipid that activates inflammatory pathways (Bazinet and Laye 2014). AA is located in the cell membranes and it is found mainly in neurons. Its function is associated with cell signaling, favoring neuronal activity and long-term potentiation (LTP) a cell model for learning and memory (Bazinet and Laye 2014). Pharmacological inhibitors of AA decrease memory and learning capacity in behavioral tests such as the Morris water maze, and the mechanism by which AA and PLA2 (AA regulatory enzyme)

activates neurons is through the recruitment and stabilization of receptors AMPA to the synaptic membrane surface, which favors the increase of calcium currents increasing the firing rate of neuronal action potentials. Opposite effects are observed by inhibiting PLA2, or by decreasing AA levels. These results do not negate the possibility that excess AA and PLA2 cause abnormal activity in neurons, in fact, over activity of PLA2 is reported in AD (Zárate et al. 2017).

AA directly modulates neuronal activity and indirectly through its metabolites. When a lesion occurs in the membrane of neurons, AA is released and PLA2 produces two metabolites, such as Prostaglandins and Leukotrienes (Chen et al. 2008). These metabolites exert pro-inflammatory activities, glutamate excitotoxicity, decreased synaptic plasticity and oxidative stress. Which agrees with the increased levels of cytosolic PLA2 immunoreactivity, decreased levels of AA in the brain and the loss of learning capacity and memory retention (Rao et al. 2012; Luo, Long, and Chen 2015). In AD model mice, deletion of GIVA-PLA2 (a phospholipase specific for neurons) improved the performance of mice in behavioral tests. The probable mechanism that regulates synaptotoxicity generated by hyperactivity of GIVA-PLA2 is through the excess of calcium influx since the membrane NMDA receptors levels increase drastically the activity of this phospholipase during chronic exposure to the oligomers of A β (Bate 2017; Sanchez-Mejia et al. 2008).

The polyunsaturated fatty acid DHA, commonly known as omega-3 (C22: 6 n-3), is essential for the maturation of the central nervous system and the preservation of brain functions during the course of life, however is a lipid that should be ingested in the diet, in fact the satisfactory intake DHA has become a challenging goal, due to the deficiency in the western diet of n-3 fatty acids, which can be considered as a risk factor for the AD (Mohaiibes et al. 2017; Cunnane et al. 2013). Therefore, DHA is considered as a neuroprotective molecule, several authors point out that AA and DHA have antagonistic effects, when DHA increases AA decreases exerting an anti-inflammatory role, its activity depends on PLA enzymes, they process DHA from α -Linoleic acid (Abdullah et al. 2017).

Enhancing the activity of these enzymes is proposed as a therapeutic strategy that reduces the levels of amyloid peptides, since epidemiological studies showed that populations with high intake of DHA reduce the risk of suffering some dementia, consequently low levels of DHA have been found in the blood of patients with AD (Yassine et al. 2017; Freund-Levi et al. 2006). In-vitro and in-vivo studies show that the presence of DHA in the nervous system reduces the levels of A β , improves the synapse and cognitive capacity in murine models. Additionally, it has been shown DHA increase the expression of genes that promote cell survival through the regulation of lipid function such as cellular communication, signal transduction, neuronal plasticity, neurotransmitter release, and vesicle formation, providing homeostasis (Zhao et al. 2011). However, it is necessary to perform studies in appropriate

models for AD, which allow to fully understand the molecular mechanism that regulates these neuroprotective effects, since DHA is a potential anti-inflammatory therapeutic target (Heras-Sandoval, Pedraza-Chaverri, and Pérez-Rojas 2016).

AD and Neuroinflammation

Inflammation in the brain (Neuroinflammation) is a biological response which involves several changes of local immune cells such as microglia and astrocytes around the damaged or inflamed area, with release of inflammatory molecules and increased oxidative stress (Glass et al. 2010a). Neuroinflammation has been described as a pathological contributor in many neurodegenerative diseases. Some of these diseases are defined as proteinopathies, due to abnormal aggregation of specific protein species. In vitro and in vivo models have been shown that proteinopathies increased the immune response at the brain. The brain has been considered an “immunologically privileged” organ, since, the immune cells from peripheral are unable to cross the Blood-brain barrier, then the glia cells (microglia and astrocytes) are the neuroimmune system.

In the CNS, microglia constantly regulate the tissue environment by producing factors that influence their surrounding brain cells, particularly in response to stimuli such as pathogen invasion or tissue damage, promoting tissue repair. However, this inflammation response may become detrimental to the tissue if it persists resulting in excessive production of neurotoxic molecules, such as cytokines and interleukins amplifying the inflammatory processes (W. Y. Wang et al. 2015). In fact neurotoxic molecules have been associated with neurodegenerative diseases like AD and Parkinson Disease. These diseases have proteinopathies, defects in axonal transport, mitochondrial dysfunction and apoptosis in common. The inflammatory response in neurodegenerative diseases is not clearly described as an initiating factor, nonetheless, increasing evidence shows a close relation with disease progression suggesting important roles of glia cells and their effectors of inflammatory processes at the brain.

Microglia cells

Microglia are the macrophages cell population in the central nervous system (CNS) making up around 10% of all glia cells. In 1932, Pio Hortege del Rio described the morphology of microglia in the CNS, as cells that extend long processes and branches from the soma, and was called “ramified microglia”. However, later it was shown that the morphology of microglia is diverse and that these processes are highly motile. In fact, that properties help to sense signals, and interactions with epithelial cells, neurons, astrocytes, oligodendrocytes, and blood vessels. This is essential to function as a primary innate immune cell in the CNS. Microglia cells respond to any alteration in brain homeostasis, such as infections or proteinopathies, for instance amyloid- β aggregations. The response is called microglia activation

and it includes morphological, gene expression, and functional changes. Activated microglia can adopt an amoeboid morphology which is usually accompanied by microglia proliferation (microgliosis) (Keren-Shaul et al. 2017). While on the molecular level have been described the overexpression of specific molecules, such recognition receptors, e.g., toll-like receptors (TLRs), CD14, CD36, triggering receptor expressed by myeloid cells 2 (TREM2), and CD33 (Hickman, Allison, and El Khoury 2008a).

The proinflammatory response is followed by myeloid differentiation primary protein 88 (MYD88) and Nuclear factor- κ B (NF- κ B) pathway, triggering cytokines production such as IL-1 β and IL-6. This cellular process involves multiple molecular mechanisms such as inflammasome to control the inflammatory response (Wu et al. 2017).

Microglia function

Microglia are play critical roles in brain development by supporting neurogenesis, pruning synapses, and removal of excess neurons and inflammatory response. During development the Microglia cells arrive in the brain around embryonic day 9 in mice. guiding the neural circuit formation through realizing neurotrophins like Bdnf. It was demonstrated since microglia Bdnf depletion, induced impaired learning and memory behavior by reduction of synapse formation on mice (Parkhurst et al. 2013). Microglia are also involved in synapses engulfed and elimination (pruning) during normal brain development, as well as phagocytosing apoptotic cells, modulating neurogenesis, and regulating synapse plasticity and myelin formation. All these different functions are often accompanied by regional differences in microglia morphology and region distribution, which change depending of maturation stage of the brain (Schafer and Stevens 2015; Hammond et al. 2018).

On the other hand has been reported that immune activation in the CNS, present in infections, immune disorders, and neurodegeneration, involves microglia and astrocytes which constitute the resident immune cells of the CNS of the brain during development, adulthood, and aging (Akiyama et al. 2000). Like macrophages, the microglia cells destroy strange materials in the CNS as innate immune player through the phagocytic and cytotoxic mechanism. Furthermore, microglia can act as antigen-presenting and contribute to inflammation by secreting molecules, such as cytokines (Interleukins), Tumoral Necrosis Factor- α (TNF- α), free radicals (Nitric Oxide), and polyunsaturated fatty acids (Eicosanoids) (Heppner, Ransohoff, and Becher 2015b). In the CNS, the microglia constantly produce molecules that influence surrounding cells such as astrocytes and neurons, however, peak activity occurs in response to homeostasis alterations commonly due to viral or bacterial infections, free radicals, loss of ion homeostasis, as well as neurotoxins and innate immune dysfunction, furthermore it has been shown that protein aggregations such as beta-amyloid also activate the fully phagocytic form of microglia (Glass et al. 2010b).

Although the inflammation is a response to recover homeostasis, it can also produce neuro-toxic factors, which can amplify the alteration, generating pathological tissue, depending on the persistence of inflammatory stimuli or failure in compensation mechanisms (Hepner, Ransohoff, and Becher 2015a). The main neurotoxic factors are cytokines and interleukins. These neurotoxic mediators, in general, are associated with several neurodegenerative diseases including AD, which are commonly linked to intracellular mechanisms failures such as protein degradation, mitochondria dysfunction, defects on axonal transport, and apoptosis. Inflammation is not related as initiating factor of the neurodegenerative disease. However, the emerging evidence on the sustained inflammatory response from microglia and astrocytes in disease progression, suggest important roles of neuroinflammation effectors in neuronal dysfunction and death (Heneka et al. 2015a). Neurodegeneration induced by protein aggregations is relevant, since it refers an imbalance in homeostasis mechanism kept by CNS, recent evidence suggests an important role of immune response in neurodegeneration.

Microglia in AD

Chronic activation of microglia affects brain homeostasis, inducing injuries and death of neurons through constant release of cytotoxic factors, including inflammatory cytokines, complement components, chemokines, reactive nitrogen species (RNS), reactive oxygen species (ROS), and fatty acids. All those mediators have been suggested to contribute to AD pathogenesis. The cytokines are divided into two main groups according to the effect: pro-inflammatory and anti-inflammatory, basically, both have antagonist effects as pro-inflammatory cytokines amplify the inflammatory response while anti-inflammatory limit the inflammatory response. IL-1, IL-6, and TNF- α are the most common pro-inflammatory cytokines, which have been found increased in the CSF and brain of AD patients (Zheng, Zhou, and Wang 2016), being described as a relevant factor in AD pathophysiology. On the other hand, anti-inflammatory cytokines such as interleukins (IL's) 4, 10 and 13, are also elevated to negatively modulate the inflammatory response. Interestingly has been reported the increasing levels of those IL's reduced the A β aggregation and inflammatory response observed surrounding senile plaques (Szczepanik et al. 2001) (figure 4).

In AD, microglia is binding to all types of A β such as soluble amyloid β , oligomers, and A β fibrils via microglia receptors including class A scavenger receptor A (SR-A) 1, CD36, CD14, α 6 β 1 integrin, CD47 and toll-like receptors (TLR2, TLR4, TLR6 and TLR9) which are involved with inflammatory response in AD (Bamberger et al. 2003). The binding process is followed by engulfment of A β by microglia to degrade it via endosomes/lysosomes. Although early microglial recruitment promotes A β clearance, the constant accumulation of microglia on the time exacerbate the cytokines release, due to two events: increasing levels of SR-A

Figure 4. Inflammation in Alzheimer's Disease. Amyloid- β peptide, produced by cleavage of the amyloid precursor protein (APP), aggregates forms that activate microglia, in part by signaling through Toll-like receptors (TLRs). These receptors induce the transcription factors NF- κ B, which produce reactive oxygen species (ROS) and drive the expression of inflammatory mediators such as cytokines. These inflammatory factors act directly on neurons and also stimulate astrocytes, which amplify proinflammatory signals to induce neurotoxic effects.; resulting in neuronal apoptosis. Microglia also play protective roles by mediating clearance of A β through phagocytosis mechanisms. Graphic source (CREATIVE COMMONS LICENCE and Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License n.d.).

at the cell membrane (Hickman, Allison, and El Khoury 2008b), and A β plaques are highly resistant to enzymatic degradation, both trigger a general failure of A β clearance (Heneka et al. 2015a; Hickman, Allison, and El Khoury 2008a). (Mawuenyega et al. 2010). This has been suggested, that increased cytokine release has an antagonist effect on phagocytosis by microglia cells via negative regulation of A β phagocytosis receptors. Recently have been described mutations on the microglia receptor TREM2 (triggering receptor expressed in myeloid cells 2) and apoE4, which increased the AD risk (Yuan et al. 2016). TREM2 is a surface receptor highly expressed by microglia and it regulates the phagocytosis of brain debris. TREM2 activity is mainly detected on microglia and reactive astrocytes surrounding apoptotic brain cells and A β plaques (Jay, von Saucken, and Landreth 2017). On the other hand, the overexpression of apoE4 has been described to reduce the clearance of A β and increase its deposition in plaques, however, the precise mechanisms by which apoE4 confers increased

AD risk are not well understood yet (Castellano et al. 2011). Interestingly the activity of both receptors together plays a key role to facilitate microglial phagocytosis of apoptotic neurons, debris, and aggregates such as A β (Atagi et al. 2015). c by differentially regulating clearance of A β from the brain, which is directly related with inflammatory cytokines-mediated neurotoxicity induced by excess A β (Carmona et al. 2018).

Transcriptomic analysis of A β -plaque-associated microglia has revealed a special phenotype, which is related with disease environmental. This phenotype is not observed in microglia in non A β -plaque areas, indicating that the neuroinflammation phenotype mainly occurs in plaque-associated microglia, also known as disease-associated microglia (DAM) (Butovsky and Weiner 2018). These microglia cells have up-regulated genes included Axl, Apoe, Clec7a, Itgax, Lgals3 and Cst7, which have been reported by several groups to be part of common neurodegenerative diseases. Microglia lose the homeostatic molecular functions, related with synaptic stability and become into a chronical inflammatory source, however, more investigations are needed to probe if the microglia can show specific phenotypes and functions depending of the disease and the stages of the disease (Butovsky and Weiner 2018; Yuan et al. 2016).

Mediators in AD inflammation

As it has mentioned before, A β aggregation alone can induce inflammatory response; mainly by microglia over activate which release pro-inflammatory cytokines during long-term that subsequently inducing neuronal apoptosis which contributes to cognitive decline and AD progression (Shabab et al. 2017). These results were also supported in AD mice models, where the pro-inflammatory cytokines such as IL-1 IL-1 β and TNF- α increased proportionally with the A β plaques burden. Furthermore, these cytokines levels were elevated in cerebrospinal fluid (CSF) and plasma of AD patients compared with healthy controls or patients with mild cognitive decline. In addition, in vitro experiments showed that exposition of microglia primary cultures to A β 41-42 oligomers increased the production of IL-1, IL-1 β , and TNF- α (Spangenberg and Green 2017). To produce these cytokines, the microglia activates several molecular pathways which involve enzymes such as caspases. IL-1 β maturation requires the activation of caspase-1; interestingly high levels of caspase-1 are reported in the brains of patients with AD (Flores et al. 2018). Consequently, the levels of IL-1 β are also increased especially around the A β plaques and CSF, as well as the in vitro evidence showed the release of IL-1 β after the stimulation with A β oligomers (W. Y. Wang et al. 2015). Furthermore, has been shown those cytokines can affect the neuronal function altering the action potentials of synaptic transmission in AD model mice. Recently has been reported microglia, macrophages, and dendritic cells coexist in the CNS at steady state which change to activated state after an inflammatory stimulus, during aging and in Alzheimer animal models (Mrdjen et al. 2018).

A β plaques activates NLRP3 inflammasome complex via lysosome disruption, the NLRP3 regulates the caspase-1 dependent maturation and secretion of pro-inflammatory cytokines (Nakanishi et al. 2018). Since during the oligomerization of NLRP3 two adaptor molecules such as ASC and the pro-form of caspase-1 are recruited, leading to the activation (cleavage) of caspase-1. Activated caspase-1, in turn, catalyzes the maturation of IL-1 β . Consequently, has been shown that deficient of caspases-1 and NLRP3 reduce A β plaque burden, furthermore reduce the altered synaptic plasticity, the spatial memory affection and behavioral alteration in AD mice models (Nakanishi et al. 2018). Caspase 3 is also activated in over-reactive microglia which have a positive regulation over the nuclear factor- κ B (NF- κ B), which is a transcription factor for pro-inflammatory mediators such as IL-1 β , TNF α , and nitric oxide (NO) (Wu et al. 2017). Consequently, deletion of caspases 1 and 3 in mice models also showed negative regulation on microglia activation and AD progress (de Calignon et al. 2010). These results suggest that pro-inflammatory cytokines from microglial activate surrounding the A β plaques are potentially detrimental for brain homeostasis increasing the AD pathology.

The pro-inflammatory cytokines via surface receptors activate the enzyme inducible nitric oxide synthase (iNOS) in microglia, which increase the production of NO that in high concentration is neurotoxic (Sierra et al. 2014). The levels of iNOS are up-regulated in AD patients favoring the posttranslational nitration on tyrosine amino acids of A β peptides which increase their disposition to aggregate, interestingly the nitration has been found in the core of A β plaques, suggesting nitrated A β is able to initiate the plaque formation and transforming the plaque into insoluble stage being NO relevant during the early stage of development of AD (Heneka et al. 2015b). In contrast, the genetic ablation of iNOS reduces the A β burden and inflammatory response in AD mice models. This indicates posttranslational modification causes structural damage to AD brain (Nathan et al. 2005).

Therapeutic strategies to treat AD.

Diagnose Alzheimer's dementia, is the first obstacle to understand the disease since is difficult to have an accurate diagnosis. The clinical diagnosis needs the association of neurological and psychiatric history of the patient; furthermore, neuropathology, neuroimaging, computed tomography and the clinical phase called moderate cognitive impairment (MCI, Mild Cognitive Impairment), are determining factors to differentiate Alzheimer's disease from other causes of dementia (dos Santos Picanco et al. 2018). Currently, new promising diagnostic tests are emerging, such as positron emission tomography (PET), biomarkers and determination of atrophy of medial temporal lobe by magnetic resonance imaging (MRI), which basically seeks identify with a high sensitivity, patients with Alzheimer's disease, however still without clear results as well as the clinical treatments (dos Santos Picanco et al. 2018).

There is a high unmet medical need for effective treatment of AD. Despite all the therapeutic approaches, the treatments are still palliative, without blocking the cause of the disease. In recent years, some drugs have been proposed for the treatment of AD, the goal of current pharmacologic therapies is to delay the progression of symptoms of neurocognitive and physical decline mainly focusing on early stages of AD. However most of them are in the experimental phase and currently, the approved treatments have different molecular brain targets (Cummings et al. 2018).

AD brains have reduced the levels of Acetylcholine neurotransmitter and their function which is involved on cognitive impairment. The overactivity of cholinesterase enzyme (AChE) degrade the acetylcholine from the synaptic cleft, which decreases the signal transmission between neurons. There have been developed specific inhibitors of cholinesterase avoiding reversibly their activity on Acetylcholine, thereby increasing cholinergic transmission between neurons. However, after several years of clinical trials, the benefits of this treatment on clinical symptoms of AD patients seems not too relevant. Since, the cognitive decline decreased 3.15 points less than placebo on a 78-point assessment scale over 4 years with a delay on the AD progression of 6 months, as long as vitamin E is included with the treatment (Epperly, Dunay, and Boice 2017). There is also approved partial inhibitor of N-methyl-D-aspartate (NMDA) receptors, Memantine, which reduce the toxic effect from Glutamate excess on hippocampal neurons, this inhibitor has been used on several dementia types, showing benefits in cognition functional and global status, however the benefits can be classified as moderate and not consistent results between patients. The magnitude of benefit was: 2.97 points on a 100-point cognitive assessment scale, 1.27 points on a 54-point ADL scale, and 2.76 points on a 144-point behavior scale (Epperly, Dunay, and Boice 2017).

Other medications that have been investigated include statins, non-steroidal anti-inflammatory to limit the aggregation and inflammation induced by of A β plaques in AD patients, however, were not found a beneficial effect on the cognitive process and functional status. On the other hand, the poly-unsaturated fatty acid Omega-3 has been tested on AD patients and healthy controls having not beneficial effects on AD patients, nonetheless this fatty acid is still a potential target because observational studies have suggested the intake of dietary omega-3 reduce the incidence of dementia, being a potential preventive treatment against dementia due to their anti-inflammatory effects (Ajith 2018).

To control the inflammatory processes that result from the disease, several therapeutic strategies have been tried, which are basically aimed at inhibiting cyclooxygenase 2 (COX2), prostaglandin E2 receptors (PGE2), stimulation of phagocytosis by microglia, immunization with A β , blocking complement factors and TNF- α , among others; the results of those therapies are still controversial. The possible explanations for the absence of success with anti-inflammatory therapy with more established dementia include a failure to stop the spread of fully established tauopathy and failure to rescue neuron loss (Heneka et al. 2015b).

Figure 5. RNAi natural process. Drosha cleaves the Pri-miRNA to generate a pre-miRNA or short hairpin RNA (shRNA) it requires the activity of endogenous Exportin 5 for nuclear export. Ago2 (Argonaute 2) is recruited by the role of DICER binding partner (TRBP), that forms a dimer with Dicer, and then receives the pre-miRNA. The shRNA is cleaved in one step by DICER generating a 19-22 nucleotides duplex siRNA. After identification of the “guide strand” in the siRNA duplex, the “passenger strand” is cleaved by Ago2. The “guide strand” is integrated into the RISC that contains different argonautes and argonaute-associated proteins. The siRNA guides RISC to the target mRNA. The target mRNA is cleaved by Ago2 and degraded. Graphic template source (CREATIVE COMMONS LICENCE and Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License n.d.).

In addition, some drugs have been designed to act on the activity of amyloid-producing enzymes, however most of them are in the experimental phase; within these we have: the activators of α -secretase and the inhibitors of β and γ secretase, as therapeutic candidates, which prevent production and aggregation of A β and favoring their elimination; A β -immunotherapy, either by passive or active immunization, seeks to generate anti-A β antibodies, which bind to the senile plaques, inducing elimination by the microglia or alternatively joining the soluble A β in the periphery, facilitating its exit from the brain; inhibitors of fibrillation of A β , base their mechanism of action, by interfering with A β -A β and A β -ApoE interactions, preventing conformational changes in their structure and subsequent fibrillation; however, the results are controversial. Inhibitors of BACE-1 such as Verubecestat showed efficiency to reduce the A β level in the cerebrospinal fluid, but no effect on cognitive or functional decline of patients with Alzheimer’s disease (Egan et al. 2018), in contrast, AZD3293- BACE1 inhibitor had prolonged suppression of plasma and cerebrospinal fluid A β , and Two “Phase III” studies are now ongoing to know the effects on cognitive decline (Cebers et al. 2016). Furthermore, comparative studies have been shown BACE1 inhibitor treatments more effective than immune therapies, since, results showed that AZD3293 is a better therapeutic agent for treating AD than Solanezumab (Hassan et al. 2018). Currently, clinical trials of

gamma-secretase inhibitors, aggregation blockers, vaccination with A β , vaccination with monoclonal antibodies against A β epitopes are ongoing. Finally, other candidates have been proposed for their potential as a therapy, such as genetic therapies by neutralizing targeted mRNA molecules of β or Ω secretase, however, they are still in development stages (Diego Piedrahita et al. 2016; Villamil-Ortiz et al. 2016).

RNA interference (RNAi) a therapeutic alternative to treat Alzheimer's disease

A wide variety of factors can induce AD and it is hard to understand what the functions of these factors are and how they work together to triggering AD; however, RNAi may make it easy to determine these. Since RNAi can be used to bind and allow the degradation of specific mRNA, which make evident endogenous biological pathways, it can be a potential therapeutic strategy, by decreasing the expression of genes and observe the changes on a specific molecular pathway, as well as biological and pharmacological effects in cell cultures and animal models for several diseases (Chakraborty et al. 2017b; Bobbin and Rossi 2016). Compared with other therapeutics, the advantage of RNAi is that the targets include those that are not easy to be targeted with drugs might be amenable for RNAi targeting. This is because in theory any transcript may be targeted by RNAi. However, stability and the release of RNAi in vivo, low intracellular uptake and non-specific stimulation of the immune system are critical points to be resolved in order to develop therapeutic RNAi for clinical use. Currently, RNAi is being used in basic bioscience, to solve the specific problem of several diseases such as AD. To date, approximately 20 clinical trials have been initiated using RNAi based therapeutics. Several other RNAi therapeutics are in the preclinical stage and aiming to enter clinical trials (Chakraborty et al. 2017a).

RNAi regulates the synthesis of protein with a posttranscriptional modification, which is a gene-silencing mechanism. miRNA maturation involves two cleavages mediated by RNAseIII enzymes DROSHA and DICER (Ipsaro and Joshua-Tor 2015). DROSHA works inside the nucleus on pri-miRNA releasing a hairpin of 65 nucleotides, being transported into the cytoplasm as a double strand pre-miRNA. There pre-miRNA is cleaved by DICER enzyme liberating ~22-nucleotides mature miRNA duplex, called small interfering RNA (siRNA), later the siRNA is incorporated in an enzymatic complex called RNA-induced silencing complex (RISC), where the sense strand of siRNA is cleavage by Argonaute 2 (Ago2). The RISC complex beside containing DICER, is also constituted by other proteins such as Argonauts, TRBP, PACT, Gemin3 and KSRP, the last one, is part of both Dicer and the Drosha complex, joining the precursors of the miRNAs with a high affinity, facilitating its maturation. The anti-sense RNA strand induces RISC complex to bind with the mRNA target, which is complementary to the antisense strand and degrading it. In addition, the activated RISC-siRNA complex can then be recycled for the degradation of identical mRNA targets by RNases (Li and Patel 2016) (figure 5).

Currently, Pharma companies are designing clinical trials for RNAi therapeutics. Based on several biomedical studies, which has been developed different computer models and mathematical algorithms for the prediction and design of interference RNA, establishing general principles; such as: 1) both the sense and anti-sense strand are composed of 21 nucleotides, with 2 protruding nucleotides in the 3'; 2) to select the target sequence, it must be taken from 50 nucleotides downstream of the stop codon of the target mRNA; 3) the sequence must have low Guanine/Cytosine content (not exceeding 50%); 4) the sense band must have a low internal stability in the 3'.

These rules are important to create RNAi with high efficiency; for example, silencing effects in *C. elegans* can be disseminated and transmitted to several generations. The conversion of dsRNA to a siRNA fragment of 21-23 nucleotide can by itself provide amplification, which can explain the potential of RNAi strategy. Another possible explanation is the RISC complex, where a simple active center can catalyze multiple reactions with catalytic targets and cleavage activities. The amplification process also involves RNA-dependent RNA polymerase (RdRP) (Chakraborty et al. 2017b). As the primers can be linked in a random manner to target sequences, where siRNA, serves as a primer for a RdRP reaction, where siRNA-primer converts the mRNA into dsRNA so that DICER degrades it to siRNA and thus starts a chain reaction. Therefore, target specificity and wide spectrum give RNAi advantages respect to traditional small molecules and protein drugs. However, the biological barriers are present especially when the brain cells are the target, which raised function versatility requirements to RNAi therapies, since; the right design and efficacy of RNAi are determinants to break down the barriers to target tissues in local or systemic therapies delivery (Chakraborty et al. 2017a).

BACE1 is considered an excellent therapeutic target for the treatment of AD, since, in addition to being a key enzyme in the production of A β , BACE1 knockdown reduces APP levels, catalytic activity is reduced and mice have no detectable abnormalities (D Piedrahita et al. 2015). Recently, RNAi appears as an attractive mechanism to induce a knockdown and overcome the problems of specificity presented by different approaches, such as, the design of BACE1 inhibitors which has presented undesirable side effects (Hu et al. 2008; Moussa 2017). Consequently, our most recent findings have validated the potential of BACE1 silencing in neuroprotection, since it causes specific BACE1 decreasing levels and it has been correlated with the decrease in protein aggregation (D Piedrahita et al. 2015). Additionally, macro-autophagy was establishing how the main NFT degradation pathway after shBACE-1miR treatment. Interestingly the BACE1miR-mediated soluble hyperphosphorylated tau downregulation was only blocked by 3-MA (a class III PI3K inhibitor) in vitro. Together, these data suggest a role for BACE1miR in the double-membrane vesicles and in membrane fusion to form autophagosomes, which requires phosphatidylethanolamine, suggesting an interaction between BACE1 activity and lipid metabolism to induce the reduction of NFT (Piedrahita et al. 2016). Supporting that the silencing of BACE1 is a good target to prevent the formation of A β and NFT.

The alteration in lipid metabolism can influence the development of AD, influencing the production and accumulation of A β in membrane microdomains rich in saturated fatty acids (SFA) (H.-L. Wang et al. 2016a; Panchal et al. 2014). In addition, cellular SFA levels modulate the metabolism of APP; through increasing the stability of APP-BACE1 interaction, leading to increasing in A β levels and accelerates the AD pathology (Xiang, Lam, and Shui 2015). In rodents, high cholesterol levels in neurons cause an increase in BACE1 levels, and accumulation of A β 1-42 peptide; furthermore, it is correlated with tau hyperphosphorylation (Moussa 2017). Have been reported in mice AD model and AD human that A β is finding in cellular structures with a high content of SFA such as lipid rafts, indicating that lipids are an important player involve on AD development mainly influencing the plaque formation (Hong 2015).

In addition, recent studies show direct correlation between AD and neuroinflammation showing that this correlation is being modulated by lipid metabolism. Due to several lipid species have diverse biological activities, in order to maintain tissue homeostasis including regulation of inflammation and its resolution (Heppner, Ransohoff, and Becher 2015b; Han-nun and Obeid 2008). The alteration of lipid homeostasis, trigger, thereby increasing the pro-inflammatory response in the CNS (Gualtierotti et al. 2017). Have been reported that families of bioactive lipids like lysophospholipids and sphingolipids as well as several elements of their complex metabolism and signaling (i.e., enzymes and receptors) are mainly dysregulated chronic inflammatory diseases and AD (Di Paolo and Kim 2011; Buckley, Gilroy, and Serhan 2014). Indicating that lipids are essential on inflammatory processes as well as in protein aggregation disorders, which suggest that the modulation of lipids; could be a future and promising therapeutic strategy to be undertaken.

For all the above, we propose as an alternative to potentiate the homeostasis recovery of the brain affected by AD, developing a knockdown of BACE1 mRNA at the CA1 hippocampal area using RNA interference as a therapeutic strategy, which would reduce the A β aggregation levels. On the other hand, the homeostasis recovery involves several molecular factors, including lipid metabolism and inflammatory response when our therapy is in the CNS. BACE1 protein levels are reduced could it modify the lipid composition of cell membranes? And whether the membrane lipid composition is modified, would these lipid changes modulate the neuro-inflammatory mediators at the AD mice brain and primary neuron cultures?

HYPOTHESIS

BACE1 mRNA silencing induces recovery of cell homeostasis, through the regulation of fatty acid metabolism and pro-inflammatory response in the hippocampus region in the 3xTg-AD mouse model (in vivo) and primary cortical neuron cultures (in vitro).

MAIN AIM

Establish the effect of BACE1 silencing on fatty acid metabolism and neuroinflammation using an AD mice model and primary neuron cultures.

SPECIFIC AIMS

- Examine the effect of the silencing of BACE1 on the hippocampus lipid profile in 3xTg-AD mice and primary cortical neuron cultures.
- Evaluate the relationship between the lipid profile induced by the silencing of BACE1 and pro-inflammatory mediators in 3xTg-AD model and cortex primary neuron cultures.
- Establish the ratio of membrane lipid profiles in the brains of patients diagnosed with Alzheimer's disease. In order to propose critical points of therapeutic intervention for the early-onset AD and late-onset AD.

References

- Abdullah, L. et al. APOE ϵ 4 specific imbalance of arachidonic acid and docosahexaenoic acid in serum phospholipids identifies individuals with preclinical Mild Cognitive Impairment/Alzheimer's Disease. *Aging (Albany, NY)* 9, 964–985 (2017).
- Ajith, T. A. A recent update on the effects of omega-3 fatty acids in Alzheimer's disease. *Curr. Clin. Pharmacol.* 13, (2018).
- Akiyama, H. et al. Inflammation and Alzheimer's disease. *Neurobiol Aging* 21, 383–421 (2000).
- Alonso, R., Mata, N. & Mata, P. Benefits and risks assessment of simvastatin in familial hypercholesterolemia. *Expert Opin Drug Saf* 4, 171–181 (2005).
- Atagi, Y. et al. Apolipoprotein E Is a Ligand for Triggering Receptor Expressed on Myeloid Cells 2 (TREM2). *J. Biol. Chem.* 290, 26043–26050 (2015).
- Bamberger, M. E., Harris, M. E., McDonald, D. R., Husemann, J. & Landreth, G. E. A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. *J. Neurosci.* 23, 2665–74 (2003).
- Barage, S. H. & Sonawane, K. D. Amyloid cascade hypothesis: Pathogenesis and therapeutic strategies in Alzheimer's disease. *Neuropeptides* 52, 1–18 (2015).
- Barao, S., Moechars, D., Lichtenthaler, S. F. & De Strooper, B. BACE1 Physiological Functions May Limit Its Use as Therapeutic Target for Alzheimer's Disease. *Trends Neurosci* 39, 158–169 (2016).
- Bate, C. Breaking the cycle, cholesterol cycling, and synapse damage in response to amyloid- β . *J. Exp. Neurosci.* 11, (2017).
- Bazinet, R. P. & Laye, S. Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat Rev Neurosci* 15, 771–785 (2014).
- Ben Halima, S. et al. Specific Inhibition of β -Secretase Processing of the Alzheimer Disease Amyloid Precursor Protein. *Cell Rep.* 14, 2127–2141 (2016).
- Bharadwaj, P. et al. Role of the cell membrane interface in modulating production and uptake of Alzheimer's beta amyloid protein. *Biochim. Biophys. Acta - Biomembr.* (2018). doi:10.1016/j.bbmem.2018.03.015
- Bignante, E. A., Heredia, F., Morfini, G. & Lorenzo, A. Amyloid β precursor protein as a molecular target for amyloid β -induced neuronal degeneration in Alzheimer's disease. *Neurobiol Aging* 34, 2525–2537 (2013).
- Bloom, G. S. Amyloid- β and Tau. *JAMA Neurol.* 71, 505 (2014).
- Bloom, G. S. Amyloid- β and Tau. *JAMA Neurol.* 71, 505 (2014).
- Bryleva, E. Y. et al. ACAT1 gene ablation increases 24(S)-hydroxycholesterol content in the brain and ameliorates amyloid pathology in mice with AD. *Proc Natl Acad Sci U S A* 107, 3081–3086 (2010).
- Bu, G. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat Rev Neurosci* 10, 333–344 (2009).
- Butovsky, O. & Weiner, H. L. Microglial signatures and their role in health and disease. *Nat. Rev. Neurosci.* 19, 622–635 (2018).
- Cai, D. et al. Presenilin-1 uses phospholipase D1 as a negative regulator of beta-amyloid formation. *Proc Natl Acad Sci U S A* 103, 1941–1946 (2006).
- Castellano, J. M. et al. Human apoE Isoforms Differentially Regulate Brain Amyloid- Peptide Clearance. *Sci. Transl. Med.* 3, 89ra57-89ra57 (2011).
- Cebers, G. et al. AZD3293: Pharmacokinetic and Pharmacodynamic Effects in Healthy Subjects and Patients with Alzheimer's Disease. *J. Alzheimer's Dis.* 55, 1039–1053 (2016).
- Chakraborty, C., Sharma, A. R., Sharma, G., Doss, C. G. P. & Lee, S.-S. Therapeutic miRNA and siRNA: Moving from Bench to Clinic as Next Generation Medicine. *Mol. Ther. - Nucleic Acids* 8, 132–143 (2017).
- Chang, T. Y., Chang, C. C., Ohgami, N. & Yamauchi, Y. Cholesterol sensing, trafficking, and esterification. *Annu Rev Cell Dev Biol* 22, 129–157 (2006).
- Chen, C. T., Green, J. T., Orr, S. K. & Bazinet, R. P. Regulation of brain polyunsaturated fatty acid uptake and turnover. *Prostaglandins, Leukot. Essent. Fat. Acids* 79, 85–91 (2008).
- Choi, G. E. et al. Membrane-associated effects of glucocorticoid on BACE1 upregulation and A β generation: involvement of lipid raft-mediated CREB activation. *J. Neurosci.* 37, 0074-17 (2017).
- Congdon, E. E. & Sigurdsson, E. M. Tau-targeting therapies for Alzheimer disease. *Nat. Rev. Neurol.* (2018). doi:10.1038/s41582-018-0013-z
- Congdon, E. E. & Sigurdsson, E. M. Tau-targeting therapies for Alzheimer disease. *Nat. Rev. Neurol.* (2018). doi:10.1038/s41582-018-0013-z
- CREATIVE COMMONS LICENCE & Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License. SMART - Servier Medical ART - 3000 free medical images. Available at: <https://smart.servier.com/>. (Accessed: 1st October 2018)
- Cuervo, A. M., Wong, E. S. & Martinez-Vicente, M. Protein degradation, aggregation, and misfolding. *Mov Disord* 25 Suppl 1, S49-S4 (2010).
- Cunnane, S. C., Chouinard-Watkins, R., Castellano, C. A. & Barberger-Gateau, P. Docosahexaenoic acid homeostasis, brain aging and Alzheimer's disease: Can we reconcile the evidence? *Prostaglandins Leukot Essent Fat. Acids* 88, 61–70 (2013).
- de Calignon, A. et al. Caspase activation precedes and leads to tangles. *Nature* 464, 1201–1204 (2010).
- Desbène, C. et al. Critical role of cPLA2 in A β oligomer-induced neurodegeneration and memory deficit. *Neurobiol Aging* 33, 1123.e17-29 (2012).
- Di Paolo, G. & Kim, T. W. Linking lipids to Alzheimer's disease: cholesterol and beyond. *Nat Rev Neurosci* 12, 284–296 (2011).
- Díaz, M. et al. Biophysical Alterations in Lipid Rafts from Human Cerebral Cortex Associate with Increased BACE1/A β PP Interaction in Early Stages of Alzheimer's Disease. *J. Alzheimer's Dis.* 43, 1185–1198 (2014).
- Dinkins, M. B., Wang, G. & Bieberich, E. Sphingolipid-Enriched Extracellular Vesicles and Alzheimer's Disease: A Decade of Research. *J. Alzheimer's Dis.* 60, 757–768 (2017).
- dos Santos Picanco, L. C. et al. Alzheimer's Disease: A Review from the Pathophysiology to Diagnosis, New Perspectives for Pharmacological Treatment. *Curr. Med. Chem.* 25, 3141–3159 (2018).
- Duthey, B. Priority Medicines for Europe and the World "A Public Health Approach to Innovation Alzheimer Disease and other Dementias. (2013).
- Egan, M. F. et al. Randomized Trial of Verubecestat for Mild-to-Moderate Alzheimer's Disease. *N. Engl. J. Med.* 378, 1691–1703 (2018).
- Evin, G. & Hince, C. BACE1 as a Therapeutic Target in Alzheimer's Disease: Rationale and Current Status. *Drugs Aging* (2013). doi:10.1007/s40266-013-0099-3
- Fassbender, K. 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* 98, 5856–5861 (2001).
- Fonseca, A. C. R. G., Resende, R., Oliveira, C. R. & Pereira, C. M. F. Cholesterol and statins in Alzheimer's disease: Current controversies. *Exp. Neurol.* 223, 282–293 (2010).
- Fonteh, A. N. et al. Sphingolipid Metabolism Correlates with Cerebrospinal Fluid Beta Amyloid Levels in Alzheimer's Disease. *PLoS One* 10, e0125597 (2015).
- Freude, K. K., Penjwini, M., Davis, J. L., LaFerla, F. M. & Blurton-Jones, M. Soluble amyloid precursor protein induces rapid neural differentiation of human embryonic stem cells. *J Biol Chem* 286, 24264–24274 (2011).
- Freund-Levi, Y. 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* 63, 1402–1408 (2006).
- Frohman, M. A. The phospholipase D superfamily as therapeutic targets. *Trends Pharmacol. Sci.* 36, 137–144 (2015).
- Fukami, Y., Ariga, T., Yamada, M. & Yuki, N. Brain Gangliosides in Alzheimer's Disease: Increased Expression of Cholinergic Neuron-Specific Gangliosides. *Curr. Alzheimer Res.* 14, 586–591 (2017).
- Fukami, Y., Ariga, T., Yamada, M. & Yuki, N. Brain Gangliosides in Alzheimer's Disease: Increased Expression of Cholinergic Neuron-Specific Gangliosides. *Curr. Alzheimer Res.* 14, 586–591 (2017).
- Giudetti, A. M., Romano, A., Lavecchia, A. M. & Gattani, S. The Role of Brain Cholesterol and its Oxidized Products in Alzheimer's Disease. *Curr. Alzheimer Res.* 13, 198–205 (2016).
- Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C. & Gage, F. H. Mechanisms Underlying Inflammation in Neurodegeneration. *Cell* 140, 918–934 (2010).
- Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C. & Gage, F. H. Mechanisms Underlying Inflammation in Neurodegeneration. *Cell* 140, 918–934 (2010).

Grimm, M. O. W. et al. Intracellular APP Domain Regulates Serine-Palmitoyl-CoA Transferase Expression and Is Affected in Alzheimer's Disease. *Int. J. Alzheimers. Dis.* 2011, 695413 (2011).

Hammond, T. R. et al. Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. *Immunity* (2018). doi:10.1016/j.immuni.2018.11.004
Hannun, Y. A. & Obeid, L. M. Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Biol* 9, 139–150 (2008).

Hardy, J. The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. *Science* (80-.). 297, 353–356 (2002).

Hartmann, T., Kuchenbecker, J. & Grimm, M. O. Alzheimer's disease: the lipid connection. *J Neurochem* 103 Suppl, 159–170 (2007).

Hassan, M. et al. Molecular Docking and Dynamic Simulation of AZD3293 and Solanezumab Effects Against BACE1 to Treat Alzheimer's Disease. *Front. Comput. Neurosci.* 12, 34 (2018).

He, X., Huang, Y., Li, B., Gong, C. X. & Schuchman, E. H. Deregulation of sphingolipid metabolism in Alzheimer's disease. *Neurobiol Aging* 31, 398–408 (2010).
Heneka, M. T. et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 14, 388–405 (2015).

Heneka, M. T. et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 14, 388–405 (2015).
Heppner, F. L., Ransohoff, R. M. & Becher, B. Immune attack: the role of inflammation in Alzheimer disease. *Nat. Rev. Neurosci.* 16, 358–372 (2015).

Heppner, F. L., Ransohoff, R. M. & Becher, B. Immune attack: the role of inflammation in Alzheimer disease. *Nat. Rev. Neurosci.* 16, 358–372 (2015).

Heras-Sandoval, D., Pedraza-Chaverri, J. & Pérez-Rojas, J. M. Role of docosahexaenoic acid in the modulation of glial cells in Alzheimer's disease. *J. Neuroinflammation* 13, 61 (2016).

Hickman, S. E., Allison, E. K. & El Khoury, J. Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. *J. Neurosci.* 28, 8354–60 (2008).

Hickman, S. E., Allison, E. K. & El Khoury, J. Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. *J. Neurosci.* 28, 8354–60 (2008).

Hirsch-Reinshagen, V., Burgess, B. L. & Wellington, C. L. Why lipids are important for Alzheimer disease? *Mol Cell Biochem* 326, 121–129 (2009).

Hong, H. Role of Lipids in Folding, Misfolding and Function of Integral Membrane Proteins. *Adv Exp Med Biol* 855, 1–31 (2015).

Hong, H. Role of Lipids in Folding, Misfolding and Function of Integral Membrane Proteins. *Adv Exp Med Biol* 855, 1–31 (2015).

Hu, X. et al. Genetic deletion of BACE1 in mice affects remyelination of sciatic nerves. *FASEB J* 22, 2970–2980 (2008).

Huttunen, H. J. et al. The acyl-coenzyme A: cholesterol acyltransferase inhibitor CI-1011 reverses diffuse brain amyloid pathology in aged amyloid precursor protein transgenic mice. *J. Neuropathol. Exp. Neurol.* 69, 777–88 (2010).

Iacono, D. et al. Mild Cognitive Impairment and Asymptomatic Alzheimer Disease Subjects. *J. Neuropathol. Exp. Neurol.* 73, 295–304 (2014).

Jang, H. J. et al. Phospholipase C- γ 1 involved in brain disorders. *Adv Biol Regul* 53, 51–62 (2013).

Jay, T. R., von Saucken, V. E. & Landreth, G. E. TREM2 in Neurodegenerative Diseases. *Mol. Neurodegener.* 12, 56 (2017).

Jimenez, S. et al. Age-dependent Accumulation of Soluble Amyloid β (A β) Oligomers Reverses the Neuroprotective Effect of Soluble Amyloid Precursor Protein- α (sAPP α) by Modulating Phosphatidylinositol 3-Kinase (PI3K)/Akt-GSK-3 β Pathway in Alzheimer Mouse Model. *J. Biol. Chem.* 286, 18414–18425 (2011).

Kalvodova, L. et al. Lipids as modulators of proteolytic activity of BACE: involvement of cholesterol, glycosphingolipids, and anionic phospholipids in vitro. *J Biol Chem* 280, 36815–36823 (2005).

Kamal, A., Almenar-Queral, A., LeBlanc, J. F., Roberts, E. A. & Goldstein, L. S. Kinesin-mediated axonal transport of a membrane compartment containing beta-secretase and presenilin-1 requires APP. *Nature* 414, 643–648 (2001).

Keren-Shaul, H. et al. A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell* 169, 1276–1290.e17 (2017).

Kivipelto, M. et al. Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, popula-

tion based study. *BMJ* 322, 1447–1451 (2001).

Koelsch, G. BACE1 Function and Inhibition: Implications of Intervention in the Amyloid Pathway of Alzheimer's Disease Pathology. *Molecules* 22, 1723 (2017).

Lai, M. K. P. et al. Selective effects of the APOE epsilon4 allele on presynaptic cholinergic markers in the neocortex of Alzheimer's disease. *Neurobiol. Dis.* 22, 555–61 (2006).

Landman, N. et al. Presenilin mutations linked to familial Alzheimer's disease cause an imbalance in phosphatidylinositol 4,5-bisphosphate metabolism. *Proc Natl Acad Sci U S A* 103, 19524–19529 (2006).

LeBlanc, A. C., Koutroumanis, M. & Goodyer, C. G. Protein kinase C activation increases release of secreted amyloid precursor protein without decreasing Abeta production in human primary neuron cultures. *J Neurosci* 18, 2907–2913 (1998).

Lefort, R., Pozueta, J. & Shelanski, M. Cross-linking of cell surface amyloid precursor protein leads to increased β -amyloid peptide production in hippocampal neurons: implications for Alzheimer's disease. *J Neurosci* 32, 10674–10685 (2012).

Li, S. & Patel, D. J. Drosha and Dicer: Slicers cut from the same cloth. *Cell Res.* 26, 511–512 (2016).

Lichtenthaler, S. F. Alpha-secretase cleavage of the amyloid precursor protein: proteolysis regulated by signaling pathways and protein trafficking. *Curr Alzheimer Res* 9, 165–177 (2012).

Lingwood, D. & Simons, K. Lipid Rafts As a Membrane-Organizing Principle. *Science* (80-.). 327, 46–50 (2010).

Liu, Y. et al. The Role of Phospholipase D in Modulating the MTOR Signaling Pathway in Polycystic Kidney Disease. *PLoS One* 8, e73173 (2013).

Luo, D., Long, Y. & Chen, G.-J. Cyclooxygenase-2 gene polymorphisms and risk of Alzheimer's disease: A meta-analysis. *J. Neurol. Sci.* 359, 100–105 (2015).

Ma, H. et al. Involvement of beta-site APP cleaving enzyme 1 (BACE1) in amyloid precursor protein-mediated enhancement of memory and activity-dependent synaptic plasticity. *Proc Natl Acad Sci U S A* 104, 8167–8172 (2007).

Mahfoud, R. et al. Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins. *J Biol Chem* 277, 11292–11296 (2002).

Marin, R. et al. Anomalies occurring in lipid profiles and protein distribution in frontal cortex lipid rafts in dementia with Lewy bodies disclose neurochemical traits partially shared by Alzheimer's and Parkinson's diseases. *Neurobiol. Aging* 49, 52–59 (2017).

Marquer, C. et al. Local cholesterol increase triggers amyloid precursor protein-Bace1 clustering in lipid rafts and rapid endocytosis. *FASEB J* 25, 1295–1305 (2011).

Mattson, M. P., Guo, Z. H. & Geiger, J. D. Secreted form of amyloid precursor protein enhances basal glucose and glutamate transport and protects against oxidative impairment of glucose and glutamate transport in synaptosomes by a cyclic GMP-mediated mechanism. *J Neurochem* 73, 532–537 (1999).

Mawuenyega, K. G. et al. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science* 330, 1774 (2010).

Mielke, M. M. et al. Plasma Sphingomyelins are Associated with Cognitive Progression in Alzheimer's Disease. *J. Alzheimer's Dis.* 27, 259–269 (2011).

Mohaibes, R. J. et al. The hydroxylated form of docosahexaenoic acid (DHA-H) modifies the brain lipid composition in a model of Alzheimer's disease, improving behavioral motor function and survival. *Biochim. Biophys. Acta - Biomembr.* 1859, 1596–1603 (2017).

Moussa, C. E.-H. Beta-secretase inhibitors in phase I and phase II clinical trials for Alzheimer's disease. *Expert Opin. Investig. Drugs* 26, 1131–1136 (2017).

Mrdjen, D. et al. High-Dimensional Single-Cell Mapping of Central Nervous System Immune Cells Reveals Distinct Myeloid Subsets in Health, Aging, and Disease. *Immunity* 48, 380–395.e6 (2018).

Murphy, S. R. et al. Acat1 Knockdown Gene Therapy Decreases Amyloid- β in a Mouse Model of Alzheimer's Disease. *Mol. Ther.* 21, 1497–1506 (2013).

Nalivaeva, N. N. & Turner, A. J. The amyloid precursor protein: a biochemical enigma in brain development, function and disease. *FEBS Lett* 587, 2046–2054 (2013).

Noel, A., Ingrand, S. & Barrier, L. Ganglioside and related-sphingolipid profiles are altered in a cellular model of Alzheimer's disease. *Biochimie* 137, 158–164 (2017).

Obregon, D. et al. Soluble amyloid precursor protein- α modulates β -secretase activity and amyloid- β generation. *Nat Commun* 3, 777 (2012).

Oliveira, T. G. et al. Phospholipase d2 ablation amelio-

rates Alzheimer's disease-linked synaptic dysfunction and cognitive deficits. *J Neurosci* 30, 16419–16428 (2010).

Ostrowski, S. M., Wilkinson, B. L., Golde, T. E. & Landreth, G. Statins reduce amyloid-beta production through inhibition of protein isoprenylation. *J Biol Chem* 282, 26832–26844 (2007).

Panchal, M. et al. Ceramides and sphingomyelinases in senile plaques. *Neurobiol. Dis.* 65, 193–201 (2014).
Parkhurst, C. N. et al. Microglia Promote Learning-Dependent Synapse Formation through Brain-Derived Neurotrophic Factor. *Cell* 155, 1596–1609 (2013).

Pfriege, F. W. Cholesterol homeostasis and function in neurons of the central nervous system. *Cell Mol Life Sci* 60, 1158–1171 (2003).

Piedrahita, D. et al. beta-Secretase 1's Targeting Reduces Hyperphosphorylated Tau, Implying Autophagy Actors in 3xTg-AD Mice. *Front Cell Neurosci* 9, 498 (2015).

Piedrahita, D. et al. β -Secretase 1's Targeting Reduces Hyperphosphorylated Tau, Implying Autophagy Actors in 3xTg-AD Mice. *Front. Cell. Neurosci.* 9, (2016).

Rao, J. S., Kellom, M., Kim, H. W., Rapoport, S. I. & Reese, E. A. Neuroinflammation and synaptic loss. *Neurochem Res* 37, 903–910 (2012).

Rebeck, G. W. The role of APOE on lipid homeostasis and inflammation in normal brains. *J. Lipid Res.* 58, 1493–1499 (2017).

Reid, P. C., Urano, Y., Kodama, T. & Hamakubo, T. Alzheimer's disease: cholesterol, membrane rafts, isoprenoids and statins. *J. Cell. Mol. Med.* 11, 383–92 (2007).

Reitz, C. & Mayeux, R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochem Pharmacol* 88, 640–651 (2014).

Rituper, B. et al. Cholesterol-mediated membrane surface area dynamics in neuroendocrine cells. *Biochim Biophys Acta* 1831, 1228–1238 (2013).

Saeedi Saravi, S. S., Saeedi Saravi, S. S., Arefidoust, A. & Dehpour, A. R. The beneficial effects of HMG-CoA reductase inhibitors in the processes of neurodegeneration. *Metab. Brain Dis.* 32, 949–965 (2017).

Salter, M. W. & Beggs, S. Sublime Microglia: Expanding Roles for the Guardians of the CNS. *Cell* 158, 15–24

(2014).

Sanchez-Mejia, R. O. & Mucke, L. Phospholipase A2 and arachidonic acid in Alzheimer's disease. *Biochim Biophys Acta* 1801, 784–790 (2010).

Sanchez-Mejia, R. O. et al. Phospholipase A2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease. *Nat Neurosci* 11, 1311–1318 (2008).

Sato, N. & Morishita, R. The roles of lipid and glucose metabolism in modulation of β -amyloid, tau, and neurodegeneration in the pathogenesis of Alzheimer disease. *Front. Aging Neurosci.* 7, 199 (2015).

Schafer, D. P. & Stevens, B. Microglia Function in Central Nervous System Development and Plasticity. *Cold Spring Harb. Perspect. Biol.* 7, a020545 (2015).

Schreiber, S. et al. Alzheimer Disease Signature Neurodegeneration and APOE Genotype in Mild Cognitive Impairment With Suspected Non-Alzheimer Disease Pathophysiology. *JAMA Neurol.* 74, 650 (2017).

Selkoe, D. & Kopan, R. Notch and Presenilin: regulated intramembrane proteolysis links development and degeneration. *Annu Rev Neurosci* 26, 565–597 (2003).

Sharpe, L. J. & Brown, A. J. Controlling cholesterol synthesis beyond 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). *J Biol Chem* 288, 18707–18715 (2013).

Stoothoff, W. H. & Johnson, G. V. Tau phosphorylation: physiological and pathological consequences. *Biochim. Biophys. Acta* 1739, 280–297 (2005).

Szczepanik, A. M., Funes, S., Petko, W. & Ringheim, G. E. IL-4, IL-10 and IL-13 modulate A β (1–42)-induced cytokine and chemokine production in primary murine microglia and a human monocyte cell line. *J. Neuroimmunol.* 113, 49–62 (2001).

Takayama, K. et al. Amyloid precursor protein is a primary androgen target gene that promotes prostate cancer growth. *Cancer Res* 69, 137–142 (2009).

Tanabe, F., Nakajima, T. & Ito, M. Involvement of diacylglycerol produced by phospholipase D activation in A β -induced reduction of sAPP α secretion in SH-SY5Y neuroblastoma cells. *Biochem. Biophys. Res. Commun.* 446, 933–939 (2014).

The Lancet, T. Alzheimer's disease: expedition into the unknown. *Lancet (London, England)* 388, 2713 (2016).

Uddin, M. S. et al. APOE and Alzheimer's Disease: Evidence Mounts that Targeting APOE4 may Combat Alzheimer's Pathogenesis. *Mol. Neurobiol.* (2018). doi:10.1007/s12035-018-1237-z

Uranga, R. M., Alza, N. P., Conde, M. A., Antollini, S. S. & Salvador, G. A. Phosphoinositides: Two-Path Signaling in Neuronal Response to Oligomeric Amyloid β Peptide. *Mol. Neurobiol.* 54, 3236–3252 (2017).

Uranga, R. M., Alza, N. P., Conde, M. A., Antollini, S. S. & Salvador, G. A. Phosphoinositides: Two-Path Signaling in Neuronal Response to Oligomeric Amyloid β Peptide. *Mol. Neurobiol.* 54, 3236–3252 (2017).

Venegas, C. et al. Microglia-derived ASC specks cross-seed amyloid- β in Alzheimer's disease. *Nature* 552, 355–361 (2017).

Venkataramani, V. et al. Histone deacetylase inhibitor valproic acid inhibits cancer cell proliferation via down-regulation of the Alzheimer amyloid precursor protein. *J Biol Chem* 285, 10678–10689 (2010).

Villamil-Ortiz, J. G. et al. BACE1 RNAi Restores the Composition of Phosphatidylethanolamine-Derivates Related to Memory Improvement in Aged 3xTg-AD Mice. *Front Cell Neurosci* 10, 260 (2016).

Walter, J. γ -Secretase, apolipoprotein E and cellular cholesterol metabolism. *Curr Alzheimer Res* 9, 189–199 (2012).

Wang, H.-L. et al. Cholesterol, 24-Hydroxycholesterol, and 27-Hydroxycholesterol as Surrogate Biomarkers in Cerebrospinal Fluid in Mild Cognitive Impairment and Alzheimer's Disease: A Meta-Analysis. *J. Alzheimer's Dis.* 51, 45–55 (2016).

Wang, H.-L. et al. Cholesterol, 24-Hydroxycholesterol, and 27-Hydroxycholesterol as Surrogate

Wang, W. Y., Tan, M. S., Yu, J. T. & Tan, L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. in *Ann Transl Med* 3, (2015).

Wolfe, M. S. Structure, mechanism and inhibition of gamma-secretase and presenilin-like proteases. *Biol Chem* 391, 839–847 (2010).

Wu, P.-J., Hung, Y.-F., Liu, H.-Y. & Hsueh, Y.-P. Deletion of the Inflammasome Sensor Nlrp3 Aims to Mitigate A β Deposition and Microglial Activation but Increases Inflammatory Cytokine Expression in an Alzheimer Disease Mouse Model. *Neuroim-*

munomodulation 24, 29–39 (2017).

Xiang, Y., Lam, S. M. & Shui, G. What can lipidomics tell us about the pathogenesis of Alzheimer disease? *Biol. Chem.* 396, 1281–91 (2015).

Yahi, N. & Fantini, J. Deciphering the Glycolipid Code of Alzheimer's and Parkinson's Amyloid Proteins Allowed the Creation of a Universal Ganglioside-Binding Peptide. *PLoS One* 9, e104751 (2014).

Yanagisawa, K. GM1 ganglioside and the seeding of amyloid in Alzheimer's disease: endogenous seed for Alzheimer amyloid. *Neuroscientist* 11, 250–260 (2005).

Yassine, H. N. et al. DHA brain uptake and APOE4 status: a PET study with [1-11C]-DHA. *Alzheimers. Res. Ther.* 9, 23 (2017).

Yuan, P. et al. TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy. *Neuron* 92, 252–264 (2016).

Yuyama, K. & Yanagisawa, K. Sphingomyelin accumulation provides a favorable milieu for GM1 ganglioside-induced assembly of amyloid beta-protein. *Neurosci Lett* 481, 168–172 (2010).

Zárate, R., el Jaber-Vazdekis, N., Tejera, N., Pérez, J. A. & Rodríguez, C. Significance of long chain polyunsaturated fatty acids in human health. *Clin. Transl. Med.* 6, 25 (2017).

Zarrouk, A. et al. Lipid Biomarkers in Alzheimer's Disease. *Curr Alzheimer Res* (2017). doi:10.2174/1567205014666170505101426

Zarrouk, A. et al. Lipid Biomarkers in Alzheimer's Disease. *Curr. Alzheimer Res.* 15, 303–312 (2018).
Zhang, H., Ma, Q., Zhang, Y. W. & Xu, H. Proteolytic processing of Alzheimer's β -amyloid precursor protein. *J Neurochem* 120 Suppl, 9–21 (2012).

Zhao, Y. et al. Docosahexaenoic acid-derived neuroprotectin D1 induces neuronal survival via secretase- and PPAR γ -mediated mechanisms in Alzheimer's disease models. *PLoS One* 6, e15816 (2011).

Zheng, C., Zhou, X.-W. & Wang, J.-Z. The dual roles of cytokines in Alzheimer's disease: update on interleukins, TNF- α , TGF- β and IFN- γ . *Transl. Neurodegener.* 5, 7 (2016).

CHAPTER 2

BACE₁ RNAi restores the composition of phosphatidylethanolamine-derivates related to memory improvement in aged 3xTg-AD mice

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Synaptic Diseases: From Biology to Potential Therapy

Abstract

β -amyloid ($A\beta$) is produced by the β -secretase 1 (BACE1)-mediated enzymatic cleavage of the amyloid precursor protein through the amyloidogenic pathway, making BACE1 a therapeutic target against Alzheimer's Disease (AD). Alterations in lipid metabolism are a risk factor for AD by an unknown mechanism. The objective of this study was to determine the effect of RNA interference against BACE1 (shBACEmiR) on the phospholipid profile in hippocampal CA1 area in aged 3xTg-AD mice after 6 and 12 months of treatment compared to aged PS1KI mice. The shBACEmiR treatment induced cognitive function recovery and restored mainly the fatty acid composition of lysophosphatidylethanolamine (LPE) and etherphosphatidylethanolamine (ePE), reduced the cPLA2's phosphorylation, down-regulated the levels of arachidonic acid (AA) and COX2 in the hippocampi of 3xTg-AD mice. Together, our findings suggest, for the first time, that BACE1 silencing restores phospholipids composition which could favor the recovery of cellular homeostasis and cognitive function in the hippocampus of triple transgenic AD mice.

Key words: Alzheimer's disease, phospholipids, BACE1, RNA interference, hippocampus, cognitive function.

Abbreviations: PA, phosphatidic acid; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; ePC etherphosphatidylcholine; PS, phosphatidylserine; ePS, etherphosphatidylserine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; ePE, etherphosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; SM, sphingomyelin; CE, cholesterol esters; AA, arachidonic acid; SCR, scrambled; B, shBACE1miR; cPLA, cytosolic phospholipase A; m, months.

Introduction

Alzheimer's disease (AD) is a disabling neurodegenerative disorder that impacts millions of people worldwide and is recognized as the most common form of dementia (Querfurth and LaFerla, 2010). The aggregation of three main neuropathological markers—senile plaques (SP), neurofibrillary tangles (NFT) and lipid granules—is characteristic of AD. Current evidence suggests that senile plaques trigger the amyloid cascade and that the deposition of β -amyloid ($A\beta$) is the first step in the development of AD, leading to the formation of SP, NFT, neuronal loss, and finally, clinical dementia. In AD, neuronal loss alters the cellular composition and macrostructure of cerebral regions, either moderately, as observed in the prefrontal cortex, or severely, as in the entorhinal cortex and hippocampus. Progressive cell death in these regions is tightly linked to dementia due to the reduction in the number of neurons found in the hippocampus and cerebral cortex of individuals with AD but not asymptomatic subjects (Andrade-Moraes et al., 2013). Accordingly, AD patients show significantly less activation in the hippocampal formation, which is associated with progressive memory loss (Sperling et al., 2003).

$A\beta$ is generated by the enzymatic processing of the amyloid precursor protein (APP). This process involves the cleavage of APP by the β -site APP cleavage enzyme 1 (BACE1) and the γ -secretase complex, which contains presenilin 1 or 2 (PS1 or PS2) as the catalytic subunit to generate the $A\beta$ 40 and $A\beta$ 42 peptides (Thinakaran and Koo, 2008). BACE1 is a type I transmembrane aspartic protease that is related to pepsins and retroviral proteases. The subcellular localization of BACE1 is within the trans-Golgi network and the endosomal compartment. Although BACE1 reaches the plasma membrane due to vesicle trafficking, it is quickly recycled. Only a limited amount of APP cleavage by BACE1 takes place at the plasma membrane; the primary BACE1-mediated APP processing occurs in endocytic vesicles where the low pH environment favors the enzymatic activity (Kandalepas and Vassar, 2012).

Cellular lipid composition also regulates the catalytic activity of enzymes such as BACE1, as observed in cholesterol-rich membrane domains known as lipid rafts (Ehehalt et al., 2003). The cholesterol content of these membrane domains also seems to affect the enzymatic activity of BACE1 on APP (Kalvodova et al., 2005). However, although cholesterol is the best-studied brain lipid in AD, many other lipids are involved in the $A\beta$ -lipid regulatory system, and some of these lipids exhibit stronger effects than cholesterol on $A\beta$ production (Di Paolo and Kim, 2011).

The association between an aberrant lipid balance and AD is supported by the fact that the neuronal lipid composition regulates the trafficking and activity of the membrane proteins involved in $A\beta$ production, such as APP, BACE1 and presenilin 1 and 2 (Grimm et al., 2005). It has also been found that high levels of $A\beta$ modulate the activity of enzymes such as phospholipases A2, C and D and thus alter membrane homeostasis (Berman et al., 2008; Oliveira

and Di Paolo, 2010; Sanchez-Mejia and Mucke, 2010). In addition, several genes related to AD, such as APOE, ABCA7, CLU, and BIN1, are tightly connected to lipid metabolism and cellular membrane dynamics (Hollingworth et al., 2011; Karch and Goate, 2014; Rosenthal and Kamboh, 2014).

Although the link between lipid metabolism and AD was suggested years ago, many aspects of this relationship have remained elusive due to the lack of technology and analytical methodologies to understand the molecular mechanisms of lipid alterations in AD. The advent of lipidomics has allowed researchers to detect and quantify the lipid species and FAs of cerebral structures, thus facilitating the analysis of the lipid classes involved in cell signaling, membrane structure and trafficking. These techniques have been used to establish the lipid compositions of brain tissues from healthy individuals and AD patients (Han et al., 2001; Fabelo et al., 2014), revealing changes in the levels of plasmalogens, such as, ether-phosphatidylethanolamine (ePE), sulfatide, ceramide, galactosylceramide, cholesterol and alkyl-acylglycerophosphocholine (Han et al., 2001; Han et al., 2002; Cutler et al., 2004).

Nevertheless, there is also evidence suggesting that some of enzymes involved in AD, can regulate lipid homeostasis. Thus, postmortem analysis of frontal cortices from AD patients revealed a 47% increased activity of BACE1 compared with samples from cognitively normal individuals. They found a positive significant linear correlation between BACE1 activity and the levels of 4-hydroxynonenal (HNE) and malondialdehyde (MDA) (Borghi et al., 2007). This study hints to the fact BACE1 may have a direct effect on regulating lipid homeostasis, which is also supported by the work of Meakin and colleagues who used mice lacking BACE1 gene to evaluate the effect of this enzyme on the body weight, as well as lipid and glucose metabolism. They found that the knockout animals had decreased production of lipids in the body and showed increased insulin sensitivity compared to their littermate controls, which may be an indicative of high energy expenditure. Furthermore experiments demonstrated that the absence of BACE1 in brown adipose tissue and skeletal muscle led to increased levels of the uncoupling protein 1, which seems to be involved in protection against oxidative stress and fatty acid handling (Gallagher et al., 2012).

In addition, our previous results showed that the silencing of BACE1 produces reduction of tauopathy in old 3xTg-AD mice, and part of the effect was autophagosome lipidation-dependent (Piedrahita et al., 2015). Therefore, supported in those previous evidences about the role of BACE1 in lipid homeostasis; our hypothesis is that BACE1 down-regulation affects the phospholipid composition of hippocampus in an AD model. In this study, we present the lipidomic profiles of hippocampal tissue obtained from a commonly used transgenic mouse model of AD (3xTg-AD) treated with RNA interference (RNAi) against BACE1 for 6 and 12 months and compared them with control groups, using mass spectrometry, we detected and analyzed 12 different lipid classes and subclasses, including phospholipids (PLs), sphingolipids, lysophospholipids, plasmalogens, covering over 402 lipid subspecies.

Materials And Methods

RNAi design

We designed the shRNAi sequences for silencing BACE1 (shBACE1miR). The scrambled RNAi sequences used as control (shSCRmiR) were based on previously published sequences (Chang et al., 2006). These sequences were cloned into human miR-30-based stem-loops by polymerase extension of overlapping DNA oligonucleotides. The following primers were used for polymerase extension to clone the RNAi into the lentiviral shuttle plasmid (pCMV-GIN-ZEO.GFP) for transfection in HEK-293T cells: shBACE1miR forward primer, 5'...CAGAAGGCTCGAGAAGGTATATGCTGTTGACAGTGAGCGCGGACTGCAAGGAGTACAACTATAGTGAAGCCACAGATGTA...3' and shBACE1miR reverse primer, 5'...CTAAAGTAGCCCCCTGAATTCCGAGGCAGTAGGCATGGACTGCAAGGAGTACAACTATACATCTGTGGCTTCAC...3'; shSCRmiR forward primer, 5'...CAGAAGGCTCGAGAAGGTATATGCTGTTGACTAGCACACATCAGGAAGCGCTCGACAGTGATAGTGAAGCCACAGATGTA...3', and shSCRmiR reverse primer, 5'...CTAAAGTAGCCCCCTGAATTCCGAGGCAGTAGGCATGGACTGCAAGGAGTACAACTATACATCTGTGGCTTCAC...3'. The extension products were digested with XhoI and EcoRI for directional cloning into the pCMV-GIN-ZEO.GFP vector (Open Biosystems, Pittsburgh, USA). To clone the RNAi vectors for adeno-associated virus (AAV) production, the following primers were used for polymerase extension: shBACE1miR forward primer, 5'...AAAAGCTCGAGGAGCTCGTGAGCGCTGGACTGCAAGGAGTACAACTCTGTGAAGCCACAGATGGG...3' and shBACE1miR reverse primer 5'...TTTGGATCCATTAATAGGCAATGGACTGCAAGGAGTACAACTCCCATCTGTGGCTTCACAG...3'; shSCRmiR forward primer, 5'...AAAAGCTCGAGGAGCTCGAGGAGCTCGTGAGCGCTGGACTGCAAGGAGTACAACTCTGTGAAGCCACAGATGGG...3' and shSCRmiR reverse primer, 5'...AAAAGCTAGTAGGCGTACCATCGAACCCTGAGAGTTACCCATCTGTGGCTTTACAG...3'. These extension products were digested with XhoI and SpeI for directional cloning into a U6 expression plasmid that was cut with XhoI and XbaI (Boudreau et al., 2009).

Viral particle production and neuronal culture transduction

The AAV particles were obtained by the large-scale production of heterologous proteins from Sf9 insect cell cultures that were co-infected with recombinant baculovirus derived from the *Autographa californica* nuclear polyhedrosis virus (Urabe et al., 2002). The shBACE1miR expression cassettes—driven by the mouse U6 promoter—were cloned into pAAV-CMV.hrGFP, which contained AAV serotype 2/5 inverted terminal repeats and a CMV-humanized Renilla GFP (hrGFP)-simian virus 40 poly (A) reporter cassette (Urabe et al., 2002;). The AAV titers were determined using quantitative PCR and/or DNA slot blot analysis. The AAV particles were dialyzed before use.

Animal procedures

We used 3xTg-AD mice containing PS1 (M146V), APP (Swe), and tau (P301L) mutations (Oddo et al., 2003), as well as the PS1KI mice containing the PS1 (M146V) mutation, named as “control group”, because PS1KI is the genetic background of the 3xTg-AD mice, do not develop intracellular and extracellular β -Amyloid and do not exhibit LTP deficits. Furthermore, those mice are viable, fertile, normal in size and do not display any gross physical or behavioral abnormalities (Oddo et al., 2003). Both mice strains were kindly donated by Dr. Frank M LaFerla from the University of California (Irvine, CA, USA). The animals were bred in-house in a specific pathogen-free (SPF) colony at the vivarium at SIU (Sede de Investigación Universitaria, University of Antioquia, Medellin, Colombia), maintained with a 12 h:12 h dark:light cycle, and received food and water ad libitum. The animals were handled in accordance with the Colombian standards (Law 84/1989 and resolution 8430/1993) and NIH guidelines for animal welfare and care (Public Law 99-158, November 20, 1985, “Animals in Research”). Specific care was taken to minimize animal suffering and minimize the number of animals used. A total of 42 3xTg-AD mice (6 and 12 months old, 20-30 g), 20 PS1KI mice (6 and 12 months old, 20-30 g) were used. 28 male and 14 female mice were aleatorily assigned, using three males and two-three females per group. One side of the hippocampi was used for lipidomic analysis and the other for biochemical analysis.

The animals were anesthetized (5% ketamine and 2% xylazine, 50:5 dosage mg/kg) and bilaterally injected with 1 μ L of AAV2-shSCRmiR (shSCRmiR) or AAV2-shBACE1miR (shBACE1miR) into both hippocampi (bregma coordinates were -1.7 anteroposterior, 0.8 (right) and -0.8 (left) lateral, and 2.5 mm dorsoventral). The injections were performed with a 10- μ L syringe (Hamilton, Reno, USA) at 0.1 μ L/min, and 10 min elapsed after the infusion before the syringe was withdrawn. The following experimental groups were used: Short-term treatment group, PS1KI and 3xTg-AD mice were injected with shBACE1miR (B12m) or scrambled miR (Scr12m) at an age of 12 months old and evaluated 6 months later; and Long-term treatment group, PS1KI and 3xTg-AD mice injected with shBACE1miR (B6m) and scrambled miR (Scr6m) at an age of 6 months and evaluated one year after injection (Fig. 1). The animals were evaluated by the Morris water maze test and later sacrificed for biochemical measurements (Fig. 1). The hippocampi were dissected, immediately frozen on dry ice and stored at -80°C until use.

Morris water maze test

Morris water maze testing was performed at 6 and 12 months following the shBACE1miR and shSCRmiR injection in the PS1KI and 3xTg-AD mice. A circular pool (1-m diameter and 0.5-m height) was filled with water at 22 $\pm$ 2°C, with an escape platform with a diameter of 20 cm submerged 2 cm below the water surface. The platform was hidden in the water,

which was painted with non-toxic white color and opaque paint to facilitate video recording. The test procedure consisted of four stages: 1) Visible: 2 days, 2 trials per day, 60 seconds per trial with the platform 2 cm above the water level; 2) Learning: 5 days, 2 trials per day (10 trials in total), 60 seconds per trial to find the hidden platform; 3) Retention: 48 hours after the last training day (1 day, 1 trial per day, 60 seconds per trial without platform); and 4) Transference (changing location of the platform to the opposite side of the pool): A second learning trial of 2 days, 2 trials per day (4 trials in total), 60 seconds per trial to find the hidden platform (Fig. 1). The animal behavioral tests were recorded and analyzed individually using View Point software (Lyon, France).

Lipid analyses

The total lipids from the mouse hippocampus were extracted according to the FOLCH technique (FOLCH et al., 1957) using a mixture of 2 mL of chloroform (CHCl₃) and 1 mL of methanol (MeOH) in a 2:1 (v/v) ratio. Then, 0.005% butylated hydroxytoluene (BHT) was added, and this mixture was used to homogenize the hippocampus. Subsequently, 1 mL of 0.9% NaCl was added, and the mixture was centrifuged at 3000 rpm for 3 minutes. The organic layer (lower layer) was removed and transferred to a new glass tube. This procedure was performed in an oxygen-free using enriched nitrogen environment to avoid lipid oxidation processes and was repeated three times. The solvents were evaporated, and the extract was lyophilized to remove the excess of humidity. Finally, the lipid composition was analyzed by mass spectrometry.

Figure 1. Experimental design. AAVshSCRmiR and AAVshBACEmiR were injected in the hippocampus of 6 and 12 months-old PS1KI and 3xTg-AD mice. After 6 and 12 months' post-injection respectively, behavioral analyses using Morris water maze test were performed. First and second days' visible test; learning and memory were evaluated during 5 days (10 trials); after 48 h retention test was realized. Afterward, the transfer test was performed over 2 days (four trials).

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Mass spectrometry

An automated ESI-MS/MS approach was used and data acquisition and analysis carried out at the Kansas Lipidomics Research Center using an API 4000™ and Q-TRAP (4000Qtrap) detection system as described previously (Sparkes et al., 2010; Zhou et al., 2011). This protocol allowed the detection and quantification of low concentrations of the polar lipid compounds. The molecules were determined by the mass/charge ratios, which were compared with the respective internal standard to determine which species of lipids were present in the evaluated extract: 0.66 nmol di14:0-PC, 0.66 nmol di24:1-PC, 0.66 nmol 13:0-lysoPC, 0.66 nmol 19:0-lysoPC, 0.36 nmol di14:0-PE, 0.36 nmol di24:1-PE, 0.36 nmol 14:0-lysoPE, 0.36 nmol 18:0-lysoPE, 0.36 nmol 14:0-lysoPG, 0.36 nmol 18:0-lysoPG, 0.36 nmol di14:0-PA, 0.36 nmol di20:0(phytanoyl)-PA, 0.24 nmol di14:0-PS, 0.24 nmol di20:0(phytanoyl)-PS, 0.20 nmol 16:0-18:0-PI, 0.16 nmol di18:0-PI, and 1 nmol 15:0 fatty acid. The system detected a total of 12 different lipid species and their respective sub-species, which were identified by the number of carbons and degree of unsaturation of the chain. Lipid concentration was normalized by molar concentration across all species for each sample, and the final data are presented as mean mol%.

Western blotting analysis

The animals were sacrificed, and the cerebral cortices were dissected, immediately frozen in liquid nitrogen, and stored at -80°C prior to use. The samples were lysed in 10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1% NP40, 1 nM orthovanadate, 5 mM NaF, 1 mM phenylmethylsulfonyl fluoride, and a protease inhibitor cocktail (Sigma-Aldrich) (Cardona-Gomez et al., 2004). The lysates (containing approximately 40 µg of proteins, quantified using the Bradford method) were loaded onto 8% and 10% polyacrylamide gels and transferred onto nitrocellulose membranes (GE Healthcare) at 250 mA for 2 h using an electrophoretic transfer system. The membranes were incubated overnight at 4°C in anti-phospho-cPLA2 (Ser505) (rabbit polyclonal. 1:1000; Cell Signaling), anti-COX2 (rabbit polyclonal. 1:500; abcam), and mouse anti-βIII tubulin (1:5000; Promega). IRDye 800CW goat anti-rabbit (LI-COR; 1:5000) were used as the secondary probes. The blots were developed using the Odyssey Infrared Imaging System. To minimize inter-assay variation, samples from all of the experimental groups were processed in parallel.

Statistical analysis

In the behavioral test were used 10-15 animals/group. The escape latency during the hidden platform training sessions and transference tests were determined using repeated-measures ANOVA. The latency and the number of platform crosses in the hidden platform trials and in the probe, trials were analyzed by One-way ANOVA, also ANOVA per day followed by Fisher's post hoc test were realized. The analyses were performed using SPSS 18.0 software (Chicago, IL, USA). The values were expressed as the means ± SEM. The results were considered to be significant at * $p < 0.05$, and ** $p < 0.01$. All of the sample groups were processed in parallel to reduce inter-assay variation.

The lipid levels for each sample were calculated by summing the total number of moles of all lipid species measured and then normalizing that total to mol%. Comparisons between groups were assessed either by one-way ANOVA, followed by the Tukey post-hoc test or the Kruskal-Wallis test, depending on the homoscedasticity and normality of the experimental data. Multivariate statistics were performed using principal component analysis (PCA) and a partial least squares-discriminant analysis (PLS-DA) (Barker et al., 2003). PLS-DA was included because it is particularly suitable for the analysis of datasets with a small number of samples and a large number of variables. The PLS-DA analysis was carried out using the routines described in (Ballabio and Consonni, 2013). For both techniques, an index representing the importance of the variables according to the first components was estimated. PCA used an index (denoted as p) that considers the entries of the principal directions and the variance of each component to determine the weight that every lipid species has in the estimation of the principal directions (Jolliffe, 2002). In contrast, for PLS-DA, the index used is called the variable importance in projection (VIP) (Mehmood, 2012), which analogously to p , determines the importance of each variable that is reflected by each component and also considers the variance explained by each PLS component (for a detailed description of VIP, please refer to Mehmood and the references therein). The confidence ellipsoids per group and treatment are also included. The data from the univariate and bivariate statistics are expressed as the mean ± the standard error of the mean. The statistical significance is indicated in the figures and tables.

Results

BACE1 gene silencing prevents learning and memory impairments in 3xTg-AD mice after 6 and 12 months of treatment

During the visible platform test, the shBACE1miR-treated 3xTg-AD mice did not show differences compared to the controls after either 6 or 12 months of treatment (Fig. 2A). However, the learning task performance of the shBACE1miR-treated mice at 6- and 12-months post-injection was better than that of the corresponding shSCRmiR-treated 3xTg-AD control

A. Visible **B. Learning** **C. Retention** **D. Trajectory** **E. Transference**

Figure 2. BACE1miR prevents cognitive dysfunction in 3xTg-AD mice. The learning and memory task performance of the PS1KI and 3xTg-AD mice were evaluated by the Morris water maze test after 6 months and 12 months of treatment with shBACE1miR and compared with the respective animal controls. A- Visible test, B- Learning test (First position of the platform), C-, D- Retention and representative trajectory images of the animals during the retention test, E- Transference test (Second position of the platform). The data are expressed as the group mean $\pm$ S.E.M. *, **Significant differences: * $P < 0.05$, ** $P < 0.001$; $n = 12$ to 10-15 animals/group.

mice (Fig. 2B). These observations supported a memory improvement because the shBACE-1miR-treated mice spent less time finding the hidden platform and showed preferences in the trajectory for the quadrant of the platform (Fig. 2C, D) at both post-injection time points. Additionally, reversal learning skills were improved in the 3xTgAD mice treated with shBACE1miR, which spent less time looking for the second platform location in the transfer test than the shSCRmiR-treated 3xTgAD mice (Fig. 2E). PS1KI mice treated with shBACE1miR did not show changes respect to the untreated control in both evaluated time lines (Fig. 2).

The phospholipid profile of the hippocampus is altered in 3xTg-AD mice

To determine whether the transduction with shBACE1miR or the scrambled version induced lipid changes in the 3xTg-AD mice, we characterized the hippocampal phospholipid composition of the transgenic animals at two different post-injection time points (6 and 12 months). These profiles were compared with shBACE1miR or the scrambled treated PS1KI mice, which were evaluated at the same post injection times. At a glance, the analysis revealed two types of variations in the phospholipid contents of the 3xTg-AD mouse hippocampus. The first set of changes seems to be related to the pathological condition, while the second type is associated with the shBACE1miR treatment.

The lipid profiling of the all control groups PS1KI and 3xTg-AD mice hippocampus shows that the lipidome is primarily composed of high abundance glycerophospholipids, such as PC (47.5% and 47.2%), PS (5.1% and 5.8%), PE (18.6% and 20.0%), and PI (1.9% and 1.8%); sphingolipids, such as SM-DSM (8.0% and 8.3%); low abundance glycerophospholipids, such as PA (0.3% and 0.4%) and PG (0.07% and 0.6%); lysophospholipids, such as LPC (0.2% and 0.4%) and LPE (0.9% and 1.0%); and etherphospholipids, such as ePC (1.4% and 1.6%), ePS (0.02% and 0.11%) and ePE (1.4% and 1.6%) (Fig. 3A).

The treatment of 3xTg-AD mice with the shBACE1miR or shSCRmiR for 6 or 12 months resulted in distinctive lipid profiles compared with the control animals (Fig. 3A). The disease-associated changes in the phospholipid composition involve an overall decrease in PC (control vs AD mice, $p < 0.001$), PS (control vs AD mice, $p < 0.01$) PE (control vs AD mice, $p < 0.01$), PI (control vs AD mice, $p < 0.01$) and SM-DSM (control vs AD mice, $p < 0.05$) (Fig. 3A). In contrast, the lipid profile obtained from the shBACE1miR-treated 3xTg-AD mice showed that the treatment restored the LPE ($p < 0.001$) and ePE ($p < 0.05$) content to basal levels (Fig. 3A and 4A3), suggesting a regulatory role for BACE1 in the biosynthesis of these particular plasmalogens.

Figure 3. Lipid composition of the hippocampus from 3xTg-AD and control mice, grouped according to the time of treatment. Various lipid changes can be seen in the hippocampal tissue. A - The lipid class profiles are expressed as %Mol composition. All lipid species were measured (means), and the error bars represent the SEM. B. Multivariate analyses of the lipid profiles from the hippocampus. PCA, Principal component analyses for the lipid classes and PLS-DA, Partial least squares analysis to discriminate between the lipid classes. The left panels illustrate the factor loadings for PC1 and PC2, with the indices of variance explained for each component. The right panels show the factor score plots for PLS-DA. The data for the 3xTg-AD mice were significantly different from the control groups (* $p < 0.05$, ** $p < 0.01$; * $p < 0.001$ ANOVA followed by the Tukey post hoc test or Kruskal-Wallis test) Abbreviations: PA, phosphatidic acid; PC, phosphatidylcholine; LPC, Lysophosphatidylcholine; ePC etherphosphatidylcholine; PS, phosphatidylserine; ePS, etherphosphatidylserine; PE, phosphatidylethanolamine; LPE, Lysophosphatidylethanolamine; ePE, etherphosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; SM, sphingomyelin. $n = 3-5$ per group.**

Figure 4. A- Multivariate analyses of the lipid profiles from the hippocampus. PCA, Principal component analyses for the lipid classes and PLS-DA, Partial least squares analysis to discriminate between the lipid classes. The left panels illustrate the factor loadings for PC1 and PC2, with the indices of variance explained for each component. The right panels show the factor score plots for PLS-DA. The variables in the analyses are the controls, AD (3xTg-AD without treatment) and AD+shBACE1-miR (3xTg-AD with 6 and 12 months of treatment). $n = 3-5$ per group.

The results of the PCA analyses from the detected lipids indicate that nearly 82% of the total variance may be explained by the first 2 principal components (PC1 and PC2) (Fig. 4A). The most relevant variables for these two components were related to the PC subclasses 32:0 and PE subclasses 40:6, which demonstrated higher p indexes (13.6236 and 12.1049 respectively) (Fig. 4A). PC 32:0 is composed of the saturated FA Palmitic acid (Fig. 4A). In contrast, PE 40:6 is composed of the polyunsaturated FAs DHA and saturated FAs stearic acid (Fig. 4A). The PLS-DA analyses indicate that nearly 86% of the total variance may be explained by factor 1. In this case, the most relevant feature according to VIP are PI 40:00 (VIP: 2.0965) and PC 44:8 (VIP = 1.9355). Moreover, three of the first five most relevant features according to VIP belong to the LPE species. (Fig. 4A). This LPE subclass is composed of the monounsaturated FA such as Palmitoleic (16:1), Oleic (18:1) and polyunsaturated AA acids, as arachidonic (20:4). Both the PCA and PLS-DA analyses indicate that the control group is located in a different quadrant of the plane compared with the AD and treated AD animals, which are in the right quadrant of the graphic (Fig. 4A). In addition, the AD and treated AD groups also show a different distribution pattern, indicating that BACE1 silencing modifies the course of certain PLs in the disease (Fig. 4A).

shBACE1miR restores the basal levels of LPE and ePE of 3xTg-AD at 6- and 12-months post-injection

We demonstrated that shBACE1miR has a regulatory effect on the lipid profile in two specific lipid classes: LPE and ePE. The LPE levels increased ($p < 0.001$) in the AD groups compared

Figure 5. shBACE1miR restores the basal levels of LPE and ePE at 6- and 12-months post-injection. A - PCA, Principal component analyses for the LPE and ePE subclasses and PLS-DA, Partial least squares method to discriminate between the LPE and ePE subclasses. The left panels illustrate the factor loadings for PC1 and PC2, with the indices of variance explained for each component. The right panels show the factor score plots for PLS-DA. The groups in the analyses are controls, AD (3xTg-AD without treatment) and AD+shBACE1-miR (3xTg-AD with 6 and 12 months of treatment). B - Contour plots of the more influential subclasses of LPE and ePE (variables) in the discriminant analyses for each evaluated variable; all LPE and ePE subclasses were measured (means), and the error bars represent the SEM. n=3-5 per group.

with the AD mice treated with the shBACE1miR and the control groups (Fig. 3A and 5A). The PCA analysis showed that the AD+shBACE1miR group had a displacement to the left quadrant close to the control group, while the AD mice occupy a different region that is shifted to the right (Fig. 5A). This result indicates that nearly 97% of the total variance may be explained by the first 2 principal components (PC1 and PC2), which is LPE 18:1 according to Rho=1.0208. However, three of the first five most relevant features according to VIP belong to unsaturated lipid species (22:6, 20:1 and 20:4) (Fig. 5A). In addition, these observations were confirmed by counter plot analyses, where the LPE content was increased in the AD groups (3xTg-Scr6m and 3xTg-Scr12m) and restored to the basal levels in the shBACE1miR-treated AD mice at 6- and 12-months post-injection (Fig. 5B). The PLS-DA analysis also revealed that the AD+shBACE1miR group on the graph had a displacement from AD to control group; however, the AD group exhibited a different distribution. The subclasses with major importance in data distribution, based on higher p index, were LPE 22:5 (docosapentaenoic acid) (1.0858) and LPE 20:2 (Eicosadienoic Acid) (1.0494) (Fig. 5A).

In addition, ePE also seems to be affected by the treatment because the levels of this plasmalogen decreased ($p < 0.05$) in AD compared with the AD+shBACE1miR and control groups (Fig. 3A). The PCA analysis confirmed this result and showed that all experimental groups occupy different areas in the quadrants, i.e., ePE species were able to differentiate among the experimental groups. The most relevant molecular species, such as ePE 40:6 (18:0 stearic/22:6 DHA) and 38:6 (16:0 palmitic/22:6 DHA), had a higher r index (0.01470 and 1.1170,

respectively) (Fig. 5C). Interestingly, the contour graphic showed changes in the same ePE subclasses and showed a decrease in AD groups, although their levels were recovered in the AD+shBACE1miR groups at 6- and 12-months post-treatment (Fig. 5D). The recovery of the basal levels may represent a modulatory effect of the shBACE1miR on the phospholipid profile in the 3xTg-AD mice. The PLS-DA analysis also demonstrated different distribution patterns for all groups on the plot. The subclasses with major importance in the data distribution based on a high VIP index were ePE 38:6 (16:0 palmitic acid/22:6 DHA) (1.5995), ePE 40:6 (22:6 DHA/18:0 stearic) (1.4983) and (Fig. 5C).

A detailed analysis of the LPE's FA composition revealed the presence of large chain fatty acyls (16C to 22C) with either monounsaturated or polyunsaturated FAs bearing 1, 4 or 6 bonds. Interestingly, the shBACE1miR treatment modified the carbon-chain length and the degree of unsaturation of the LPE subspecies. shBACE1miR seemed to restore the basal levels of long fatty acyls chains, such as 16C, 18C, 20C and 22C, which were altered in the untreated AD groups (3xTg-Scr6m and 3xTg-Scr12m) (Fig. 6A). The same effect was also found for the degree of unsaturation, particularly in polyunsaturated FAs with 1, 4 or 6 bonds, compared with the control groups (Fig. 6B). In contrast, the FA composition analysis of ePE showed that this plasmalogen was formed by very long fatty acyls chains (32C to 40 C), whose levels did not show statistically significant differences among the experimental groups (Fig. 6C). In contrast, the analysis of the FA unsaturation number revealed changes in ePE FAs with 6 bonds (Fig. 6D). It was evident that the shBACE1miR treatment (3xTg-B6m and 3xTg-B12m) partially restored this type of unsaturation in the ePE FAs to that observed in all control groups (Fig. 6D).

BACE1 silencing reduces active cPLA2, ARA and COX2 levels.

The LPE and ePE level changes suggested a potential implication of a PLA2 isoform (Dennis et al., 2011), to understand this effect we evaluated the phosphorylation of cPLA, AA levels and COX2 protein levels in all experimental groups, after confirming the decreased BACE1 protein levels ($p < 0.01$) in the hippocampi from shBACE1miR treated mice respect to those treated with shSCRmiR (Fig. 7A). BACE1 protein levels tended to increase under PS1KI condition, but without significant effect, as reported Giliberto et al., 2009. cPLA2 is the main enzyme involved in the production of both lipids species (Makide et al., 2009), inducing AA release, which trigger proinflammatory signalling through the cyclooxygenases, as COX2 (Lin et al., 1993; Kuwata et al., 1998). Our findings evidenced that the levels of phosphorylated cPLA2 in ser505 were decreased at 6 and 12 months post-injection in the shBACE1miR groups compared with Scr ($p < 0.01$) and PS1KI controls group ($p < 0.05$) (Fig. 7B). The AA levels were increased in AD (3xTg-Scr6 and Scr12m) groups, how has been previously described (Sanchez-Mejia et al., 2010), while shBACE1miR6 and shBACE1miR12 decreased to control levels ($p < 0.001$) (Fig. 7C). Finally, we found that COX2 levels increased significantly in all

Figure 6. Altered fatty acid composition of LPE and ePE in the hippocampal region from the 3xTg-AD mouse models and recovery by BACE1miR. Independent analyses of changes in lysophospholipid (LPE) and plasmalogen (ePE) chain lengths equal to the total number of carbon atoms in the fatty acid moieties A- and C-, and the LPE and ePE saturation equal to the total number of double bonds in the fatty acid moieties B- and D-. The data for the 3xTg-AD mice were significantly different from those of the control groups (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ANOVA followed by the Tukey post hoc test or Kruskal-Wallis test). # $p < 0.05$, ANOVA with Tukey's tests compared between 3xTg-AD groups. Data are expressed as Mol %. $n = 3-5$ per group.

the 3xTg-AD groups; however, this decreased only at the long-term shBACE1miR- treatment (12m) (Fig. 7D). Taken together, these results suggest that the BACE1 silencing reduces the composition of AA in LPE at 6 and 12 months of treatment, with a clear repercussion on anti-inflammatory response at 12 months by decrease of cPLA2 and COX2 levels in the hippocampus from 3xTg-AD mice.

Discussion

Recently, we validated the silencing of BACE1 in wild type and triple transgenic AD mice, where shBACE1miR treatment induced neuroprotection by reduction of CTF (carboxy-terminal fragment) and 1-42 A β levels. In addition, hyperphosphorylated tau was reduced through the regulation of some autophagy actors, which were blocked by phosphatidylethanolamine (PE) lipidation inhibitor (3 Methyl-Adenine, 3MA) (Piedrahita et al., 2016). Therefore, in the present study, we performed a deep lipid profile analysis of the hippocampal region of 3xTg-AD mice to evaluate the effect of BACE1 silencing in this AD model, whose treatment improved the spatial learning and memory skills at 6- and 12-months post-injection. Although, BACE1 inhibition has several substrates involved in neurotransmission, excitability and synapses, as neuroregulin 1, APP, between others (Munro et. al., 2016), and

Figure 7. shBACEmiR decrease the levels of phosphorylated cPLA2, ARA and COX2 levels in the hippocampus of old 3xTg-AD mice. A- Representative bands from BACE1 protein levels and B- p-cPLA2 levels at long and short-term shBACE1miR's post-treatment (6 and 12 months) by Western blotting. C- Arachidonic Acid composition levels in LPE lipid. Results are expressed as Mol % of total fatty acids. D- Representative immunoblot of COX2 protein levels. Results are expressed as relative units (RU) and. Data are shown as means $\pm$ SEM. For 3xTg-AD, results were significantly different from the controls group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ANOVA followed by Tukey post hoc test) $n = 3-5$ per group.

its inhibition could be adverse (Vassar 2014, Ohno 2016); however, a reduction of BACE1 protein under a pathological over-expressed BACE1 activity condition, could suggest an important benefit in neuroprotection, remyelination and plasticity (Farah et al., 2011; Piedrahita et al., 2016), supported by the recovery of the cognitive function in the shBACE1miR treated aged 3xTg-AD mice. Also, BACE1 silencing did not affect to PS1KI mice and had a lipid profile very similar than C57BL6 mice (data not shown).

We detected 12 lipid classes that covered over 402 lipid subclasses, observing changes in 6 PL classes in the hippocampus of the 3xTg-AD mice compared to the control groups. Interestingly, we observed that treatment with shBACE1miR modifies the levels of LPE and ePE, restoring the amounts of these plasmalogens to the basal levels found in the control

animals, which may be associated with tissue homeostasis and cognitive function recovery. In addition, there were also changes in the individual lipid species of different subclasses, suggesting that the FA carbon chain and unsaturation number may play an important role in AD pathogenesis. These results suggest that the numerous changes that occurred in the PLs could be used as potential molecular target that facilitate understanding the biochemical mechanism involved in the neurodegenerative process in AD.

Alterations in membrane lipids are widely described in brains from AD and several neurodegenerative disorders (Sagin and Sozmen, 2008). A tight relationship has been established between lipid metabolism and lipotoxicity in neurons (Du et al., 2009). Trafficking and secretase activity of the key membrane-bound proteins controlling A β levels, including APP, BACE1, and presenilins, are regulated by membrane levels of cholesterol and sphingomyelin (SM). According to early studies, a general decline of SM content in AD brains has been described (Cunane et al., 2012), which was confirmed by decreased levels of SM in the 3xTgAD mice groups in our data. Also, different studies have reported decreased brain PL levels in neurodegenerative diseases, mainly PI (Prasad et al., 1998; Berman et al., 2008; Whiley et al., 2014); PC (Whiley et al., 2014) PE and its derivatives, such as LPE and ePE (Ginsberg et al., 1993; Ginsberg et al., 1995; Nesic et al., 2012); and PS (Shea, 1997), seeming to have multifactorial origins, including the hyperactivation of phospholipases, peroxisomal dysfunction and irregular FA composition of PLs. Our observations confirm those results, where alterations in PL metabolism were close related to AD condition, whose aberrant metabolism could be reflected in the animal's behavior alteration.

In the present study, we observed decreased levels of PC in all AD groups compared with the control groups, specially the subspecies 34:1 and 32:0. The alterations in the PC levels have been described as a deregulation in the diacylglycerol cholinotransferase-mediated biosynthesis and turnover of PC from DAG, which affects neurite and axonal outgrowth and synaptic plasticity by DAG accumulation and low levels of PC (Araki and Wurtman, 1997; Mateos et al., 2010; Gaudin et al., 2012). However, the biological activity depends on the type of FA linked to the molecular structure. Previous works reported that these PCs are decreased in micro-extracted senile plaques from the post-mortem AD brain, and it could be linked to the roles of PLA2 and PLD1 in A β activation (Gaudin et al., 2012; Whiley et al., 2014;). Additionally, these species are composed of monounsaturated omega-9 oleic acid (18:1 n-9) and saturated palmitic acid (16:0), and this composition of membrane lipids influences their biophysical properties, including fluidity, permeability and charge (Finkelstein et al., 2014). The loss of oleic acid has been reported to favor BACE1 activity and increase the A β 1-42/A β 1-40 ratio, whereas dietary oleic acid supplements increased the A β 1-40/A β 1-42 ratio and reduced the levels of BACE and presenilin, as well as the number of amyloid plaques in AD brains (Amtul et al., 2011). Interestingly, our results confirm the aberrant biosynthesis of PC and the imbalance between the levels of saturated/unsaturated FAs affecting to the structure of PC could favor the abnormal destabilization of membrane in AD brains.

Also, in our work, we found decreased PS and PE levels in the hippocampus from all AD's experimental groups. Interestingly, we observed that the main decreased FA in both species was 40:6, which is composed of 22:6 (DHA) and 18:0 (stearic acid) chains. DHA is the best substrate for PS biosynthesis, and a decrease in this polyunsaturated acid has been related to cognitive impairment (Grimm et al., 2013;). Reduction in the DHA levels of hippocampal PS was demonstrated in senescence accelerated mice model, which has a short life span, memory and learning alterations, and increased amounts of hippocampal A β plaques. In addition, DHA content of PS and PE are 12% and 14% reduced in the human AD's cortex respectively, and also it has been found less amount of PE subspecies 22:6 n-3 (DHA) in the cortex of 9-month-old AD mice (Cunnane et al., 2012;). In general, a reduction of PE, PE-derived PUFAs in the brain (Ginsberg et al., 1993; Conquer et al., 2000; Dorninger et al., 2015;) and in plasma (Conquer et al., 2000) has been associated to AD. Therefore, our results are in agreement with the previous findings and suggest that altered PS and PE metabolism in the hippocampus could be implied in the cognitive impairment described in AD by affecting the signaling pathways modulated by PUFAs, such as DHA and AA.

PE accounts for approximately 25% of mammalian PLs and is significantly enriched in the brain, where the PE content is 45% of the total PLs. PE has been recently involved in the positive regulation of autophagy and longevity (Rockefeller et al., 2015). PEs and/or PE-derived (LPE and ePE) are essentials in the cell function (Vance and Tasseva, 2013), LPE can be generated from PE via a phospholipase A-type reaction (Vance and Tasseva, 2013). Currently, the physiological significance of LPE in the brain is unknown. However, in non-mammalian species, it has been attributed to certain functions. For example, in the housefly, LPE has antifungal and antibacterial activity (Meylaers et al., 2004), and in the mushroom *Grifola frondosa*, it stimulates mitogen-activated protein kinase (MAPK) signaling. Furthermore was reported that LPE induced neuronal differentiation of PC12 cells (Nishina et al., 2006). And recently it has been reported that LPE has a direct relationship with the calcium influx (Lee et al., 2015), which is close related to cell death (Bezprozvanny and Mattson, 2008). Interestingly, our data indicated increased LPE levels in the hippocampus of AD animals (Scr6 m and Scr12 m), specifically, the AA (20:4), the DHA (22:6) and the oleic acid (18:1) composition. Surprisingly, BACE1 silencing for 6 and 12 months reestablished the levels of these FAs to the control levels, which suggested that LPE is accumulated in AD. In addition, The PLS-DA analysis of the phospholipid species, also suggested that, the shBACE1miR treatment generated a displacement of lipid profile from AD group to control group profile. According to PLS-DA, such displacement is explained in an important part by LPE (16:1, 18:1, 20:4), meaning a significant role of BACE1 silencing in neuroprotection.

Although DHA is widely reported as being beneficial for cells, the excess levels likely trigger a negative effect on the membrane balance because the DHA content in the plasma membrane of T27A cells may be detrimental. This prediction was confirmed by observations of the reduced viability of cells incubated with 22:6/18:0 (Zerouga et al., 1996). In contrast, the

key enzyme involved in the turnover and degradation of LPE, phospholipase A2 (PLA2), is markedly increased in brains from AD patients (Farooqui, 2010;) and associated to AA production in AD (Sanchez-Mejia et al., 2010). Also, PLA2 activation has been linked to inflammation process in a genetically model of neurodegeneration, since it showed that specific lysophospholipids were responsible for microglial activation and the cytosolic PLA2 (cPLA2) inhibition had neuroprotective effect (Lee et al., 2011a; Sundaram et al., 2012). According to our results, BACE1 silencing down-regulated the composition of AA in LPE at 6 and 12 months' post-treatment, and decreased the phosphorylation state of cPLA2, which could be involved in the cell membrane homeostasis of treated AD mice at 12 months post-injection, with the consequent down-regulation of AA levels and COX2 pro-inflammatory pathway in the same time-line (Fig. 7C and 7D), suggesting a more effective anti-inflammatory regulation by the long-term BACE1 silencing. Which are in concordance with our recent data were BACE1 silencing reduced not only b-amyloid and Carboxy-terminal fragment (CTF), but also reduced MAPK activity and phosphorylated soluble tau (Piedrahita et al., 2016). MAPK regulates cPLA2 activity down-stream under lipid peroxidation condition (Lee et al., 2011b). Also, cPLA2 activity has been involved in the hyperphosphorylation of tau during neuroinflammation (Sundaram et al., 2012). AA through COX2 activity is the precursor of pro-inflammatory eicosanoids, prostaglandin E2 and leukotriene B4 and also increases the production of interleukin-1, tumor necrosis factor- α and IL-6 (SanGiovanni and Chew, 2005; Freund-Levi et al., 2014).

On the other side, also it has been reported that 22:6 FA suppresses the production of AA-derived eicosanoids and thus exerts anti-inflammatory and immunosuppressive effects (Calder, 2012;). Decreased levels of 22:6 trigger oxidative stress, which is known as a primary factor in the pathogenesis of AD because of the high-metabolic rate of the brain, which makes it particularly susceptible to ROS (Migliore et al., 2005). Also, oxidative stress is related to the progressive degradation of brain PLs in AD because they are rich in readily oxidizable AAs and DHAs (SanGiovanni and Chew, 2005; Calder, 2012). However, our data suggest a membrane destabilization process showing an up-regulation of DHA 22:6 in the LPE composition, maybe contributing to the pathogenesis of AD. Interestingly our data suggest the BACE1 knock-down reverse this pathogenic environment, generating homeostasis on FA composition of LPE, and reducing pro-inflammatory pathway cPLA2/AA/COX-2.

On the complementary side, a deficiency in brain ePE, which constitutes up to 70% of the total plasmalogens, has been traditionally associated with AD development, and low levels have been detected in the brain (Braverman and Moser, 2012), in the cerebrospinal fluid (Brites et al., 2009; Andrade-Moraes et al., 2013;) and in the serum of patients (Lessig and Fuchs, 2009); supporting the importance of those ones to control the membrane instability during oxidative stress in AD. According to our results, the reduction in ePE levels resulted from a significant decrease in the polyunsaturated 22:6 (DHA) and 18:0 (oleic acid) FAs. Because plasmalogens function as "scavengers" of ROS in biological membranes, low levels of ePE are associated with reduced cell viability because of the cell loss of antioxidant capacity.

Decreased concentrations of ePE may be correlated with peroxisomal alterations because the enzymes required for its biosynthesis are found in these organelles (Lizard et al., 2012;). Several studies have reported alterations in peroxisomes isolated from AD brains and reduced biosynthesis of plasmalogens composed of DHA (Kou et al., 2011; Fanelli et al., 2013). Furthermore, the main ePE degradation enzyme is PLA2, which is significantly increased in AD. Interestingly, in this work, we demonstrated that BACE1 silencing restored the amount of ePE in the hippocampus of 3xTg-AD mice to the control levels, suggesting that BACE1 silencing may play a role in the regulation of PLA2 activity restoring the homeostasis of this plasmalogen and allowing it to reach effective concentrations for antioxidant activity.

In summary, our study suggests that AD is associated with a general disruption of membrane properties and plasmalogens composition disbalance. The length and saturation number are important determinants of many membrane characteristics including membrane, fluidity, thickness and the local curvature, even molecular packing, which in turn regulate the activities of the enzyme membrane. Intramembrane proteolysis is likely to be exquisitely regulated by the thickness of the membrane (Hong 2015), which could have implications for β -secretase-mediated cleavage of APP. However, this work suggests by the first time that BACE1 is involved in FA composition of PE-derivates (LPE and ePE), and that the shBACE1miR treatment in 3xTg-AD mice restores the levels of lipid subspecies that are altered by the disease animal model, which could be associated with learning and memory improvement. However, further research is necessary to establish whether there is a direct or indirect correlation between the BACE1 levels and the metabolic pathways of PE, LPE and ePE and to explain how BACE1 silencing favors the appearance of the PUFAs involved in neuroprotection or if the reduction of BACE1 attenuated cytotoxic bA production for restoring cellular function including lipid metabolism, anti-inflammatory pathway and cognitive function, and to verify which would be the correspondence of those data with the human AD pathogenesis.

Author Contributions

JV, "design, acquisition of data, analyses and interpretation of data, write the manuscript"; AB, "design, acquisition of data, analysis and interpretation of data"; DP, "design, acquisition of data, analysis and interpretation of data"; CV, "analysis data, critical revision"; JA, "analysis data, critical revision"; GC-G, "design, acquisition of data, analysis and interpretation of data, write the manuscript".

Disclosure statement

The authors have no actual or potential conflicts of interest.

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References

1. Akbar, M., Calderon, F., Wen, Z., and Kim, H.Y. (2005). Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. *Proc Natl Acad Sci U S A* 102, 10858-10863.
2. Amtul, Z., Westaway, D., Cechetto, D.F., and Rozmahel, R.F. (2011). Oleic acid ameliorates amyloidosis in cellular and mouse models of Alzheimer's disease. *Brain Pathol* 21, 321-329.
3. Andrade-Moraes, C.H., Oliveira-Pinto, A.V., Castro-Fonseca, E., Da Silva, C.G., Guimarães, D.M., Szczupak, D., Parente-Bruno, D.R., Carvalho, L.R., Polichiso, L., Gomes, B.V., Oliveira, L.M., Rodriguez, R.D., Leite, R.E., Ferretti-Rebustini, R.E., Jacob-Filho, W., Pasqualucci, C.A., Grinberg, L.T., and Lent, R. (2013). Cell number changes in Alzheimer's disease relate to dementia, not to plaques and tangles. *Brain* 136, 3738-3752.
4. Araki, W., and Wurtman, R.J. (1997). Control of membrane phosphatidylcholine biosynthesis by diacylglycerol levels in neuronal cells undergoing neurite outgrowth. *Proc Natl Acad Sci U S A* 94, 11946-11950.
5. Ballabio, D., and Consonni, V. (2013). Classification tools in chemistry. Part 1: linear models. PLS-DA.
6. Barker, M., Biometrics and Statistical Sciences, T.P.A.G.C., Mason, Oh 45040, Usa, Rayens, W., Department of Statistics, U.O.K., Lexington, Ky 40506, Usa, and Department of Statistics, U.O.K., Lexington, Ky 40506, Usa. (2003). Partial least squares for discrimination. *Journal of Chemometrics* 17, 166-173.
7. Berman, D.E., Dall'armi, C., Voronov, S.V., McIntire, L.B., Zhang, H., Moore, A.Z., Staniszewski, A., Arancio, O., Kim, T.W., and Di Paolo, G. (2008). Oligomeric amyloid-beta peptide disrupts phosphatidylinositol-4,5-bisphosphate metabolism. *Nat Neurosci* 11, 547-554.
8. Bezprozvanny, I., and Mattson, M.P. (2008). Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci* 31, 454-463.
9. Borghi, R., Robertaborghi@yahoo.it, Department of Neuroscience, O.A.G., University of Genova, Via De Toni 5, 16132 Genova, Italy, Patriarca, S., Department of Experimental Medicine, U.O.G., Via Alberti 2, 16132 Genova, Italy, Traverso, N., Department of Experimental Medicine, U.O.G., Via Alberti 2, 16132 Genova, Italy, Piccini, A., Department of Neuroscience, O.A.G., University of Genova, Via De Toni 5, 16132 Genova, Italy, Storace, D., Department of Internal Medicine and Medical Specialties, U.O.G., Viale Benedetto Xv 6, 16132 Genova, Italy, Garuti, A., Department of Internal Medicine and Medical Specialties, U.O.G., Viale Benedetto Xv 6, 16132 Genova, Italy, Cirmena, G., Department of Internal Medicine and Medical Specialties, U.O.G., Viale Benedetto Xv 6, 16132 Genova, Italy, Odetti, P., Department of Internal Medicine and Medical Specialties, U.O.G., Viale Benedetto Xv 6, 16132 Genova, Italy, Tabaton, M., and Department of Neuroscience, O.A.G., University of Genova, Via De Toni 5, 16132 Genova, Italy (2007). The increased activity of BACE1 correlates with oxidative stress in Alzheimer's disease. *Neurobiology of Aging* 28, 1009-1014.
10. Boudreau, R.L., McBride, J.L., Martins, I., Shen, S., Xing, Y., Carter, B.J., and Davidson, B.L. (2009). Nonallelic-specific silencing of mutant and wild-type huntingtin demonstrates therapeutic efficacy in Huntington's disease mice. *Mol Ther* 17, 1053-1063.
11. Braverman, N.E., and Moser, A.B. (2012). Functions of plasmalogen lipids in health and disease. *Biochim Biophys Acta* 1822, 1442-1452.
12. Brites, P., Mooyer, P.A., El Mrabet, L., Waterham, H.R., and Wanders, R.J. (2009). Plasmalogens participate in very-long-chain fatty acid-induced pathology. *Brain* 132, 482-492.
13. Calder, P.C. (2012). Long-chain fatty acids and inflammation. *Proc Nutr Soc* 71, 284-289.
14. Cardona-Gomez, P., Perez, M., Avila, J., Garcia-Segura, L.M., and Wandosell, F. (2004). Estradiol inhibits GSK3 and regulates interaction of estrogen receptors, GSK3, and beta-catenin in the hippocampus. *Molecular and cellular neurosciences* 25, 363-373.
15. Chan, R.B., Oliveira, T.G., Cortes, E.P., Honig, L.S., Duff, K.E., Small, S.A., Wenk, M.R., Shui, G., and Di Paolo, G. (2012). Comparative lipidomic analysis of mouse and human brain with Alzheimer disease. *J Biol Chem* 287, 2678-2688.
16. Chang, K., Elledge, S.J., and Hannon, G.J. (2006). Lessons from Nature: microRNA-based shRNA libraries. *Nature methods* 3, 707-714.
17. Cohen, P. (2001). The role of protein phosphorylation in human health and disease. The Sir Hans Krebs Medal Lecture. *Eur J Biochem* 268, 5001-5010.
18. Cohen, P., Miyazaki, M., Socci, N.D., Hagge-Greenberg, A., Liedtke, W., Soukas, A.A., Sharma, R., Hudgins, L.C., Ntambi, J.M., and Friedman, J.M. (2002). Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science* 297, 240-243.
19. Cohen, P., Ntambi, J.M., and Friedman, J.M. (2003). Stearoyl-CoA desaturase-1 and the metabolic syndrome. *Curr Drug Targets Immune Endocr Metabol Disord* 3, 271-280.

20. Conquer, J.A., Tierney, M.C., Zecevic, J., Bettger, W.J., and Fisher, R.H. (2000). Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. *Lipids* 35, 1305-1312.
21. Cunnane, S.C., Schneider, J.A., Tangney, C., Tremblay-Mercier, J., Fortier, M., Bennett, D.A., and Morris, M.C. (2012). Plasma and brain fatty acid profiles in mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis* 29, 691-697.
22. Cutler, R.G., Kelly, J., Storie, K., Pedersen, W.A., Tamara, A., Hatanpaa, K., Troncoso, J.C., and Mattson, M.P. (2004). 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* 101, 2070-2075.
23. Da Silva, T.F., Sousa, V.F., Malheiro, A.R., and Brites, P. (2012). The importance of ether-phospholipids: a view from the perspective of mouse models. *Biochim Biophys Acta* 1822, 1501-1508.
24. Dennis, E.A., Cao, J., Hsu, Y.H., Magrioti, V., and Kokotos, G. (2011). Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. *Chem Rev* 111, 6130-6185.
25. Di Paolo, G., and Kim, T.W. (2011). Linking lipids to Alzheimer's disease: cholesterol and beyond. *Nat Rev Neurosci* 12, 284-296.
26. Dörninger, F., Brodde, A., Braverman, N.E., Moser, A.B., Just, W.W., Forss-Petter, S., Brügger, B., and Berger, J. (2015). Homeostasis of phospholipids - The level of phosphatidylethanolamine tightly adapts to changes in ethanolamine plasmalogens. *Biochim Biophys Acta* 1851, 117-128.
27. Du, L., Hickey, R.W., Bayir, H., Watkins, S.C., Tyurin, V.A., Guo, F., Kochanek, P.M., Jenkins, L.W., Ren, J., Gibson, G., Chu, C.T., Kagan, V.E., and Clark, R.S. (2009). Starving neurons show sex difference in autophagy. *J Biol Chem* 284, 2383-2396.
28. Ehehalt, R., Keller, P., Haass, C., Thiele, C., and Simons, K. (2003). Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. *J Cell Biol* 160, 113-123.
29. Fabelo, N., Martín, V., Marín, R., Moreno, D., Ferrer, I., and Díaz, M. (2014). Altered lipid composition in cortical lipid rafts occurs at early stages of sporadic Alzheimer's disease and facilitates APP/BACE1 interactions. *Neurobiol Aging* 35, 1801-1812.
30. Fanelli, F., Sepe, S., D'amelio, M., Bernardi, C., Cristiano, L., Cimini, A., Cecconi, F., Ceru', M.P., and Moreno, S. (2013). Age-dependent roles of peroxisomes in the hippocampus of a transgenic mouse model of Alzheimer's disease. *Mol Neurodegener* 8, 8.
31. Farah, M.H., Pan, B.H., Hoffman, P.N., Ferraris, D., Tsukamoto, T., Nguyen, T., Wong, P.C., Price, D.L., Slusher, B.S., Griffin, J.W. (2011) Reduced BACE1 activity enhances clearance of myelin debris and regeneration of axons in the injured peripheral nervous system. *J Neurosci.* 31(15):5744-54.
32. Farooqui, A.A. (2010). Studies on plasmalogen-selective phospholipase A2 in brain. *Mol Neurobiol* 41, 267-273.
33. Finkelstein, J., Heemels, M.T., Shadan, S., and Weiss, U. (2014). Lipids in health and disease. *Nature* 510, 47.
34. Folch, J., Lees, M., and Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226, 497-509.
35. Freund-Levi, Y., Vedin, I., Hjorth, E., Basun, H., Faxén Irving, G., Schultzberg, M., Eriksdotter, M., Palmblad, J., Vessby, B., Wahlund, L.O., Cederholm, T., and Basu, S. (2014). Effects of supplementation with omega-3 fatty acids on oxidative stress and inflammation in patients with Alzheimer's disease: the OmegAD study. *J Alzheimers Dis* 42, 823-831.
36. Gallagher, J., Latham, J., and Hussain, I. (2012). Reduction in BACE1 decreases body weight, protects against diet-induced obesity and enhances insulin sensitivity in mice.
37. Gaudin, M., Panchal, M., Auzeil, N., Duyckaerts, C., Brunelle, A., Laprévote, O., and Touboul, D. (2012). Choline-containing phospholipids in microdissected human Alzheimer's disease brain senile plaque versus neuropil. *Bioanalysis* 4, 2153-5159.
38. Giliberto L, Borghi R, Piccini A, Mangerini R, Sorbi S, Cirmena G, Garuti A, Ghatti B, Tagliavini F, Mughal MR, Mattson MP, Zhu X, Wang X, Guglielmotto M, Tamagno E, Tabaton M. (2009) Mutant presenilin 1 increases the expression and activity of BACE1. *J Biol Chem.* 284, 9027-38.
39. Ginsberg, L., Atack, J.R., Rapoport, S.I., and Gershfeld, N.L. (1993). Evidence for a membrane lipid defect in Alzheimer disease. *Mol Chem Neuropathol* 19, 37-46.
40. Ginsberg, L., Rafique, S., Xuereb, J.H., Rapoport, S.I., and Gershfeld, N.L. (1995). Disease and anatomic specificity of ethanolamine plasmalogen deficiency in Alzheimer's disease brain. *Brain Res* 698, 223-226.
41. Grimm, M.O., Grimm, H.S., Pätzold, A.J., Zinser, E.G., Halonen, R., Duering, M., Tschäpe, J.A., De Strooper, B., Müller, U., Shen, J., and Hartmann, T. (2005). Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. *Nat Cell Biol* 7, 1118-1123.
42. Grimm, M.O., Zimmer, V.C., Lehmann, J., Grimm, H.S., and Hartmann, T. (2013). The impact of cholesterol, DHA, and sphingolipids on Alzheimer's disease. *Biomed Res Int* 2013, 814390.
43. Han, X., Holtzman, D.M., and Mckeel, D.W. (2001). Plasmalogen deficiency in early Alzheimer's disease subjects and in animal models: molecular characterization using electrospray ionization mass spectrometry. *J Neurochem* 77, 1168-1180.
44. Han, X., M Holtzman, D., Mckeel, D.W., Kelley, J., and Morris, J.C. (2002). Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. *J Neurochem* 82, 809-818.
45. Hodgkin, M.N., Pettitt, T.R., Martin, A., Michell, R.H., Pemberton, A.J., and Wakelam, M.J. (1998). Diacylglycerols and phosphatidates: which molecular species are intracellular messengers? *Trends Biochem Sci* 23, 200-204.
46. Hong H. (2015) Role of Lipids in Folding, Misfolding and Function of Integral Membrane Proteins. *Adv Exp Med Biol.* 2015;855:1-31.
47. Hollingworth, P., Harold, D., Sims, R., Gerrish, A., Lambert, J.C., Carrasquillo, M.M., Abraham, R., Hamshere, M.L., Pahwa, J.S., Moskvina, V., Dowzell, K., Jones, N., Stretton, A., Thomas, C., Richards, A., Ivanov, D., Widdowson, C., Chapman, J., Lovestone, S., Powell, J., Proitsi, P., Lupton, M.K., Brayne, C., Rubinsztein, D.C., Gill, M., Lawlor, B., Lynch, A., Brown, K.S., Passmore, P.A., Craig, D., McGuinness, B., Todd, S., Holmes, C., Mann, D., Smith, A.D., Beaumont, H., Warden, D., Wilcock, G., Love, S., Kehoe, P.G., Hooper, N.M., Vardy, E.R., Hardy, J., Mead, S., Fox, N.C., Rossor, M., Collinge, J., Maier, W., Jessen, F., Ruther, E., Schürmann, B., Heun, R., Kölsch, H., Van Den Bussche, H., Heuser, I., Kornhuber, J., Wiltfang, J., Dichgans, M., Frölich, L., Hampel, H., Gallacher, J., Hüll, M., Rujescu, D., Giegling, I., Goate, A.M., Kauwe, J.S., Cruchaga, C., Nowotny, P., Morris, J.C., Mayo, K., Sleegers, K., Bettens, K., Engelborghs, S., De Deyn, P.P., Van Broeckhoven, C., Livingston, G., Bass, N.J., Gurling, H., McQuillin, A., Gwilliam, R., Deloukas, P., Al-Chalabi, A., Shaw, C.E., Tsolaki, M., Singleton, A.B., Guerreiro, R., Mühleisen, T.W., Nöthen, M.M., Moebus, S., Jöckel, K.H., Klopp, N., Wichmann, H.E., Pankratz, V.S., Sando, S.B., Aasly, J.O., Barcikowska, M., Wszolek, Z.K., Dickson, D.W., Graff-Radford, N.R., Petersen, R.C., et al. (2011). Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet* 43, 429-435.
48. Huang, B.X., Akbar, M., Kevala, K., and Kim, H.Y. (2011). Phosphatidylserine is a critical modulator for Akt activation. *J Cell Biol* 192, 979-992.
49. Hunt, W.T., Kamboj, A., Anderson, H.D., and Anderson, C.M. (2010). Protection of cortical neurons from excitotoxicity by conjugated linoleic acid. *J Neurochem* 115, 123-130.
50. Jolliffe, I.T. (2002). Principal component analysis.
51. Kalvodova, L., Kahya, N., Schwille, P., Ehehalt, R., Verkade, P., Drechsel, D., and Simons, K. (2005). Lipids as modulators of proteolytic activity of BACE: involvement of cholesterol, glycosphingolipids, and anionic phospholipids in vitro. *J Biol Chem* 280, 36815-36823.
52. Kandalepas, P.C., and Vassar, R. (2012). Identification and biology of β -secretase. *J Neurochem* 120 Suppl 1, 55-61.
53. Karch, C.M., and Goate, A.M. (2014). Alzheimer's Disease Risk Genes and Mechanisms of Disease Pathogenesis. *Biol Psychiatry*.
54. Kim, H.Y., Huang, B.X., and Spector, A.A. (2014). Phosphatidylserine in the brain: Metabolism and function. *Prog Lipid Res* 56C, 1-18.
55. Kim, K., Yang, J., and Kim, E. (2010). Diacylglycerol kinases in the regulation of dendritic spines. *J Neurochem* 112, 577-587.
56. Kou, J., Kovacs, G.G., Höftberger, R., Kulik, W., Brodde, A., Forss-Petter, S., Hönigschnabl, S., Gleiss, A., Brügger, B., Wanders, R., Just, W., Budka, H., Jungwirth, S., Fischer, P., and Berger, J. (2011). Peroxisomal alterations in Alzheimer's disease. *Acta Neuropathol* 122, 271-283.
57. Kuwata, H., Nakatani, Y., Murakami, M., and Kudo, I. (1998). Cytosolic Phospholipase A2 Is Required for Cytokine-induced Expression of Type IIA Secretory Phospholipase A2 That Mediates Optimal Cyclooxygenase-2-dependent Delayed Prostaglandin E2 Generation in Rat 3Y1 Fibroblasts.
58. Lee, J.C., Simonyi, A., Sun, A.Y., and Sun, G.Y. (2011a). Phospholipases A2 and neural membrane dynamics: implications for Alzheimer's disease. *J Neurochem* 116, 813-819.
59. Lee, J.C.M., Simonyi, A., Sun, A.Y., and Sun, G.Y.

(2011b). Phospholipases A2 and neural membrane dynamics: Implications for Alzheimer's disease. *J Neurochem* 116, 813-819.

60. Lee, J.M., Park, S.J., and Im, D.S. (2015). Lysophosphatidylethanolamine increases intracellular Ca(2+) through LPA(1) in PC-12 neuronal cells. *Biochem Biophys Res Commun* 461, 378-382.

61. Lessig, J., and Fuchs, B. (2009). Plasmalogens in biological systems: their role in oxidative processes in biological membranes, their contribution to pathological processes and aging and plasmalogen analysis. *Current medicinal chemistry* 16, 2021-2041.

62. Lin, L.L., Wartmann, M., Lin, A.Y., Knopf, J.L., Seth, A., and Davis, R.J. (1993). cPLA2 is phosphorylated and activated by MAP kinase. *Cell* 72, 269-278.

63. Listenberger, L.L., Han, X., Lewis, S.E., Cases, S., Farese, R.V., Ory, D.S., and Schaffer, J.E. (2003). Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci U S A* 100, 3077-3082.

64. Lizard, G., Rouaud, O., Demarquoy, J., Cherkaoi-Malki, M., and Iuliano, L. (2012). Potential roles of peroxisomes in Alzheimer's disease and in dementia of the Alzheimer's type. *J Alzheimers Dis* 29, 241-254.

65. Makide, K., Kitamura, H., Sato, Y., Okutani, M., and Aoki, J. (2009). Emerging lysophospholipid mediators, lysophosphatidylserine, lysophosphatidylthreonine, lysophosphatidylethanolamine and lysophosphatidylglycerol. *Prostaglandins Other Lipid Mediat* 89, 135-139.

66. Mateos, M.V., Salvador, G.A., and Giusto, N.M. (2010). Selective localization of phosphatidylcholine-derived signaling in detergent-resistant membranes from synaptic endings. *Biochim Biophys Acta* 1798, 624-636.

67. Mehmood, T. (2012). A review of variable selection methods in Partial Least Squares Regression. 118, 62-69.

68. Meylaers, K., Clynen, E., Daloz, D., Deloof, A., and Schoofs, L. (2004). Identification of 1-lysophosphatidylethanolamine (C(16:1)) as an antimicrobial compound in the housefly, *Musca domestica*. *Insect Biochem Mol Biol* 34, 43-49.

69. Migliore, L., Fontana, I., Cognato, R., Coppede, F., Siciliano, G., and Murri, L. (2005). Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. *Neurobiol Aging* 26, 587-595.

70. Munro, K.M., Nash, A., Pignoni, M., Lichtenthaler,

S.F., Gunnersen, J.M. (2016) Functions of the Alzheimer's Disease Protease BACE1 at the Synapse in the Central Nervous System. *J Mol Neurosci.* in press.

71. Nestic, I., Guix, F.X., Vennekens, K., Michaki, V., Van Veldhoven, P.P., Feiguin, F., De Strooper, B., Dotti, C.G., and Wahle, T. (2012). Alterations in phosphatidylethanolamine levels affect the generation of Aβ. *Aging Cell* 11, 63-72.

72. Nishina, A., Kimura, H., Sekiguchi, A., Fukumoto, R.H., Nakajima, S., and Furukawa, S. (2006). Lysophosphatidylethanolamine in *Grifola frondosa* as a neurotrophic activator via activation of MAPK. *J Lipid Res* 47, 1434-1443.

73. Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kaye, R., Metherate, R., Mattson, M.P., Akbari, Y., and Laferla, F.M. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. *Neuron* 39, 409-421.

74. Ohno, M. (2016) Alzheimer's therapy targeting the β-secretase enzyme BACE1: Benefits and potential limitations from the perspective of animal model studies. *Brain Res Bull.* 126(Pt 2):183-198.

75. Oliveira, T.G., and Di Paolo, G. (2010). Phospholipase D in brain function and Alzheimer's disease. *Biochim Biophys Acta* 1801, 799-805.

76. Piedrahita, D., Castro-Alvarez, J.F., Boudreau, R.L., Villegas-Lanau, A., Kosik, K.S., Gallego-Gomez, J.C., and Cardona-Gomez, G.P. (2016). beta-Secretase 1's Targeting Reduces Hyperphosphorylated Tau, Implying Autophagy Actors in 3xTg-AD Mice. *Front Cell Neurosci* 9, 498.

77. Prasad, M.R., Lovell, M.A., Yatin, M., Dhillon, H., and Markesbery, W.R. (1998). Regional membrane phospholipid alterations in Alzheimer's disease. *Neurochem Res* 23, 81-88.

78. Querfurth, H.W., and Laferla, F.M. (2010). Alzheimer's disease. *N Engl J Med* 362, 329-344.

79. Raghu, P., Coessens, E., Manifava, M., Georgiev, P., Pettitt, T., Wood, E., Garcia-Murillas, I., Okkenhaug, H., Trivedi, D., Zhang, Q., Razzaq, A., Zaid, O., Wakelam, M., O'kane, C.J., and Ktistakis, N. (2009). Rhabdomere biogenesis in *Drosophila* photoreceptors is acutely sensitive to phosphatidic acid levels. *J Cell Biol* 185, 129-145.

80. Rockenfeller, P., Koska, M., Pietrocola, F., Minois, N.,

Knittelfelder, O., Sica, V., Franz, J., Carmona-Gutierrez, D., Kroemer, G., and Madeo, F. (2015). Phosphatidylethanolamine positively regulates autophagy and longevity. *Cell Death Differ* 22, 499-508.

81. Rosenthal, S.L., and Kamboh, M.I. (2014). Late-Onset Alzheimer's Disease Genes and the Potentially Implicated Pathways. *Curr Genet Med Rep* 2, 85-101.

82. Sagin, F.G., and Sozmen, E.Y. (2008). Lipids as key players in Alzheimer disease: alterations in metabolism and genetics. *Curr Alzheimer Res* 5, 4-14.

83. Sanchez-Mejia, R.O., and Mucke, L. (2010). Phospholipase A2 and arachidonic acid in Alzheimer's disease. *Biochim Biophys Acta* 1801, 784-790.

84. Sangiovanni, J.P., and Chew, E.Y. (2005). The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog Retin Eye Res* 24, 87-138.

85. Seo, J., Kim, K., Jang, S., Han, S., Choi, S.Y., and Kim, E. (2012). Regulation of hippocampal long-term potentiation and long-term depression by diacylglycerol kinase ζ. *Hippocampus* 22, 1018-1026.

86. Shea, T.B. (1997). Phospholipids alter tau conformation, phosphorylation, proteolysis, and association with microtubules: implication for tau function under normal and degenerative conditions. *J Neurosci Res* 50, 114-122.

87. Sorger, D., and Daum, G. (2003). Triacylglycerol biosynthesis in yeast. *Appl Microbiol Biotechnol* 61, 289-299.

88. Sparkes, B.L., Slone, E.E., Roth, M., Welti, R., and Fleming, S.D. (2010). Intestinal lipid alterations occur prior to antibody-induced prostaglandin E2 production in a mouse model of ischemia/reperfusion. *Biochim Biophys Acta* 1801, 517-525.

89. Sperling, R.A., Bates, J.F., Chua, E.F., Cocchiarella, A.J., Rentz, D.M., Rosen, B.R., Schacter, D.L., and Albert, M.S. (2003). fMRI studies of associative encoding in young and elderly controls and mild Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 74, 44-50.

90. Sundaram, J.R., Chan, E.S., Poore, C.P., Pareek, T.K., Cheong, W.F., Shui, G., Tang, N., Low, C.M., Wenk, M.R., and Kesavapany, S. (2012). Cdk5/p25-induced cytosolic PLA2-mediated lysophosphatidylcholine production regulates neuroinflammation and triggers neurodegeneration. *J Neurosci* 32, 1020-1034.

91. Thinakaran, G., and Koo, E.H. (2008). Amyloid precursor protein trafficking, processing, and function. *J Biol Chem* 283, 29615-29619.

92. Urabe, M., Ding, C., and Kotin, R.M. (2002). Insect cells as a factory to produce adeno-associated virus type 2 vectors. *Hum Gene Ther* 13, 1935-1943.

93. Vance, J.E., and Tasseva, G. (2013). Formation and function of phosphatidylserine and phosphatidylethanolamine in mammalian cells. *Biochim Biophys Acta* 1831, 543-554.

94. Vassar, R. (2014) BACE1 inhibitor drugs in clinical trials for Alzheimer's disease. *Alzheimers Res Ther.* 6(9):89.

95. Whitley, L., Sen, A., Heaton, J., Proitsi, P., García-Gómez, D., Leung, R., Smith, N., Thambisetty, M., Kłoszewska, I., Mecocci, P., Soininen, H., Tsolaki, M., Vellas, B., Lovestone, S., Legido-Quigley, C., and Consortium, A. (2014). Evidence of altered phosphatidylcholine metabolism in Alzheimer's disease. *Neurobiol Aging* 35, 271-278.

96. Yao, J.K., Wengenack, T.M., Curran, G.L., and Poduslo, J.F. (2009). Reduced membrane lipids in the cortex of Alzheimer's disease transgenic mice. *Neurochem Res* 34, 102-108.

97. Zerouga, M., Stillwell, W., Stone, J., Powner, A., and Jenks, L.J. (1996). Phospholipid class as a determinant in docosahexaenoic acid's effect on tumor cell viability. *Anticancer Res* 16, 2863-2868.

98. Zhou, Z., Marepally, S.R., Nune, D.S., Pallakollu, P., Ragan, G., Roth, M.R., Wang, L., Lushington, G.H., Visvanathan, M., and Welti, R. (2011). LipidomeDB data calculation environment: online processing of direct-infusion mass spectral data for lipid profiles. *Lipids* 46, 879-884.

CHAPTER 3

Differential pattern of phospholipid profile in the temporal cortex from E280A-familial and sporadic Alzheimer's disease brains

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Running title: Phospholipid profile in Alzheimer's disease

Abstract

Lipids are considered important factors in the pathogenesis of Alzheimer Disease (AD). In this study we realized a comparative analysis of the phospholipid profile and phospholipid composition of the temporal cortex from E280A-familial AD (FAD), sporadic AD (SAD) and healthy human brains. Findings showed a significant decrease of lysophosphatidylcholine and phosphatidylethanolamine formed by low levels of polyunsaturated fatty acids (20:4, 22:6) in the AD brains. However, Phosphatidylethanolamine-Ceramide (PE-Cer) and Phosphoglycerol (PG) were significantly increased in SAD, conformed by high levels of (18:0/18:1) and (30/32/36:0/1/2) respectively. Together, the findings suggest deficiency in LPC, PE, and alteration in the balance between poly and unsaturated fatty acids in both types of AD, and a differential pattern of phospholipid profile and fatty acid composition between E280A FAD and SAD human brains.

Key words: Alzheimer's disease, phospholipids, fatty acids, temporal cortex, polyunsaturated fatty acids.

Abbreviations: DAG, diacylglycerol; TG, triacylglycerol; FA, fatty acid; FFA, free fatty acid; PL, phospholipid; PA, phosphatidic acid; PC, phosphatidylcholine; LPC, lysophosphacholine; ePC ether phosphatidylcholine; PS, phosphatidylserine; ePS, ether phosphatidylserine; PE, phosphatidylethanolamine; PE-Cer, phosphatidylethanolamine ceramide; LPE, lysophosphatidylethanolamine; ePE, ether phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; SM, sphingomyelin; CE, cholesterol esters; AA, arachidonic acid; cPLA, cytosolic phospholipase. SAD, Sporadic Alzheimer Disease; FAD, Familial Alzheimer Disease

Introduction

Alzheimer disease (AD) currently is suffered by 15 million of individuals worldwide [1], being the most common cause of dementia and one of the main sources of morbidity in the aging population. Its etiopathogenesis is multifactorial, with a very slow progression in the sporadic type of AD, affecting 10% of the population over 65 years of age, and approximately 50% over 85 years old. AD is characterized by severe neuronal loss, which alters the tissue composition and macrostructure of cerebral regions, in a moderate mode in the cerebral cortex, and severe mode in the hippocampus [2].

Most frequent of AD is sporadic type (SAD), however AD variants as autosomal dominant Familial Alzheimer Disease (FAD) have been described [3]. FAD explain the 0.5% of all AD cases, which is characterized by an early onset (<65 years old) and a fast-increased severity; around 50% of FAD patients presenting autosomal dominant inheritance of mutations in one of the associated three genes, namely, preseniline-1 (PS1), preseniline-2 (PS2) and APP (Amyloid precursor protein). These mutations are closely related with the increase of A β 1-42 production, which is triggered in a younger age generating different types of early onset FAD (EO-FAD) [3]. Although FAD and SAD share some features, it is not clear if these are equivalent diseases. In facts, metabolomics, lipidomics, transcriptomics, and epigenomics are new approach searching complementary criteria for AD diagnosis, because some features changes for FAD, in many but not all cases, seem similar to SAD [4].

Several lines of evidence suggest that lipid metabolism participate in the pathogenesis of AD. Epidemiologic studies suggest that increased plasma cholesterol levels are associated with the disease. Furthermore, cellular studies have shown that the levels and distribution of intracellular lipids affect the processing of amyloid precursor protein into A beta peptides, being toxic species for the brain [5][6]. Fatty acid composition of phospholipids, as polyunsaturated fatty acids (PUFAs), mainly docosahexaenoic acid (DHA) and arachidonic acid (AA) have crucial roles in the preservation of physiology and maintenance of central nervous system [7]; therefore, their alteration increases the risk for developing AD [8][9]. Also, saturated fatty acids (SFA) provide stability in the cell membrane structure and membrane proteins. But, altered levels are related with neurodegenerative diseases, and those high levels of SFA are associated with a high level of serum cholesterol, which is strongly correlated with AD [10]. Nevertheless, the PUFA effects on the regulation of lipid metabolism and in the prevention of neurodegenerative diseases appear to be diverse [11]. Balanced PUFA and saturated fatty acids ratio was thought to be very important in regulating serum cholesterol [12]. However, excessive increase of PUFA has undesirable effects on oxidative stress, because of the high susceptibility to lipid peroxidation [13][14]. Those evidences suggest that a sensitive balance between PUFAs and SFAs ratio allows a membrane homeostasis avoiding the cell death.

In the present study we realized a comparative analysis of the phospholipid profile and phospholipid composition of the inferior temporal cortex from E280A familial AD (FAD), sporadic AD (SAD) and healthy human brains.

Materials And Methods

Human brain samples

All samples from AD and healthy individuals provided informed consent for research, and this study was approved by the bioethical committee for human studies from the University of Antioquia. We included 5 cases of Sporadic Alzheimer Disease (SAD), 5 cases of Familial Alzheimer Disease (FAD) (presenilin 1 mutation E280A) and 3 control cases obtained from the University of Antioquia's Brain Bank (Table 1). On the table, we showed the post-mortem index, as time lapse between patient's death and sampling; Braak stage, to classify the degree of AD [15]; NIA-RI criteria, as pathological diagnosis of AD's "high likelihood" [16], and Consortium to Establish a Registry for Alzheimer's Disease (CERAD), is a neuropsychological battery developed for screening of Alzheimer's disease and recently validated [17]. It comprises the following eight sub-tests: Mini-Mental State Examination (MMSE/30), Verbal Fluency (VF/24), Modified Boston Naming Test (BNT/15), Word List Learning (WLL/30), Word List Recall (WLR/10), Word Recognition Discriminability (WLRD/10), Constructional Praxis Copy (CP/11), and Constructional Praxis Recall (PR/11) [17]. Frozen samples were obtained from the inferior temporal cortex for biochemical analysis.

Lipid analysis

The total lipids from the inferior temporal cortex were extracted according to the FOLCH technique [18] using a mixture of 2 mL of chloroform (CHCl₃) and 1mL of methanol (MeOH) in a 2:1 (v/v) ratio. Then, 0.005% butylated hydroxytoluene (BHT) was added, and this mixture was used to homogenize the tissue. Subsequently, 1 mL of 0.9% NaCl was added, and the mixture was centrifuged at 3000 rpm for 3 minutes. The organic layer (lower layer) was removed and transferred to a new glass tube. This procedure was performed in an oxygen-free using enriched nitrogen environment to avoid lipid oxidation processes and was repeated three times. The solvents were evaporated, and the extract was lyophilized to remove the excess of humidity. Finally, the lipid composition was analyzed by mass spectrometry.

Mass spectrometry

An automated ESI-MS/MS approach was used and data acquisition and analysis carried out at the Kansas Lipidomics Research Center using an API 4000™ and Q-TRAP (4000Qtrap) detection system as it was described previously [19]. This protocol allowed the detection

and quantification of low concentrations of the polar lipid compounds. The molecules were determined by the mass/charge ratios, which were compared with the respective internal standard to determine which species of lipids were present in the evaluated extract: 0.66nmol di14:0-PC, 0.66 nmol di24:1-PC, 0.66 nmol 13:0-lysoPC, 0.66 nmol 19:0-lysoPC, 0.36 nmol di14:0-PE, 0.36 nmol di24:1-PE, 0.36 nmol 14:0-lysoPE, 0.36 nmol 18:0-lysoPE, 0.36 nmol 14:0-lysoPG, 0.36 nmol 18:0- lysoPG, 0.36 nmol di14:0-PA, 0.36 nmol di20:0 (phytanoyl)-PA, 0.24 nmol di14:0-PS, 0.24 nmol di20:0 (phytanoyl)-PS, 0.20 nmol 16:0-18:0-PI, 0.16 nmol di18:0-PI, and 1 nmol 15:0 fatty acid. The system detected a total of 12 different lipid species and their respective subspecies, which were identified by the number of carbons and degree of unsaturation of the chain. Lipid concentration was normalized by molar concentration across all species for each sample, and the final data are presented as mean mol%.

Table 1. Description of brain tissue samples.

DIAGNOSTIC	GENDER	AGE OF ONSET	AGE OF DEATH	POST-MORTEM INDEX (H)	BRAAK NF STAGE	NIA-RI	CERAD SCORE
SAD	F	82	92	5,5	VI	3	C
SAD	F	65	74	2,5	IV	3	C
SAD	F	69	76	3,75	VI	3	C
SAD	M	NA	83	4,3	VI	3	C
SAD	M	70	83	4,3	VI	3	C
FAD	M	49	55	2,8	VI	3	C
FAD	F	50	60	2,8	VI	3	C
FAD	F	45	55	2,5	VI	3	C
FAD	M	40	53	4,1	VI	3	C
FAD	F	62	74	5,5	V	3	C
CONTROL	F	NAP	43	>24	0	0	0
CONTROL	M	NAP	60	>24	0	0	0
CONTROL	F	NAP	84	4,3	2	1	A

NA= not available; NAP= not applicable. 0 = No histological evidence of Alzheimer's disease. A = Histological findings are UNCERTAIN evidence of Alzheimer's disease. B = Histological findings SUGGEST the diagnosis of Alzheimer's disease. C = Histologic findings INDICATE the diagnosis of Alzheimer's disease.

Statistical analysis

The lipid levels for each sample were calculated by summing the total number of moles of all lipid species measured and then normalizing that total to %mol. Comparisons between groups were assessed either by one-way ANOVA, followed by the Tukey post-hoc test or the Kruskal-Wallis test, depending on the homoscedasticity and normality of the experimental data. Multivariate statistics were performed using a partial least squares-discriminant anal-

ysis (PLS-DA) [20]. PLS-DA was included because it is particularly suitable for the analysis of datasets with a small number of samples and a large number of variables. The PLS-DA analysis was carried out using the routines described in [21]. An index representing the importance of the variables according to the first components was estimated, the PLS-DA index used is called the variable importance in projection (VIP) [22], which determines the importance of each variable that is reflected by each component and, similar to PCA, it also considers the variance explained by each PLS component (for a detailed description of VIP [22]). The confidence ellipsoids per group and treatment are also included. The data from the univariate and bivariate statistics are expressed as the mean $\pm$ the standard error of the mean. The statistical significance is indicated in the figures and tables.

Figure 1. Lipid composition of the temporal cortex from human FAD, SAD and healthy brains. A - The lipid class profiles are expressed as %mol composition. All lipid species were measured (means), and the error bars represent the SEM. The data for the FAD and SAD tissue were significantly different from the control groups (* $p < 0.05$, ** $p < 0.01$, ANOVA followed by the Tukey post hoc test or Kruskal-Wallis test). B - Multivariate analysis of the lipid profiles from the temporal cortex of FAD, SAD and healthy brains. PLS-DA, Partial least squares analysis to discriminate between the lipid classes. The right panels show the factor score plots for PLS-DA and VIP index. DAG, diacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; LPC, Lysophosphatidylcholine; ePC ether phosphatidylcholine; PS, phosphatidylserine; ePS, ether phosphatidylserine; PE, phosphatidylethanolamine; PE-Cer, Phosphatidylethanolamine Ceramide; LPE, Lysophosphatidylethanolamine; ePE, ether phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; SM, sphingomyelin. $n = 3-4$ per group. ** $p < 0.01$. Data are expressed as %mol. $n = 3-5$ per group.

Results

At a glance, the lipidomic analysis of the temporal cortex revealed a type of changes related with the neurodegenerative condition of SAD and FAD compared with healthy brains, while a second type of changes presented differences between the both Alzheimer variants respect to the control group.

Temporal cortex lipid profile was detected in fifteen lipid species, where PC (47%), PE (23%) and SM (20%) showed the higher Mol%. Although, LPC and PE concentrations showed a clear tendency or significant reduction in both AD entities. Interestingly, a lipid specie derived from PE, the phosphosphingolipid PE-Cer, also known as N-(octadecanoyl)-tetradecasphing-4-enine-1-phosphoethanolamine (<http://www.lipidmaps.org/data/LMSDRecord.php?LMID=LMSP03020001>), showed a significant increased concentration in SAD, whereas its concentration in FAD did not present changes compared with the control group. Also, PG concentration increased significantly in SAD, respect to a similar concentration in FAD and in the control group (Fig. 1A).

PLS-DA analysis indicated that the control group is located in a different quadrant of the

Figure 2. PE levels in the temporal cortex from FAD, SAD and healthy brains. A - Contour plot of the more influential subclasses of PE in the discriminant analysis for each evaluated variable; all PE subclasses were measured (means). B - Multivariate analysis using PLS-DA, Partial least squares analysis, to discriminate between the PE subclasses. The table show the factor score plots for PLS-DA with the indices of variance explained for each component (VIP). C - Total number of carbon's atoms in the fatty acid moieties of PE composition. D - PE species saturation equal to the total number of double bonds in the fatty acid moieties. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are expressed as %mol. $n = 3-5$ per group.

plane compared with the FAD and SAD groups (Fig. 1B). In addition, the FAD and SAD groups showed a different distribution pattern, indicating that phospholipids (PLs) profile were different for each AD's type (Fig. 1B). 60.8 % of the total variance may be explained by factor 1, that in according to the VIP the five more changing species were conformed mainly by saturated fatty acids were PG 32:0 (16:0/16:0) (VIP = 2.1285), PG (36:2) (18:1/18:1), PS 36:4 (20:4/16:0) (VIP = 1.9358), PE 34:0 (16:0/18:0) (VIP = 1.8640), PA 32:0 (16:0/16:0) (VIP = 1.8120) compared with control group (Fig. 1B).

PE and LPC levels are reduced in the temporal cortex from FAD and SAD brains

When we analysed each significant changing phospholipid, we found that PE levels presented reduced concentration in FAD and SAD, and PLS-DA analysis indicated that nearly 73% (PE) of the total variance could be explained by factor 1 (Fig. 2). The most relevant molecular sub-lipidic specie was PE 40:6 (18:0/22:6) in AD, which had a higher VIP index (2,6757) (Fig. 2A, B), also supported by the reduction in the fatty acids composition of 40C with 4 or 6

Figure 4. PE-Cer levels in the temporal cortex from FAD, SAD and healthy brains. A - Multivariate analysis using PLS-DA, Partial least squares analysis to discriminate between the PE-Cer subclasses. B - shown the Contour plot of the more influential subclasses of PE-Cer (variables) in the discriminant analysis for each variable evaluated; all PE-Cer subclasses were measured (means). C - Total number of carbon's atoms in the fatty acid moieties of PE-Cer composition. D - PE-Cer species saturation equal to the total number of double bonds in the fatty acid moieties. ** $p < 0.01$, *** $p < 0.001$. Data are expressed as %mol. $n = 3-5$ per group.

unsaturations (Fig 2C, D). Together, FAD and SAD groups had the same distribution pattern of PE on the plane compared to the different quadrant distribution showed by the control group (Fig. 2B).

Another phospholipid affected in both AD types was the LPC. In accordance with the PLS-DA analysis the main decreased lipid subclasses LPC 16:0 (VIP= 2.1285) and LPC 18:1 (VIP=2.0949), and the significant reduction of those fatty acids composition (16,18C:0,1) (Fig 3A, B). Furthermore, AD group's had the same distribution pattern on the quadrant of the graph opposed to the control group. These data were supported by the reduced dye intensity in FAD and SAD groups observed in the contour plot (Fig. 3 A). Also supported by the reduction in the fatty acids composition of 16C, 18C and 22C with 0, 1, 4 and 6 unsaturation, respect to the control group (Fig 3C, D).

Figure 3. LPC levels in the temporal cortex from FAD, SAD and healthy brains. A - the Contour plot of the more influential subclasses of LPC (variables) in the discriminant analysis for each variable evaluated; all LPC subclasses were measured (means). B - Multivariate analysis using PLS-DA, Partial least squares analysis to discriminate between the PL subclasses. The table show the factor score plots for PLS-DA with the indices of variance explained for each component (VIP). C - Total number of carbon's atoms in the fatty acid moieties of LPC composition. D - LPC species saturation equal to the total number of double bonds in the fatty acid moieties. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are expressed as %mol. $n = 3-5$ per group.

Figure 5. PG levels in the temporal cortex from FAD, SAD and healthy brains. A- The Contour plot of the more influential subclasses of PG (variables) in the discriminant analysis for each variable evaluated; all PG subclasses were measured (means). The analyzed groups are the healthy (controls), FAD and SAD. n=3-5 per group. B - Multivariate analysis using PLS-DA, Partial least squares analysis to discriminate between the PG subclasses. The table show the factor score plots for PLS-DA with the indices of variance explained for each component (VIP). C - Total number of carbons atoms in the fatty acid moieties of PG composition. D - PG species saturation equal to the total number of double bonds in the fatty acid moieties. Data are expressed as %mol. n=3-5 per group.

PE-Cer and PG have a differential profile in SAD respect to FAD brains

PLS-DA analysis presented a 72,97% of the total variance explained by factor 1 between AD types and healthy groups (Fig. 4A). PE-Cer 18:0 subclass was the most determinant in the differential distribution pattern in the plane compared to the control group (VIP=3.5201), followed by PE-Cer 38:4 (18:0/20:4) (VIP=0,7512) and PE-Cer 40:4 (18:0/22:4) (VIP=0,7512) (Fig. 4A). PE-Cer concentration was significantly higher in the SAD group in comparison with FAD and healthy groups, and specifically the higher concentration of PE-Cer (18:0) in SAD supported by the contour plot (Fig. 4B). Complementarily, the fatty acid composition by 18C with 0 or 1 unsaturation was significant higher in SAD (Fig. 4C, D). Therefore, all evaluated groups had a differential distribution patterns in the graph, suggesting a divergence between FAD and SAD in that sub-lipidic specie.

Also, PG concentration increased in SAD, which was evidence in the contour plot (Fig 5A). The main lipid subclass was the PG 32:0 (16:0/16:0) (VIP=2.0949) according to PLS-DA analysis (Fig. 5B), supported by the increased of fatty acid composition by 30C, 32C, 36C with 0 or 1 unsaturation (Fig. 5C, D). In addition, the distribution pattern in the plane showed divergent distribution between AD groups even the FAD group was closer to control group (Figure 5B).

Discussion

Our data suggest a deficiency of PE and LPC levels in the temporal cortex from AD-E280A and SAD brains respect to healthy brains, and a particular up-regulation of PE-Cer and PG levels in the SAD's samples. The temporal cortex has been identified as an affected area by NFTs and β A plaques in AD, also presenting synaptic and neuronal loss [23][24][25]. Few studies have realized a comparative analysis of lipid profile between FAD and SAD by lipidomic approach. Nevertheless, the involvement of lipid alterations in AD brains have been well-established in the last years, in particular with reference to cholesterol and polyunsaturated fatty acid contents [26] [27].

The reduction of PE and LPC levels have been previously described in AD brains [27]. PE is a precursor of several lipid sub-species, as essential component of mammalian cell membranes; it has been reported its decrease in *post-mortem* AD brains and in closed correlation with A β accumulation in Tg2527 mouse model of AD [28], confirming our observations in FAD-E280A and SAD brains (table 1). Although, our data showed a reproducible reduction of PE (15 Mol%) and a slight increased conversion to ePE (0,5%) in the inferior temporal cortex of AD, which have been demonstrated as a compensatory phenomenon [29]. Those levels were very different respect to previous studies, where ePE deficiency was up to 50% in AD mid-temporal cortex [30]. However, other recent study showed 6 and 8 Mol% of PE and ePE respectively in the prefrontal cortex from human brains [27]; suggesting an intrinsic variation per study and/or per cerebral region. Maybe, it is possible that a factor affecting the ePE levels detected in our study be because of the diacyl lipids were used as standards, and do not ether-linked lipids. Data is supported by a similar detection levels in our recent lipidomic analysis in 3xTgAD mice model [31].

Also, a decrease of PC and LPC have been reported previously in peripheral blood samples [32], human brain tissue and animal models [27]. But, PC modulation in AD is not consistent through the different studies [32] [33], as in our data, where PC levels did not change. Nevertheless, LPC could be a sensitive parameter to measure alterations in the PC turnover or breakdown, because although it is usually present in low concentration into the brain, it has more stable concentration [33]. This concentration is regulated by the catalytic enzyme phospholipase A2 (PLA2) and Lysophospholipid acyltransferase (LPCAT), which turn lysophospholipids to phospholipids. The PLA2 has been described to be significantly de-

creased in parietal and temporal cortices of patients with AD, existing a correlation between the reduction of PLA2 and the severity in AD [34].

Moreover, the failure of PE and LPC production in the temporal cortices from SAD and FAD brains have reduced composition of monounsaturated acid, as Linoleic acid (18:1) and polyunsaturated fatty acids (PUFAs), as DHA (22:6) and AA (20:4). Which have been previously reported in the hippocampus, temporal cortex and mid-frontal cortex [35] [36] [37]. Furthermore, the turnover of DHA towards the neuroprotective docosanoids, as resolvins and neuroprotectins, which are effectors of antioxidant, anti-inflammatory, and antiapoptotic actions, also are altered in AD [38]. Interestingly, a supplemental dietary of DHA (22:6n-3) reduces neurodegeneration in mouse models of AD [39] [40]. Other studies suggest a neuroprotective effect of PUFAs, as AA on cognitive function, preserving the synaptic plasticity [41][42]. Recently, it has been demonstrated that arachidonic or docosahexaenoic acid diet prevents memory impairment and reduces the A β production in Tg2576 Mice [43] [44].

On another hand, the findings showed that PE-Cer is increased in SAD brains. PE-Cer is a lipid analogue to sphingomyelin (SM), but in basal conditions is >300-fold lower [45], its increased levels are related with imbalance in SM synthase (SMS2) activity. SMS2 is a bi-functional enzyme producing both PE-Cer and SM. SM synthase-related enzyme (SMSr) is the principal PE-Cer synthase in the brain [45]. PE-Cer has been described in insects [46], expressed in Golgi apparatus, with a widespread occurrence in the animal kingdom [47]. It has been associated to synaptic vesicles [48]; and ceramide as a modulator of phosphatidylethanolamine has been correlated in chronic disease, as diabetes, obesity and some cancers [49]. Elevation of ceramide levels in AD is highly correlated in mild cognitive impairment [50] and apoptosis [51][52]. Therefore, in our study we detected that SM presented a tendency to increase in both FAD and SAD groups (Figure 1), suggesting PE-Cer is a marker of chronic impairment in SAD condition.

Another change was obtained on PG, which is found in low concentrations in mammal cells, this is mainly synthesized by the mitochondria and regulates numerous biological functions, such as activation of the protein kinase C family and the stability of mitochondrial membrane [53][54][55]. PG is catalyzed by phosphatidylglycerophosphate synthase (PGS) activity and its overexpression increase the cell content of PG and decrease the PA concentration [55], and also PG is inversely related to PE levels in synaptic dysfunction in an AD model [56], which is coincident with our data, suggesting mitochondrial dysfunction is associated to an advanced stage of SAD [57][58].

Complementarily, the main composition of PE-Cer and PG in the temporal cortex from SAD brains was composed by saturated fatty acids, as stearic (18:0), palmitic (16:0) acid and arachidonic acid (20:4). Epidemiological studies suggest that high fat diet increases the risk of AD and the degree of saturation of fatty acids is a critical risk factor for developing AD

[52], mainly in the frontal gray matter and in hippocampus [34]. In addition, neurofibrillary tangles are associated to palmitic and stearic fatty acids enriched membranes in AD brains [52]. Palmitic acid causes up-regulation of the BACE1 enzyme and its amyloidogenic processing of β -amyloid precursor protein (APP) [59][60][61], supporting the metabolic alteration in the sporadic condition. Also, this chronic neurodegeneration could be accompanied by a pro-inflammatory environment, since our data showed AA composition in PE-Cer and PG. AA is metabolized to eicosanoids, such as prostaglandins, leukotrienes, and thromboxanes, all of which are potent mediators of inflammation. This pathway also involves to cyclooxygenases (COX), particularly COX-2 leading to A β plaques formation and cognitive deficits in APP mouse model [9].

In summary, the results suggest a deficiency of PE and LPC phospholipids formed by low concentration of mono (18:1) and polyunsaturated fatty acid (22:6, 20:4) in the temporal cortex of E280A-FAD and SAD brains, suggesting a common loss of homeostasis in a neurodegenerative process and the necessity of adequate balance between saturated and unsaturated FA composition in the cerebral regions. In addition, SAD brains present a high composition of saturated fatty acid (18:0 and 16:0) in PE-Cer and PG, suggesting a context of Golgi and mitochondria dysfunction and pro-inflammatory environment during a longer chronic neurodegeneration. This differential metabolic profile could help to define a divergent pattern between AD types and healthy brains. However, more studies are necessary for understanding the role of the phospholipids in the dynamic of neurodegeneration and the recovering of cerebral homeostasis.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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References

- [1] Reitz C, Mayeux R (2014) Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers. *Biochem Pharmacol* 88, 640-651.
- [2] Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S, Van der Flier WM (2016) Alzheimer's disease. *Lancet* 388, 507-517.
- [3] Bird TD (1993) Early-Onset Familial Alzheimer Disease. In *Gene Reviews*, Pagon RA, Adam MP, Ardinger HH et al. eds. University of Washington, Seattle.
- [4] Lista S, O'Bryant SE, Blennow K, Dubois B, Hugon J, Zetterberg H, Hampel H (2015) Biomarkers in sporadic and familial Alzheimer's disease. *J Alzheimers Dis* 47, 291-317.
- [5] Kalvodova L, Kahya N, Schwille P, Eehalt R, Verkade P, Drechsel D, Simons K (2005) Lipids as modulators of proteolytic activity of BACE: Involvement of cholesterol, glycosphingolipids, and anionic phospholipids in vitro. *J Biol Chem* 280, 36815-36823.
- [6] Hirsch-Reinshagen V, Burgess BL, Wellington CL (2009) Why lipids are important for Alzheimer disease? *Mol Cell Biochem* 326, 121-129.
- [7] Diau GY, Hsieh AT, Sarkadi-Nagy EA, Wijendran V, Nathanielsz PW, Brenna JT (2005) The influence of long chain polyunsaturate supplementation on docosahexaenoic acid and arachidonic acid in baboon neonate central nervous system. *BMC Med* 3, 11.
- [8] Bazan NG, Molina MF, Gordon WC (2011) Docosahexaenoic acid signalolipidomics in nutrition: Significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. *Annu Rev Nutr* 31, 321-351.
- [9] Bazinet RP, Laye S (2014) Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat Rev Neurosci* 15, 771-785.
- [10] Simopoulos AP (2016) An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients* 8, 128.
- [11] Rao JS, Ertley RN, DeMar JC Jr, Rapoport SI, Bazinet RP, Lee HJ (2007) Dietary n-3 PUFA deprivation alters expression of enzymes of the arachidonic and docosahexaenoic acid cascades in rat frontal cortex. *Mol Psychiatry* 12, 151-157.
- [12] Siri-Tarino PW, Sun Q, Hu FB, Krauss RM (2010) Saturated fat, carbohydrate, and cardiovascular disease. *Am J Clin Nutr* 91, 502-509.
- [13] Liu JJ, Green P, Mann JJ, Rapoport SI, Sublette ME (2015) Pathways of polyunsaturated fatty acid utilization: Implications for brain function in neuropsychiatric health and disease. *Brain Res* 1597, 220-246.
- [14] Calder PC (2012) Long-chain fatty acids and inflammation. *Proc Nutr Soc* 71, 284-289.
- [15] Braak H, Braak E (1997) Staging of Alzheimer-related cortical destruction. *Int Psychogeriatr* 9(Suppl 1), 257-272.
- [16] Geddes JW, Tekirian TL, Soultanian NS, Ashford JW, Davis DG, Markesbery WR (1997) Comparison of neuropathologic criteria for the diagnosis of Alzheimer's disease. *Neurobiol Aging* 18, 99-105.
- [17] Fillenbaum GG, van Belle G, Morris JC, Mohs RC, Mirra SS, Davis PC, Tariot PN, Silverman JM, Clark CM, Welsh-Bohmer KA, Heyman A (2008) CERAD (Consortium to Establish a Registry for Alzheimer's Disease) The first 20 years. *Alzheimers Dement* 4, 96-109.
- [18] Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226, 497-509.
- [19] Zhou Z, Marepally SR, Nune DS, Pallakollu P, Ragan G, Roth MR, Wang L, Lushington GH, Visvanathan M, Welti R (2011) LipidomeDB data calculation environment: Online processing of direct-infusion mass spectral data for lipid profiles. *Lipids* 46, 879-884.
- [20] Barker M, Rayens W (2003) Partial least squares for discrimination. *J Chemometrics* 17, 166-173.
- [21] Ballabio D, Cosonni V (2013) Classification tools in chemistry. Part 1: Linear models. PLS-DA. *Anal Methods* 5, 3790-3798.
- [22] Mehmood T, Hovde Liland K, Snipen L, Sæbø S (2012) A review of variable selection methods in Partial Least Squares Regression. *Chemometr Intell Lab Syst* 118, 62-69.
- [23] Khan UA, Liu L, Provenzano FA, Berman DE, Profaci CP, Sloan R, Mayeux R, Duff KE, Small SA (2014) Molecular drivers and cortical spread of lateral entorhinal cortex dysfunction in preclinical Alzheimer's disease. *Nat Neurosci* 17, 304-311.
- [24] Davies CA, Mann DM, Sumpter PQ, Yates PO (1987) A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. *J Neurol Sci* 78, 151-164.
- [25] Scheff SW, Price DA (2006) Alzheimer's disease-related alterations in synaptic density: Neocortex and hippocampus. *J Alzheimers Dis* 9, 101-115.
- [26] Di Paolo G, Kim TW (2011) Linking lipids to Alzheimer's disease: Cholesterol and beyond. *Nat Rev Neurosci* 12, 284-296.
- [27] Chan RB, Oliveira TG, Cortes EP, Honig LS, Duff KE, Small SA, Wenk MR, Shui G, Di Paolo G (2012) Comparative lipidomic analysis of mouse and human brain with Alzheimer disease. *J Biol Chem* 287, 2678-2688.
- [28] George AJ, Holsinger RM, McLean CA, Tan SS, Scott HS, Cardamone T, Cappai R, Masters CL, Li QX (2006) Decreased phosphatidylethanolamine binding protein expression correlates with Abeta accumulation in the Tg2576 mouse model of Alzheimer's disease. *Neurobiol Aging* 27, 614-623.
- [29] Dörninger F, Brodde A, Braverman NE, Moser AB, Just WW, Forss-Petter S, Brügger B, Berger J (2015) Homeostasis of phospholipids - The level of phosphatidylethanolamine tightly adapts to changes in ethanolamine plasmalogens. *Biochim Biophys Acta* 1851, 117-128.
- [30] Ginsberg L, Rafique S, Xuereb JH, Rapoport SI, Gershfeld NL (1995) Disease and anatomic specificity of ethanolamine plasmalogen deficiency in Alzheimer's disease brain. *Brain Res* 698, 223-6.
- [31] Villamil-Ortiz JG, Barrera-Ocampo A, Piedrahita D, Velasquez-Rodriguez CM, Arias-Londono JD, Cardona-Gomez GP (2016) BACE1 RNAi restores the composition of phosphatidylethanolamine-derivates related to memory improvement in aged 3xTg-AD mice. *Front Cell Neurosci* 10, 260.
- [32] Naudi A, Cabre R, Jove M, Ayala V, Gonzalo H, Portero-Otin M, Ferrer I, Pamplona R (2015) Lipidomics of human brain aging and Alzheimer's disease pathology. *Int Rev Neurobiol* 122, 133-189.
- [33] Whitley L, Sen A, Heaton J, Proitsi P, García-Gómez D, Leung R, Smith N, Thambisetty M, Kloszewska I, Mecocci P, Soininen H, Tsolaki M, Vellas B, Lovestone S, Legido-Quigley C, AddNeuroMed Consortium (2014) Evidence of altered phosphatidylcholine metabolism in Alzheimer's disease. *Neurobiol Aging* 35, 271-278.
- [34] Lee JCM, Simonyi A, Sun AY, Sun GY (2011) Phospholipases A2 and neural membrane dynamics: Implications for Alzheimer's disease. *J Neurochem* 116, 813-819.
- [35] Soderberg M, Edlund C, Kristensson K, Dallner G (1991) Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. *Lipids* 26, 421-425.
- [36] Astarita G, Jung KM, Berchtold NC, Nguyen VQ, Gillen DL, Head E, Cotman CW, Piomelli D (2010) Deficient liver biosynthesis of docosahexaenoic acid correlates with cognitive impairment in Alzheimer's disease. *PLoS One* 5, e12538.
- [37] Cunnane SC, Chouinard-Watkins R, Castellano CA, Barberger-Gateau P (2013) Docosahexaenoic acid homeostasis, brain aging and Alzheimer's disease: Can we reconcile the evidence? *Prostaglandins Leukot Essent Fatty Acids* 88, 61-70.
- [38] Farooqui AA (2012) Lipid mediators and their metabolism in the nucleus: Implications for Alzheimer's disease. *J Alzheimers Dis* 30(Suppl 2), S163-178.
- [39] Qu MH, Yang X, Wang Y, Tang Q, Han H, Wang J, Wang GD, Xue C, Gao Z (2016) Docosahexaenoic acid-phosphatidylcholine improves cognitive deficits in an Abeta23-35-induced Alzheimer's disease rat model. *Curr Top Med Chem* 16, 558-564.
- [40] Stonehouse W, Conlon CA, Podd J, Hill SR, Minihane AM, Haskell C, Kennedy D (2013) DHA supplementation improved both memory and reaction time in healthy young adults: A randomized controlled trial. *Am J Clin Nutr* 97, 1134-1143.
- [41] Kotani S, Nakazawa H, Tokimasa T, Akimoto K, Kawashima H, Toyoda-Ono Y, Kiso Y, Okaichi H, Sakakibara M (2003) Synaptic plasticity preserved with arachidonic acid diet in aged rats. *Neurosci Res* 46, 453-461.
- [42] Kotani S, Sakaguchi E, Warashina S, Matsukawa N, Ishikura Y, Kiso Y, Sakakibara M, Yoshimoto T, Guo J, Yamashita T (2006) Dietary supplementation of arachidonic and docosahexaenoic acids improves cognitive dysfunction. *Neurosci Res* 56, 159-164.
- [43] Hosono T, Mouri A, Nishitsuji K, Jung CG, Kontani M, Tokuda H, Kawashima H, Shibata H, Suzuki T, Nabeshima T, Michikawa M (2015) Arachidonic or docosahexaenoic acid diet prevents memory impairment in Tg2576 mice. *J Alzheimers Dis* 48, 149-162.
- [44] Hosono T, Nishitsuji K, Nakamura T, Jung CG, Kontani M, Tokuda H, Kawashima H, Kiso Y, Suzuki T, Michikawa M (2015) Arachidonic acid diet attenuates brain Abeta deposition in Tg2576 mice. *Brain Res* 1613, 92-99.
- [45] Bickert A, Ginkel C, Kol M, vom Dorp K, Jastrow H, Degen J, Jacobs RL, Vance DE, Winterhager E, Jiang XC, Dörmann P, Sommerharju P, Holthuis JC, Willecke K (2015) Functional characterization of enzymes catalyzing ceramide phosphoethanolamine biosynthesis in mice. *J Lipid Res* 56, 821-835.
- [46] Hildenbrandt GR, Abraham T, Bieber LL (1971) Metabolism of ceramide phosphorylethanolamine, phosphatidylinositol, phosphatidylserine and phosphatidylglycerol by housefly larvae. *Lipids* 6, 508-516.
- [47] Vacaru AM, van den Dikkenberg J, Ternes P, Holthuis JC (2013) Ceramide phosphoethanolamine biosynthesis in *Drosophila* is mediated by a unique ethanolamine phosphotransferase in the Golgi lumen. *J Biol Chem* 288, 11520-11530.
- [48] Maurice A, Malgat M (1993) Phosphatidylethanolamine

olamine: Ceramide-ethanolaminephosphotransferase activity in synaptic plasma membrane vesicles. Influence of some cations and phospholipid environment on transferase activity. Further proof of its location. *Int J Biochem* 25, 1183-1187.

[49] Doroudgar M, Lafleur M (2017) Ceramide-C16 is a versatile modulator of phosphatidylethanolamine polymorphism. *Biophys J* 112, 2357-2366.

[50] Bartke N, Hannun, YA (2009) Bioactive sphingolipids: Metabolism and function. *J Lipid Res* 50(Suppl), S91-96.

[51] Vethakanraj HS, Babu TA, Sudarsanan GB, Duraisamy PK, Ashok Kumar S (2015) Targeting ceramide metabolic pathway induces apoptosis in human breast cancer cell lines. *Biochem Biophys Res Commun* 464, 833-839.

[52] Patil S, Chan C (2005) Palmitic and stearic fatty acids induce Alzheimer-like hyperphosphorylation of tau in primary rat cortical neurons. *Neurosci Lett* 384, 288-293.

[53] Osman C, Voelker DR, Langer T (2011) Making heads or tails of phospholipids in mitochondria. *J Cell Biol* 192, 7-16. [54] Murray NR, Fields AP (1998) Phosphatidylglycerol is a physiologic activator of nuclear protein kinase C. *J Biol Chem* 273, 11514-11520.

[55] Morita SY, Terada T (2015) Enzymatic measurement of phosphatidylglycerol and cardiolipin in cultured cells and mitochondria. *Sci Rep* 5, 11737.

[56] Monteiro-Cardoso VF, Oliveira MM, Melo T, Domingues MR, Moreira PI, Ferreiro E, Peixoto F, Videira RA (2015) Cardiolipin profile changes are associated to the early synaptic mitochondrial dysfunction in Alzheimer's disease. *J Alzheimers Dis* 43, 1375-1392.

[57] Horvath SE, Daum, G (2013) Lipids of mitochondria. *Prog Lipid Res* 52, 590-614.

[58] WangX, SuB, LeeHG, LiX, PerryG, SmithMA, ZhuX (2009) Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. *J Neurosci* 29, 9090-9103.

[59] Patil S, Sheng L, Masserang A, Chan C (2006) Palmitic acid-treated astrocytes induce BACE1 upregulation and accumulation of C-terminal fragment of APP in primary cortical neurons. *Neurosci Lett* 406, 55-59.

[60] Marin R, Fabelo N, Fernandez-Echevarria C, Canerina- Amaro A, Rodriguez-Barreto D, Quinto-Aleman D, Mesa-Herrera F, Diaz M (2016) Lipid raft alterations in aged-associated neuropathologies. *Curr Alzheimer Res* 13, 973-984.

[61] Fabelo N, Mart'in V, Mar'in R, Moreno D, Ferrer I, Diaz M (2014) Altered lipid composition in cortical lipid rafts occurs at early stages of sporadic Alzheimer's disease and facilitates APP/BACE1 interactions. *Neurobiol Aging* 35, 1801-1812.

CHAPTER 4

cPLA2 and desaturases underlie the tau hyperphosphorylation offset induced by BACE knock-down in neuronal primary cultures

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Abstract

Inflammation has been suggested to play early and perhaps causative roles in Alzheimer's disease (AD) pathogenesis possibly in part by the overactivation of the aspartic acid protease named β -site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), which is responsible for the β -amyloid cascade. We have described that BACE1 is involved in the lysophosphatidylethanolamine (LPE) (18:1/20:4/22:6) upregulation associated with tauopathy and inflammation signaling (cPLA2/arachidonic acid/COX2) in a triple transgenic model of Alzheimer's disease, where BACE1 silencing reversed the imbalanced profile and produced cognitive function improvement. In this study, we analyze the role of cPLA2 and desaturases (SCD1, FAD6) in the BACE1 knockdown-induced protective action under a glutamate excitotoxicity model. Glutamate (125 μ M) produced hyperphosphorylation of tau in cortical primary cultures along with increased apoptotic nuclei, LDH release, and cPLA2 expression, which were all reversed by BACE1-KD. This beneficial effect was reinforced by the silencing of cPLA2 but attenuated by the reduction in SCD1 and partially attenuated by the reduction in FAD6. Inversely, overexpression SCD1 and FAD6 recovered the neuroprotective effect produced by BACE1-KD, which was not achieved by the overexpression of each desaturase alone. These findings suggest that the hyperphosphorylation of tau and the creation of a pro-inflammatory cell environment are blocked in a desaturase-dependent manner by targeting BACE1.

Key words: BACE1, hyperphosphorylated tau, excitotoxicity, cPLA2, desaturases, inflammation.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by senile plaques composed of amyloid- β (A β) peptides, intracellular paired helical filaments (PHFs) caused by abnormally phosphorylated tau protein, neuroinflammation and neuronal loss. A β is generated by the enzymatic processing of the amyloid precursor protein (APP). This process involves the cleavage of APP by β -site APP cleavage enzyme 1 (BACE1) and the γ -secretase complex, which contains presenilin 1 or 2 (PS1 or PS2) as the catalytic subunit to generate the A β 40 and A β 42 peptides (Karran, Mercken, and De Strooper 2011; D. J. Selkoe and Hardy 2016). The amyloid cascade hypothesis suggests that synaptic toxicity and neurotoxicity mediate the creation of the multimeric amyloid- β peptide species and the consequent hyperphosphorylation of tau, PHF formation and cognitive impairment; however, the molecular etiopathogeneses leading to those events are not fully understood. Recent works suggest that systemic inflammation could exacerbate or even trigger neuronal dysfunction associated with dementia, including impaired insulin signaling, synapse degradation and memory disturbances associated with the release of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (Karran, Mercken, and De Strooper 2011; Qin et al. 2003; Shi et al. 2016). Elevated APP and COX2 production has been linked to calcium-dependent phospholipase A2 (cPLA2) upregulation in vivo and in vitro in cortical neurons exposed to A β (Sagy-Bross et al. 2015). cPLA2 upregulation is closely associated with inflammation (Tan, Ng, and Ong 2016), excitotoxicity in spinal cord neurons (Z. Zhao et al. 2011) and mood disorders (Kim, Rapoport, and Rao 2011). Additionally, pro-inflammatory arachidonic fatty acid released by cPLA2 is metabolized by COX2 (Leslie 2015). In addition, elevated cPLA2 activity has been associated with increased tau hyperphosphorylation during inflammation (Sundaram et al. 2012), and MAPK regulates cPLA2 activity under lipid peroxidation conditions (Lee et al. 2011).

Alternatively, our previous studies have suggested that BACE1 may have a more direct action on tauopathy because it decreases the rate of tau phosphorylation, blocking the MAPK pathway in a triple transgenic AD mice model (Diego Piedrahita et al. 2016). Additionally, the reduction in the phosphorylation of tau by shBACE1miR was blocked by an inhibitor of autophagosome lipidation of phosphatidylethanolamine (PE), 3-methyladenine (3-MA) (Diego Piedrahita et al. 2016). However, our recent research showed that BACE1 silencing prevents cognitive impairment and inflammation; reduces arachidonic acid, cPLA2 and COX2; and recovers the mono- and polyunsaturations of the fatty acid composition of PE plasmalogens, such as lyso (LPE) and ether (ePE), in the hippocampus of 3xTgAD mice (Villamil-Ortiz et al. 2016).

Therefore, considering that cPLA2 is the main enzyme involved in the production of plasmalogens (Makide et al. 2009) and that stearoyl-coenzyme A desaturase 1 (SCD) and fatty

acid desaturase 6 (FADS6) are responsible for fatty acid unsaturation; our next question was to analyze the role of cPLA2 and desaturases (SCD1, FAD6) in BACE1-KD-induced protective action and tau hyperphosphorylation reduction in a model of glutamate excitotoxicity.

Materials and methods

Primary neuronal cultures

Cortical samples from Wistar rat embryos (E18-19) were dissected, dissociated and cultured on poly-L-lysine (Sigma-Aldrich, St. Louis, MO, USA) precoated multiwell plates in Neurobasal medium (Gibco, Rockville, MD, USA), which contained B-27 supplement (Sigma-Aldrich), albumin from a chicken egg (Sigma-Aldrich), N2 human supplement (GIBCO), and a penicillin-streptomycin antibiotic mixture (GIBCO); the samples were cultured at 37°C in a 5% CO₂ humidified atmosphere for a maximum of 19 days in vitro (DIV19). Isolated primary neurons were plated at a low density (52 cell/mm²) for immunofluorescence and at a high density (1500 cell/mm²) for Western blotting to assay cPLA2, SCD1 and FADS6.

ShRNA-miR AAV viral vector, shRNA lentiviral particles, and transduction

The SCR (scrambled) or BACE1 shRNA-miRs (short hairpin RNA in a microRNA backbone) were designed and validated in our previous study (D Piedrahita, Castro-Alvarez, Boudreau, Villegas-Lanau, Kosik, Gallego-Gomez, and Cardona-Gómez 2015). The adeno-associated virus (AAV) particles were obtained from the Davidson Laboratory (University of Iowa Viral Vector Core). The AAV aliquots were dialyzed, and the cells were transduced with 2 µL AAV 2/5 (10 genomes per mL) at a titer of 1012 vg/mL for 3 h at 37°C; the media were subsequently completed with B27 supplemented Neurobasal medium. The transductions were performed at DIV7 and maintained for 12 days posttransduction prior to the administration of inhibitor and the glutamate treatments. cPLA, SCD1 and FADS6 shRNA mouse (m) viral particles containing target-specific constructs that encode 19-25-nt (plus hairpin) shRNAs were used to knock down gene expression (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Commercial control shRNA viral particles encoding a scrambled shRNA sequence that does not specifically degrade any known cellular mRNA were also used. At DIV12, the cultures were transduced for 7 days with lentiviral particles at a titer of 10⁶ infectious units of virus in Dulbecco's modified Eagle's medium (DMEM) that contained 25 mM HEPES, pH 7.3.

Transfection and cotransfection of neuron cultures

Neurons were cotransfected with cPLA2, SCD1 and FADS6 CRISPR activation plasmid (m) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or with an empty vector in DMEM with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). At 4 h, the transfection medium was

replaced with DMEM containing 10% fetal bovine serum (FBS). Hippocampal neurons were transduced with SCR or BACE1 shRNAmiR at DIV7. Clustered regularly interspaced short palindromic repeats (CRISPR) activation plasmid (m) and empty plasmid were transfected and cotransfected into cortical neurons with Lipofectamine 2000 (Invitrogen) in Neurobasal medium at DIV17. An equimolar mixture of 0.4 µg of DNA with Lipofectamine 2000 was used. At 4 h, the posttransfection medium was changed for the supplemented Neurobasal medium, and the DIV18 neurons were treated with 125 µM glutamate for 20 min (Sigma-Aldrich) to induce excitotoxicity.

Treatments

DIV18 neurons were treated with 125 µM glutamate for 20 min to induce excitotoxicity (Posada-Duque et al. 2013). After 20 min, the glutamate was washed out to evaluate the effects. At 24 h postglutamate, the media were collected for use in the LDH assay, and either the protein was collected or the cells were fixed.

Immunofluorescence

Immunofluorescence analysis was performed as previously described (Posada-Duque et al. 2013). Briefly, the cells were incubated overnight at 4°C with the following primary antibodies: anti-mouse cPLA2 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-goat SCD1 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-goat FADS6 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and anti-mouse phospho-tau (Ser202, Thr205) (AT8) (1:1000, Thermo Fisher, CA, USA) antibodies. The Alexa-594 fluorescent antibody was used as a secondary antibody (1:1000, Molecular Probes, Eugene, OR, USA). The nuclei were stained with Hoechst (1:5000, Invitrogen), and the cells were incubated with each primary antibody conjugated with Alexa 594 (1: 2000, Molecular Probes) for 1 h. The cells were washed 4 times in buffer, cover slipped using Gel Mount (Biomedex, Hatfield, PA, USA), and observed under an Olympus IX 81 epifluorescence microscope (Olympus Latin America, Inc., Miami, FL, USA) or a Disk Scan Unit (DSU) Spinning Disc Confocal microscope. No staining was observed when the primary antibodies were omitted. XY images were collected using an Olympus IX 81 microscope with 10X (NA, 0.4), 40X (NA, 1.3) or 60X (NA, 1.42) oil immersion objectives.

Quantitative image analysis

The percentage of condensed nuclei was obtained using the following formula: [condensed nuclei/ (condensed nuclei + normal nuclei)] × 100. (Posada-Duque et al., 2013) The mean area, diameter and intensity of each nucleus were quantified using the automatic measurement/spatial trace feature tool. Condensed nuclei were defined as those possessing an average area equal to or less than 40 µm², a diameter equal to or less than 6 µm and a fluo-

rescence intensity equal to or greater than 120 (AU). Images were analyzed individually and calibrated to a micrometer scale using Image Scope Pro Plus software (Media Cybernetics Inc., Rockville, MD, USA). Analyses were performed for 15 neurons per treatment in each duplicate assay and for at least four independent experiments ($n=3-5$).

LDH release

A cytotoxicity detection kit (Roche) was used to assess the presence of cytotoxicity by measuring the activity of lactate dehydrogenase (LDH) released by cultures. The culture medium was recovered one day after glutamate treatment (DIV 19); LDH activity was determined by measuring NADH absorption as the linear rate of consumption during pyruvate reduction to lactate using a spectrophotometer. The cytotoxicity was calculated for a given test condition using the following equation: cytotoxicity (%) = $[(A - \text{low control})/(\text{high control} - \text{low control})] * 100$, where A is the mean LDH activity measured in medium samples from three wells per duplicate that were subjected to the test condition, low control is the LDH activity released from untreated normal cells, and high control is the maximum LDH activity released in all cells (cells treated with 1% Triton X-100 for 24 h).

Western blotting

After treatment, neuronal cultures were homogenized in lysis buffer (150 mM NaCl, 20 mM Tris (pH 7.4), 10% glycerol, 1 mM EDTA, 1% NP40, and 1 mg/mL inhibiting cocktail proteases). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (12% SDS–PAGE) was performed using a mini-protein system (Bio-Rad) with low-range molecular-weight standards (Bio-Rad). Protein (40 μ g) was loaded into each lane with a loading buffer containing 0.375 M Tris (pH 6.8), 50% glycerol, 10% SDS, 0.5 M DTT and 0.002% bromophenol blue. Samples were heated at 95°C for 3 min prior to gel loading. After electrophoresis, the proteins were transferred to nitrocellulose membranes (Amersham, Buckinghamshire, UK) using an electrophoretic transfer system (mini Trans-blot Electrophoretic Transfer Cell) at 250 mA for 2 h. The membranes were then washed with TTBS (20 mM Tris–HCl, pH 7.5; 500 mM NaCl; 0.05% Tween-20 containing TRIS-buffered saline solution, pH 7.4) and 5% nonfat dry milk and incubated overnight at 4 °C with the following primary antibodies: anti-rabbit cPLA2 and p-cPLA2 (ser505) (1:1000; Cell Signaling, Danvers, MA, USA), anti-rabbit BACE1 (1:500; Merck, Millipore, Darmstadt, Germany), anti-rabbit Cox2 (1:1000; Merck, Millipore, Darmstadt, Germany), anti-mouse AT8 (1:1000; Thermo Fisher, CA, USA), anti-mouse TAU5 (1:1000; Thermo Fisher, CA, USA); anti-mouse SCD1 (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-rabbit iNOS2 (1:250; Abcam Cambridge, UK), anti-goat FADS6 (1:250; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Anti- β -tubulin (1:5000; Promega) and anti-mouse actin (1:1000; Sigma-Aldrich) were used as loading controls. IRDye 800CW goat anti-mouse or IRDye 680 goat anti-rabbit 1:15000 (Li-COR), as well as peroxidase-conju-

gated anti-mouse IgG and anti-rabbit IgG 1:5000 (Pierce), were used as secondary probes. Images of fluorescent blots were analyzed using Odyssey Infrared Imaging System application software version 3.0 (Li-COR). The results from each membrane were normalized to control values. Samples from all experimental groups were processed in parallel to minimize inter-assay variation.

SCD1 and FADS6 activation assays

To increase the expression of the SCD1 (mouse activation plasmid; Santa Cruz Biotechnology, Santa Cruz, CA, USA); and FADS6 (mouse activation plasmid; Santa Cruz Biotechnology, Santa Cruz, CA, USA) genes in the neuronal primary cultures, we used the CRISPR activation plasmid (m) as a system for SCD1 and FADS6 transcription activation as a synergistic activation mediator (SAM). We used the scrambled gRNA CRISPR activation plasmid as a negative control for experiments using a target-specific CRISPR activation plasmid; it encodes nontargeting scrambled gRNA that does not recognize any DNA sequence. This SAM complex will not bind DNA or activate the transcription of any specific gene. In a 24-well tissue culture plate, we seeded 2×10^4 cells in 0.5 ml of antibiotic-free standard growth medium per well 24 h prior to transfection (DIV 17). For each transfection, we diluted 1 μ g of plasmid DNA into plasmid transfection medium to bring the final volume to 100 μ l. The cells transfected with the CRISPR activation plasmid were assayed 48 h after transfection. Overexpression was measured using an immunofluorescence cell assay.

Statistical analyses

The n values used for the in vitro experiments were 3–5. The parametric data were compared using multivariable 2-way ANOVA followed by Tukey's post hoc test for comparisons between several independent groups. The nonparametric data were analyzed using Kruskal–Wallis and Dunn's multiple comparison tests. The comparisons between 2 groups were performed using Student's t-test for parametric data or the Mann–Whitney test for nonparametric data. All sample groups were processed in parallel to reduce inter-assay variation. The data are expressed as the means $\pm$ SEM. PRISM (La Jolla, CA, USA) software was used. The results were considered significant when $p < 0.05$ (* or #), $p < 0.01$ (** or ##), or $p < 0.001$ (***) or (###). The symbols * or # directly above the bars represent comparisons between each treatment and the control.

Results

BACE1 silencing provides neuroprotection and reduces tau hyperphosphorylation induced by glutamate excitotoxicity in cortical primary cultures

To confirm our model based on BACE1 silencing, we validated the effect of shBACE1miR on AAV2/5 transduced in cortical primary neurons for 12 days and observed a significant decrease in BACE1 protein levels with and without excitotoxicity by glutamate ($p < 0.01$ and $p < 0.001$, respectively) (Fig. 1A). These observations were supported by our previous studies, which demonstrated that BACE1 RNAi (BACE1-KD) reduces BACE1 protein levels in both primary neuronal cultures and the injected brain region of an Alzheimer's mice model by approximately 50% (D Piedrahita, Castro-Alvarez, Boudreau, Villegas-Lanau, Kosik, Gallego-Gomez, and Cardona-Gomez 2015; Villamil-Ortiz et al. 2016). Glutamate (125 μ M) increased hyperphosphorylated tau (AT8) levels in the shSCRmiR (control) group ($p < 0.0001$) (Fig. 1B). This increase was significantly reversed by shBACE1miR treatment ($p < 0.001$), though it was still increased compared to shBACE1miR without glutamate ($p < 0.05$) (Fig. 1B). These observations were supported by the altered morphology of the neurons (Fig. 1C), including greater percentages of condensed nuclei ($p < 0.0001$) (Fig. 1D) and increased LDH release ($p < 0.05$) (Fig. 1E) seen in glutamate-treated neurons. These effects were prevented by BACE1-KD treatment ($p < 0.001$ and $p < 0.0001$, respectively) (Fig. 1C-E). These data confirm that BACE1 silencing protects neuronal cells and reduces tau hyperphosphorylation in a glutamate-induced excitotoxicity model.

BACE1-KD decreases glutamate-upregulated cPLA2, and cosilencing with shcPLA2 accentuates the reduction in hyperphosphorylated tau and COX2 levels

Previous findings indicate that cPLA2 is involved in different forms of neurodegeneration (Villamil-Ortiz et al. 2016). cPLA2 is increased in AD brains and is associated with A β deposits and pro-inflammatory pathways, specifically, the production of arachidonic acid (AA) and the activation of the COX2 enzyme (Villamil-Ortiz et al. 2016). We observed that cPLA2 protein levels increased in a glutamate-mediated excitotoxic environment ($p < 0.001$) (Fig. 2A) together with AT8 ($p < 0.05$) (Fig. 2B) and COX2 ($p < 0.05$) (Fig. 2C) levels. cPLA2 and AT8 upregulation was prevented by the silencing of BACE1 ($p < 0.05$) but not COX2 (Fig. 2A, B, C). Under exposure to shcPLA2, the silencing of cPLA2 in all groups was confirmed ($p < 0.01$ or $p < 0.001$ respectively), including a significant reduction in cPLA2 under glutamate toxicity conditions ($p < 0.01$) (Fig. 2A). In addition, AT8 ($p < 0.01$) and COX2 ($p < 0.001$) protein levels were decreased, indicating that cPLA2 silencing per se provided neuroprotection (Fig. 2A, B, C). Interestingly, the co-silencing of cPLA2 accentuated the reduction in AT8 (Fig. 2B) achieved by BACE1-KD ($p < 0.05$), and it had a weak tendency to reduce COX2 levels (Fig. 2C).

Figure 1. BACE1-KD decreases tau hyperphosphorylation and protects against glutamate-mediated excitotoxicity. Primary cortex neurons were transduced with SCR or BACE1 shRNA-miR and treated with 125 μ M glutamate for 20 min. The proteins were collected after 24 h. In (A) and (B), the BACE1 and AT8 protein levels were detected by Western blotting. Actin and tau5 were used as the loading control. Relative Units = RU, $n = 4$. * represents comparisons for BACE, AT8, % LDH and % condensed nuclei, respectively. (C) Morphological characteristics indicate adeno-associated virus (AAV) vector viral eGFP-tagged SCR and shBACE1-miR (green) and AT8 (red). (D) Percentage of condensed nuclei to total nuclei in Hoechst-positive cells. (E) Neuronal cytotoxicity was presented as the percentage of lactate dehydrogenase (LDH) release at 24 h after glutamate application ($n = 5$), and each experiment was performed in duplicate. All values were normalized to the control neurons. ANOVA with Tukey's test was used for comparisons. Scale bar = 20 μ m, * Comparison between each treatment at 24 h. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

In contrast, INOS protein levels were reduced by shBACE1miR or shcPLA2 alone under exposure to glutamate ($p < 0.05$) but not under the co-silencing condition (Fig. 2D). Those findings were supported by the reduction in LDH release by shBACE1miR or cPLA2 in the excitotoxic environment without a synergic effect of the co-silencing of BACE1 and cPLA2 on cell death (Fig. 2E). Taken together, these data suggest a direct action of shBACE1miR in decreasing the hyperphosphorylation of tau by its effect on the reduction in cPLA2 in an excitotoxic context via a pathway other than direct control of COX2 or INOS pro-inflammatory signaling.

BACE1 silencing needs SCD1 to reduce hyperphosphorylated tau under glutamate-mediated excitotoxicity

The amount of saturated fatty acids is regulated by desaturase enzymes such as SCD1, which catalyze the synthesis of monounsaturated fatty acids, and the indices of fatty acyl-CoA desaturase activity have been associated with cognitive dysfunction in Alzheimer's disease (Fonteh et al. 2016). In this study, we confirm the upregulation of SCD1 protein levels in a glutamate-mediated excitotoxicity context ($p < 0.01$ - $p < 0.05$) (Fig. 3A), which we previously observed in aged 3xTg-AD mice (Villamil-Ortiz et al. 2016). SCD1 silencing reversed the change in SCD1 protein expression ($p < 0.01$ - $p < 0.05$) (Fig. 3A). Interestingly, SCD1 targeting did not reduce the AT8 and COX2 protein levels that were upregulated under glutamate excitotoxicity (Fig. 3B, C) and even increased COX2 in the basal condition ($p < 0.01$) (Fig. 3C). The silencing of SCD1 avoided the reduction in tau hyperphosphorylation usually resulting from BACE1-KD treatment, and the co-silencing of BACE1 and SCD1 had the clear tendency to increase or prevent the reduction in COX2 in all experimental groups (Fig. 3C). INOS was not statistically affected by any treatment (Fig. 3D); however, a clear tendency to be increased by glutamate, reversed by BACE1-KD, and slightly increased in all treatments was observed with silencing and co-silencing by SCD1-KD (Fig. 3D). Together, these data could mean that SCD1 is overregulated by glutamate, although its silencing produced a generalized pro-inflammatory cell environment, and that it affects the neuroprotection exerted by the silencing of BACE1, particularly in the reduction in hyperphosphorylated tau.

Overexpression of SCD1 reinforces the shBACE1miR-induced neuroprotection

To validate the role of SCD1 in neuroprotection, combined with BACE1 silencing, we cotreated the neuronal cultures with shBACE1miR, to knock down BACE1-mRNA (DIV 7), and with an activation plasmid (CRISP/Cas9), to induce SCD1 overexpression (DIV 17). Treatments were either in the presence or absence of glutamate, which could simulate stress. Treatment groups were compared with their respective control, which were transduced with shScRmiR and/or transfected with the SCD1 empty plasmid coupled to GFP. The control neurons exhibited typical hallmarks of glutamate excitotoxicity, including a severe loss of neuronal processes (Fig. 4A), an increased percentage of condensed nuclei ($p < 0.05$) (Fig.

Figure 2. cPLA2 silencing improved the BACE1-KD-induced reduction in tau hyperphosphorylation in a glutamate-mediated excitotoxicity model. Primary cortical neurons were doubly transduced with SCR, BACE1 shRNA-miR and cPLA2 shRNA and were treated with 125 μ M glutamate for 20 min. After 24 h, (A) cPLA2, (B) AT8, (C) COX2 and (D) INOS protein levels were detected by Western blotting. Actin was used as the loading control. (E) % LDH release. Relative Units = RU, $n = 4$, and each experiment was performed in duplicate. All values were normalized to the control neurons. ANOVA with Tukey's test was used for comparisons. * Comparison between each treatment in the same group. # Comparison between shScRmiR and shcPLA2miR groups. *, # $p < 0.05$; **, ### $p < 0.01$; ***, ### $p < 0.001$.

4C) and increased LDH release ($p < 0.05$) (Fig. 4D). The overexpression of the SCD1 CRISPR plasmid was confirmed by the increased immunoreactivity (IR) of SCD1 with or without glutamate treatment (Fig. 4A), although the overexpression did not allow for recovery from the stressed cell environment per se (Fig. 4A, C, D). However, the fluorescence data confirm that the morphology of the neurons treated with glutamate was recovered by the silencing of BACE1 with or without overexpression of SCD1 (Fig. 4A), as shown by decreased nuclear condensation and LDH release under glutamate exposure ($p < 0.05$) (Fig. 4C, D). SCD1 overexpression reinforced the protected morphology of neurons (Fig. 4A) and the reduction in cell death achieved by the silencing of BACE1 ($p < 0.01$) (Fig. 4C, D).

In contrast, the targeting of SCD1 affected the reduction in hyperphosphorylated tau by the neuroprotective action of BACE1-KD (Fig. 3B). We then measured the fluorescence pattern of AT8 in the SCD1 overexpression condition and found that AT8 fluorescence increased in all the groups transfected with the SCD1 CRISPR plasmid compared to the transfected empty plasmid groups (Fig. 4B). However, the AT8 fluorescence was mainly distributed in thin neuronal projections. When BACE1 was silenced with the overexpression of SCD1 CRISPR plasmid under a glutamate-mediated excitotoxicity context, an even healthier outcome was observed than the effect achieved by BACE1-KD alone. Inversely, an aggregation of AT8-IR was observed in the cytoplasm of neurons exposed to glutamate with or without SCD1 overexpression. These findings suggest that SCD1 is needed in the protection mechanism exerted by the targeting of BACE1 in a neuroexcitotoxic condition.

BACE1-KD requires a fine balance of FADS6 to generate neuroprotection in a glutamate-mediated excitotoxicity model.

It was recently suggested that desaturases are pro-survival enzymes in neurons since they are essential in producing fatty acids with six unsaturations called PUFAS (polyunsaturated fatty acids), which are involved in the anti-inflammatory process in neurons (Calder 2012; Janssen and Kiliaan 2014). However, other PUFAS with pro-inflammatory properties exist, such as arachidonic acid (AA), indicating a need for finely tuned levels of PUFAS (Zárate et al. 2017a). Next, we analyzed the role of FADS6 in the BACE1-KD-induced neuroprotection. We confirmed the silencing of FADS6 in all experimental groups, and we did not find increased FADS6 protein levels under the glutamate condition (Fig. 5A). Nevertheless, we found that the co-silencing of BACE1 and FADS6 with or without glutamate generated increased protein levels of AT-8 (Fig. 5B), which correlated with a clear downward trend or significant reduction in COX2 (Fig. 5C) and INOS (Fig. 5D) under the same conditions, showing apparently opposite results.

Figure 3. SCD1 silencing increased AT8 and COX2 levels after BACE1 silencing in glutamate-mediated excitotoxicity. Primary cortex neurons were doubly transduced with SCR, BACE1 shRNA-miR and cPLA2 shRNA and treated with 125 μ M glutamate for 20 min. After 24 h, (A) SCD1, (B) tAT8, (C) COX2 and (D) INOS protein levels were detected by Western blotting. Actin and β -III tubulin were used as loading controls. Relative Units = RU, $n = 4$, and each experiment was performed in duplicate. All values were normalized to the control neurons. ANOVA with Tukey's test was used for comparisons. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. # Comparison between shScrmir and shcPLA2miR groups. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

However, when we analyzed the state of neuronal cultures after the overexpression of FADS6 (Fig. 6), we found altered neuronal morphology mainly consisting of a decrease in the neuritic processes (Fig. 6A); furthermore, we found that AT8 aggregated in the cytoplasm (Fig. 6B), in addition to a major percentage of condensed nuclei (Fig. 6C). These effects were reversed by BACE1 silencing (Fig. 6A, B, C). Taken together, these findings suggest that the neuroprotective action induced by BACE1-KD requires FADS6 expression; however, the reduction in or overregulation of FADS6 alone altered the healthy cells, indicating a need for finely balanced expression in homeostasis.

Figure 4. Overexpression of SCD1 induces neuroprotection together with BACE1 silencing. The morphological characteristics are shown for the cortical neurons transduced with GFP-tagged shBACE1miR and shscrmir (green). The primary cultures were also transfected with an activation plasmid (CRISP/Cas9) for SCD1 overexpression. Nuclei were stained with Hoechst (blue), and SCD1 and AT8 were labeled with Alexa 594 dye (red). (A) SCD1 empty plasmid group, (B) SCD1 CRISP plasmid. Magnification was 40X. Scale bar is 20 μ m (n = 5) (C) Percentage of condensed nuclei relative to the total nuclei of transfected neurons. Data are presented as the mean $\pm$ SEM (n = 3), and each experiment was performed in duplicate. (D) Percentage of lactate dehydrogenase (LDH) release. All values were normalized to the control neurons. ANOVA with Tukey's test was used for comparisons. Scale bar= 20 μ m, * represents comparisons between each treatment in the same group. # Comparison between SCD1 empty plasmid and SCD1 CRISP plasmid groups. *, # p < 0.05; **, ## p < 0.01; ***, ### p < 0.001.

Figure 5. FADS6 silencing increased AT8 and COX2 levels after BACE1 silencing in glutamate-mediated excitotoxicity. Primary cortex neurons were doubly transduced with SCR, BACE1 shRNA-miR and FADS6 shRNA and treated with 125 μ M glutamate for 20 min. After 24 h, the proteins were collected. In (A) the SCD1, (B) the AT8, (C) COX2 and (D) the INOS protein levels were detected by Western blotting. Actin and β -III tubulin were used as the loading controls. Relative Units = RU, n = 4 and each experiment was performed in duplicate. All values were normalized to the control neurons. ANOVA with Tukey's test was used for comparisons. * Comparison between each treatment in the same group. * p < 0.05; **p < 0.01; ***p < 0.001.

Figure 6. Over-expression of FADS6 induces neuroprotection in combination with shBACE1miR treatment. The morphological characteristics are shown for the cortical neurons transduced with GFP-tagged shBACE1miR and shSCRmiR (green). The primary cultures were also transfected with an activation plasmid (CRISPR/Cas9) for FADS6 overexpression. Nuclei were stained with Hoechst (blue), FADS6 and AT8 were labeled with Alexa 594 dye (red). (A) FADS6 empty plasmid group and (B) FADS6 CRISPR. Magnification is 40X. The scale bar is 20 μ m (n = 5). (C) Percentage of condensed nuclei relative to the total nuclei of transfected neurons. (D) Percentage of lactate dehydrogenase (LDH) release. Data are presented as the mean $\pm$ SEM (n = 3), and each experiment was performed in duplicate. All values were normalized to the control neurons. ANOVA with Tukey's test was used for comparisons. Scale bar = 20 μ m, * represents comparisons between each treatment in the same group. # Comparison between SCD1 empty plasmid and SCD1 CRISPR plasmid groups. *, # p < 0.05; **, ### p < 0.01.

Discussion

Our study suggests for the first time a more direct action of BACE1 on the phosphorylation state of tau that is mediated through cPLA2 and desaturase modulation during glutamate excitotoxicity.

Despite the large body of evidence supporting the amyloid hypothesis of AD pathogenesis, efforts to develop agents that safely and unambiguously reduce CNS concentrations of A β in mice and humans have not been successful to date (Kennedy et al. 2016). Perhaps it is necessary to further understand the etiopathogenesis by BACE1 dysregulation and its role in the impairment of the neurovascular unit, inflammation and vascular dementia (Meakin et al. 2018; Botteri et al. 2018). Inhibition of this enzyme has been viewed as an A β -lowering therapeutic intervention, but several problems have prevented BACE1 inhibitors from advancing to pivotal clinical trials, especially because BACE1 has multiple functional subunits (Barao et al. 2016). However, we and others have found that BACE1 targeting improves long-term spatial memory and prevents A β plaques and tau aggregation (Yu et al. 2014; Sierant et al. 2009; Villamil-Ortiz et al. 2016), with a reduction of 50% compared to the upregulated condition in neurodegeneration (Barao et al. 2016).

BACE1 activity is exacerbated under a saturated lipid environment caused by metabolic disorders (Hattori et al. 2006; Marwarha et al. 2017). Additionally, BACE1 is associated with neuroinflammation disorders (Stertz et al. 2018), and systemic inflammation may trigger neuronal dysfunction associated with dementia, including neuronal impairment, insulin signaling, synapse degradation, and memory disturbances associated with the release of cyclooxygenase 2 (COX-2) and INOS (Karran, Mercken, and De Strooper 2011; Qin et al. 2003; Shi et al. 2016). High cPLA2 activity, as well as high expression of APP protein, was observed in primary rat cortical neuron cultures exposed to A β and in the cerebral cortex of mice infused with human cerebral β 1-42 amyloid. The prevention of the regulation of cPLA2 and its activity prevented the increase in APP protein levels and the high production of COX-2 (Sagy-Bross et al. 2015), emphasizing a close relationship between cPLA2 and COX-2 since the arachidonic pro-inflammatory fatty acid is released by cPLA2, which is metabolized by COX-2, and since COX-2 levels also decrease without cPLA2 activity (Leslie 2015).

Reviewing related pathological actors, cPLA2 has been widely involved in inflammatory responses, and it has been associated with tauopathy in AD since reduced activity of phospholipase A2 (PLA2), specifically of cPLA2 and calcium-independent intracellular PLA2 (iPLA2), has been positively correlated with a reduction in neurofibrillary tangle density in the cerebral cortex and hippocampus (Sundaram et al. 2012; Schaeffer et al. 2011). Additionally, the isoform of PLA2-IVA is Ca²⁺ dependent, is highly expressed in the brain and is hyperactivated in AD (Sanchez-Mejia and Mucke 2010). Genetic ablation of cPLA2 improved cognition, viability and behavior in the context of the J20 APP transgenic model, despite

the elevated A β burden (Sanchez-Mejia and Mucke 2010). This finding is in agreement with our current study where BACE1 silencing provided neuroprotection and reduced glutamate-induced tau hyperphosphorylation through a reduction in cPLA2 protein levels. These effects were greater than the downstream control of COX2 or INOS pro-inflammatory signaling. These findings support our previous observations where BACE1 silencing prevented pro-inflammatory signaling through cPLA2/AA/COX2, which was upregulated by the accumulation of the saturated fatty acid (18:0) and mono- and polyunsaturated FA (18:1, 20:4, 22:6) composition of LPE (lysophosphatidylethanolamine) and ePE (ether phosphatidylethanolamine) plasmalogens of PE (phosphatidylethanolamine), which is associated with tauopathy and cognitive impairment in old 3xTgAD mice (Villamil-Ortiz et al. 2016).

Additionally, we found a reduction in the mono- and polyunsaturated fatty acids composition of PE and PC (phosphatidylcholine) (20:4, 22:6) and LPC (16:0/18:1) in human brains with sporadic and familial Alzheimer's dementia compared with that in healthy controls, suggesting an alteration in the mono- and polyunsaturation rate associated with dementia. The synthesis and release of monounsaturated fatty acids are carried out by desaturase enzymes, such as stearoyl-CoA desaturase-1 (SCD-1), which changes with the cellular lipid metabolism (Poloni, Blom, and Schwartz 2015). Therefore, oleic acid (18:1) promotes neuron migration (Taberner et al. 2001; Polo-Hernandez et al. 2014), but there is also increased expression of SCD1 in Alzheimer's disease (Astarita et al. 2011; Fraser, Tayler, and Love 2010). Alternatively, our current study suggests the necessity of SCD1 for the neuroprotective action of BACE1-KD, but in a fine balance, as its upregulation under the excitotoxic condition was associated with tauopathy, LDH release and condensed nuclei. However, the reduction in SCD1 was also associated with high levels of COX2. Basal levels of SCD1 were necessary for the BACE1-KD action on the hyperphosphorylation of tau, although SCD1 overexpression did not protect neurons per se, suggesting the need for balanced production of monounsaturated fatty acids with respect to polyunsaturated fatty acids (18:1/20:4, 22:6) for healthy functioning of the brain (Bazinet and Laye 2014; Lei, Vacy, and Boon 2016), which could be regulated by sensible and homeostatic levels of BACE1.

PUFAs (polyunsaturated fatty acids) are synthesized by FADS6. The enzyme activity is regulated by the levels of PUFAs inside the cells, mainly DHA (docosahexaenoic acid, 22:6), (Hofacer et al. 2011), which has been linked to neuroprotective properties and which represents a potential treatment for a variety of neurodegenerative and neurological disorders, such as Alzheimer's disease (J. J. Liu et al. 2015). Consequently, we showed that the silencing of FADS6 increased the hyperphosphorylated tau levels, although shBACE1miR was present and therefore prevented the protective effect of BACE1 silencing in neuronal primary cultures under excitotoxicity. These results could be supported by previous reports about β -amyloid oligomers significantly increasing active c-Jun N-terminal kinase (JNK) and phosphorylation tau in cultured hippocampal neurons, whereas DHA inhibited JNK and the

tau phosphorylation (serine-422) in cultured hippocampal neurons (Q. L. Ma et al. 2009). Additionally, endogenous production of DHA reduced the level of phosphorylated tau, which has implications in AD pathogenesis and provides additional mechanistic arguments toward a beneficial effect of n-3 PUFA in AD (Oster and Pillot 2010). In addition, DHA treatment of cortical neuronal cultures resulted in enhanced axonal outgrowth (Mita et al. 2016). The beneficial effects of dietary DHA and n-3 PUFA have been described as decreasing the level of phosphorylated tau (Arsenault et al. 2011; Green et al. 2007), which supports the idea that FADS6 expression is necessary for the neuronal survival and neuroprotection induced by BACE1 silencing.

Furthermore, we have previously shown that AA and DHA are unbalanced in the 3xTg-AD Alzheimer mouse model, while BACE1 silencing recovered the balance between both fatty acids after 6 and 12 months of treatment, suggesting that the inflammatory pathway involving COX2 and INOS proteins could be regulated by BACE1 targeting (Villamil-Ortiz et al. 2016). Indeed, the ratio of AA to DHA may affect cellular homeostasis associated with neurodegenerative diseases (Farooqui, Horrocks, and Farooqui 2007; A Zarrouk et al. 2017). However, the BACE1 and FADS6 double silencing decreased the levels of COX2 protein mainly under an excitotoxic stimulus, suggesting that BACE1 could influence the COX2 molecular pathway and induce an inflammatory response in neurons. In fact, epidemiological evidence has demonstrated that long-term treatment with nonsteroidal anti-inflammatory drugs reduces the risk of AD by inhibiting the expression of COX-2 (Lim et al. 2000). Although the levels of COX-2 and its metabolic product, prostaglandin, are elevated in the brain of AD patients, the mechanisms for the development of AD remain unknown. However, some evidence suggests that COX-2 could have reciprocal regulation with IL-1 β and A β between glial and neuronal cells, particularly in the signaling pathways for IL-1 β and BACE-1 synthesis (P. Wang et al. 2014). It is also supporting the idea that shBACE1miR treatment not only suppresses the expression of BACE1 but also suggests that it inhibits the formation of hyperphosphorylated tau through downregulation of the pro-inflammatory pathway.

Few previous studies point toward the relevance of BACE1 in inflammation and as an immunoregulator (C. H. Chen et al. 2012). BACE1 expression is upregulated by pro-inflammatory stimuli, whereas BACE1-KD induced neuroprotection in neuron primary cells. Additionally, desaturase, COX2 and BACE1 signaling expression was increased in response to excitotoxic stimuli, and there is evidence that those molecules are relevant in immunoregulation and inflammation processes (Park et al. 2016; C. H. Chen et al. 2012; P. Wang et al. 2014). However, crosstalk between these three pathways had not been previously demonstrated.

In conclusion, our data suggest that the silencing of BACE1 controls cPLA2 and is desaturase dependent in offsetting the hyperphosphorylation of tau and cell death in

an excitotoxic context. Therefore, the potential therapeutic effects of BACE1 silencing on neurodegenerative pathologies may involve multiple mechanisms, such as fatty acid metabolism regulation, which suggests that an approach with BACE1 knockdown combined with fatty acid regulation may represent a potential strategy for cell therapy. Importantly, our study does not provide a complete mechanism by which BACE1 reduction leads to a decrease in tau hyperphosphorylation. Therefore, our results can only explain part of the molecular pathways altered in this model, and further studies are needed regarding the mechanism necessary to reestablish cellular homeostasis.

Acknowledgments and conflict of interest disclosure

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References

- E. Karran, M. Mercken, B. De Strooper, The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics, *Nat Rev Drug Discov.* 10 (2011) 698–712. doi:10.1038/nrd3505.
- D.J. Selkoe, J. Hardy, The amyloid hypothesis of Alzheimer's disease at 25 years, *EMBO Mol Med.* (2016). doi:10.15252/emmm.201606210.
- W. Qin, L. Ho, P.N. Pompl, Y. Peng, Z. Zhao, Z. Xiang, N.K. Robakis, J. Shioi, J. Suh, G.M. Pasinetti, Cyclooxygenase (COX)-2 and COX-1 potentiate beta-amyloid peptide generation through mechanisms that involve gamma-secretase activity, *J Biol Chem.* 278 (2003) 50970–50977. doi:10.1074/jbc.M307699200.
- S. Shi, D. Liang, Y. Chen, Y. Xie, Y. Wang, L. Wang, Z. Wang, Z. Qiao, Gx-50 reduces beta-amyloid-induced TNF-alpha, IL-1beta, NO, and PGE2 expression and inhibits NF-kappaB signaling in a mouse model of Alzheimer's disease, *Eur J Immunol.* 46 (2016) 665–676. doi:10.1002/eji.201545855.
- C. Sagy-Bross, K. Kasianov, Y. Solomonov, A. Braiman, A. Friedman, N. Hadad, R. Levy, The role of cytosolic phospholipase A2 alpha in amyloid precursor protein induction by amyloid beta1-42: implication for neurodegeneration, *J Neurochem.* 132 (2015) 559–571. doi:10.1111/jnc.13012.
- C.S.-H. Tan, Y.-K. Ng, W.-Y. Ong, Epigenetic Regulation of Cytosolic Phospholipase A2 in SH-SY5Y Human Neuroblastoma Cells, *Mol. Neurobiol.* 53 (2016) 3854–3872. doi:10.1007/s12035-015-9314-z.
- Z. Zhao, N. Liu, J. Huang, P.-H. Lu, X.-M. Xu, Inhibition of cPLA2 activation by Ginkgo biloba extract protects spinal cord neurons from glutamate excitotoxicity and oxidative stress-induced cell death, *J. Neurochem.* 116 (2011) 1057–1065. doi:10.1111/j.1471-4159.2010.07160.x.
- H.W. Kim, S.I. Rapoport, J.S. Rao, Altered arachidonic acid cascade enzymes in postmortem brain from bipolar disorder patients, *Mol. Psychiatry.* 16 (2011) 419–428. doi:10.1038/mp.2009.137.
- C.C. Leslie, Cytosolic phospholipase A(2): physiological function and role in disease, *J Lipid Res.* 56 (2015) 1386–1402. doi:10.1194/jlr.R057588.
- J.R. Sundaram, E.S. Chan, C.P. Poore, T.K. Pareek, W.F. Cheong, G. Shui, N. Tang, C.M. Low, M.R. Wenk, S. Kesavapany, Cdk5/p25-induced cytosolic PLA2-mediated lysophosphatidylcholine production regulates neuroinflammation and triggers neurodegeneration, *J Neurosci.* 32 (2012) 1020–1034. doi:10.1523/JNEUROSCI.5177-11.2012.
- J.C.M. Lee, A. Simonyi, A.Y. Sun, G.Y. Sun, Phospholipases A2 and neural membrane dynamics: Implications for Alzheimer's disease, *J Neurochem.* 116 (2011) 813–819. doi:10.1111/j.1471-4159.2010.07033.x.
- D. Piedrahita, J.F. Castro-Alvarez, R.L. Boudreau, A. Villegas-Lanau, K.S. Kosik, J.C. Gallego-Gomez, G.P. Cardona-Gómez, β -Secretase 1's Targeting Reduces Hyperphosphorylated Tau, Implying Autophagy Actors in 3xTg-AD Mice, *Front. Cell. Neurosci.* 9 (2016). doi:10.3389/fncel.2015.00498.
- J.G. Villamil-Ortiz, A. Barrera-Ocampo, D. Piedrahita, C.M. Velasquez-Rodriguez, J.D. Arias-Londono, G.P. Cardona-Gomez, BACE1 RNAi Restores the Composition of Phosphatidylethanolamine-Derivates Related to Memory Improvement in Aged 3xTg-AD Mice, *Front Cell Neurosci.* 10 (2016) 260. doi:10.3389/fncel.2016.00260.
- K. Makide, H. Kitamura, Y. Sato, M. Okutani, J. Aoki, Emerging lysophospholipid mediators, lysophosphatidylserine, lysophosphatidylthreonine, lysophosphatidylethanolamine and lysophosphatidylglycerol, *Prostaglandins Other Lipid Mediat.* 89 (2009) 135–139. doi:10.1016/j.prostaglandins.2009.04.009.
- D. Piedrahita, J.F. Castro-Alvarez, R.L. Boudreau, A. Villegas-Lanau, K.S. Kosik, J.C. Gallego-Gomez, G.P. Cardona-Gómez, β -Secretase 1's Targeting Reduces Hyperphosphorylated Tau, Implying Autophagy Actors in 3xTg-AD Mice, *Front Cell Neurosci.* 9 (2015) 498. doi:10.3389/fncel.2015.00498.
- R.A. Posada-Duque, D. Velasquez-Carvajal, G.P. Eckert, G.P. Cardona-Gomez, Atorvastatin requires geranylgeranyl transferase-I and Rac1 activation to exert neuronal protection and induce plasticity, *Neurochem Int.* 62 (2013) 433–445. doi:10.1016/j.neuint.2013.01.026.
- D. Piedrahita, J.F. Castro-Alvarez, R.L. Boudreau, A. Villegas-Lanau, K.S. Kosik, J.C. Gallego-Gomez, G.P. Cardona-Gomez, β -Secretase 1's Targeting Reduces Hyperphosphorylated Tau, Implying Autophagy Actors in 3xTg-AD Mice, *Front Cell Neurosci.* 9 (2015) 498. doi:10.3389/fncel.2015.00498.
- A.N. Fonteh, M. Cipolla, J. Chiang, X. Arakaki, M. Harrington, Indices of fatty acyl-CoA desaturase activity are associated with cognitive function and preclinical changes in cerebrospinal fluid beta-amyloid and tau levels in Alzheimer's disease, (2016). http://www.fasebj.org/content/30/1_Supplement/625.3.

P.C. Calder, Long-chain fatty acids and inflammation, *Proc Nutr Soc.* 71 (2012) 284–289. doi:10.1017/S0029665112000067.

C.I. Janssen, A.J. Kiliaan, Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: the influence of LCPUFA on neural development, aging, and neurodegeneration, *Prog Lipid Res.* 53 (2014) 1–17. doi:10.1016/j.plipres.2013.10.002.

R. Zárate, N. el Jaber-Vazdekis, N. Tejera, J.A. Pérez, C. Rodríguez, Significance of long chain polyunsaturated fatty acids in human health, *Clin. Transl. Med.* 6 (2017) 25. doi:10.1186/s40169-017-0153-6.

M.E. Kennedy, A.W. Stamford, X. Chen, K. Cox, J.N. Cumming, M.F. Dockendorf, M. Egan, L. Ereshefsky, R.A. Hodgson, L.A. Hyde, S. Jhee, H.J. Kleijn, R. Kuvelkar, W. Li, B.A. Mattson, H. Mei, J. Palcza, J.D. Scott, M. Tanen, M.D. Troyer, J.L. Tseng, J.A. Stone, E.M. Parker, M.S. Forman, The BACE1 inhibitor verubecestat (MK-8931) reduces CNS beta-amyloid in animal models and in Alzheimer's disease patients, *Sci Transl Med.* 8 (2016) 363ra150. doi:10.1126/scitranslmed.aad9704.

P.J. Meakin, S.M. Jaliczy, G. Montagut, D.J.P. Allsop, D.L. Cavellini, S.W. Irvine, C. McGinley, M.K. Liddell, A.D. McNeilly, K. Parmionova, Y.-R. Liu, C.L.S. Bailey, J.K. Dale, L.K. Heisler, R.J. McCrimmon, M.L.J. Ashford, Bace1-dependent amyloid processing regulates hypothalamic leptin sensitivity in obese mice., *Sci. Rep.* 8 (2018) 55. doi:10.1038/s41598-017-18388-6.

G. Botteri, L. Salvadó, A. Gumà, D. Lee Hamilton, P.J. Meakin, G. Montagut, M.L.J. Ashford, V. Ceperuelo-Mallafre, S. Fernández-Veledo, J. Vendrell, M. Calderón-Dominguez, D. Serra, L. Herrero, J. Pizarro, E. Barroso, X. Palomer, M. Vázquez-Carrera, The BACE1 product sAPPβ induces ER stress and inflammation and impairs insulin signaling, *Metabolism.* (2018). doi:10.1016/j.metabol.2018.03.005.

S. Barao, D. Moechars, S.F. Lichtenthaler, B. De Strooper, BACE1 Physiological Functions May Limit Its Use as Therapeutic Target for Alzheimer's Disease, *Trends Neurosci.* 39 (2016) 158–169. doi:10.1016/j.tins.2016.01.003.

C.J. Yu, W. Liu, H.Y. Chen, L. Wang, Z.R. Zhang, BACE1 RNA interference improves spatial memory and attenuates Abeta burden in a streptozotocin-induced tau hyperphosphorylated rat model, *Cell Biochem Funct.* 32 (2014) 590–596. doi:10.1002/cbf.3055.

M. Sierant, K. Kubiak, J. Kazmierczak-Baranska, M. Warashina, T. Kuwabara, B. Nawrot, Evaluation of BACE1

Silencing in Cellular Models, *Int J Alzheimers Dis.* 2009 (2009). doi:10.4061/2009/257403.

C. Hattori, M. Asai, H. Onishi, N. Sasagawa, Y. Hashimoto, T.C. Saido, K. Maruyama, S. Mizutani, S. Ishiura, BACE1 interacts with lipid raft proteins, *J. Neurosci. Res.* 84 (2006) 912–917. doi:10.1002/jnr.20981.

G. Marwarha, S. Rostad, J. Lilek, M. Kleinjan, J. Schommer, O. Ghribi, Palmitate Increases β-site AβPP-Cleavage Enzyme 1 Activity and Amyloid-β Genesis by Evoking Endoplasmic Reticulum Stress and Subsequent C/EBP Homologous Protein Activation, *J. Alzheimer's Dis.* 57 (2017) 907–925. doi:10.3233/JAD-161130.

L. Stertz, V. Contreras-Shannon, N. Monroy-Jaramillo, J. Sun, C. Walss-Bass, BACE1-Deficient Mice Exhibit Alterations in Immune System Pathways, *Mol. Neurobiol.* 55 (2018) 709–717. doi:10.1007/s12035-016-0341-1.

E.L. Schaeffer, V.J. De-Paula, E.R. da Silva, B. de A. Novaes, H.D. Skaf, O. V. Forlenza, W.F. Gattaz, Inhibition of phospholipase A2 in rat brain decreases the levels of total Tau protein, *J. Neural Transm.* 118 (2011) 1273–1279. doi:10.1007/s00702-011-0619-4.

R.O. Sanchez-Mejia, L. Mucke, Phospholipase A2 and arachidonic acid in Alzheimer's disease, *Biochim Biophys Acta.* 1801 (2010) 784–790. doi:10.1016/j.bba-lip.2010.05.013.

S. Poloni, H.J. Blom, I. V Schwartz, Stearoyl-CoA Desaturase-1: Is It the Link between Sulfur Amino Acids and Lipid Metabolism?, *Biol.* 4 (2015) 383–396. doi:10.3390/biology4020383.

A. Tabernero, E.M. Lavado, B. Granda, A. Velasco, J.M. Medina, Neuronal differentiation is triggered by oleic acid synthesized and released by astrocytes, *J. Neurochem.* 79 (2001) 606–616. http://dx.doi.org/.

E. Polo-Hernandez, V. Tello, A.A. Arroyo, M. Dominguez-Prieto, F. de Castro, A. Tabernero, J.M. Medina, Oleic acid synthesized by stearoyl-CoA desaturase (SCD-1) in the lateral periventricular zone of the developing rat brain mediates neuronal growth, migration and the arrangement of prospective synapses, *Brain Res.* 1570 (2014) 13–25. doi:10.1016/j.brainres.2014.04.038.

G. Astarita, K.M. Jung, V. Vasilevko, N. V Dipatrizio, S.K. Martin, D.H. Cribbs, E. Head, C.W. Cotman, D. Piomelli, Elevated stearoyl-CoA desaturase in brains of patients with Alzheimer's disease, *PLoS One.* 6 (2011) e24777. doi:10.1371/journal.pone.0024777.

T. Fraser, H. Tayler, S. Love, Fatty acid composition of

frontal, temporal and parietal neocortex in the normal human brain and in Alzheimer's disease, *Neurochem Res.* 35 (2010) 503–513. doi:10.1007/s11064-009-0087-5.

R.P. Bazinet, S. Laye, Polyunsaturated fatty acids and their metabolites in brain function and disease, *Nat Rev Neurosci.* 15 (2014) 771–785. doi:10.1038/nrn3820.

E. Lei, K. Vacy, W.C. Boon, Fatty acids and their therapeutic potential in neurological disorders, *Neurochem Int.* 95 (2016) 75–84. doi:10.1016/j.neuint.2016.02.014.

R. Hofacer, R. Jandacek, T. Rider, P. Tso, I.J. Magrisso, S.C. Benoit, R.K. McNamara, Omega-3 fatty acid deficiency selectively up-regulates delta6-desaturase expression and activity indices in rat liver: prevention by normalization of omega-3 fatty acid status, *Nutr Res.* 31 (2011) 715–722. doi:10.1016/j.nutres.2011.08.007.

J.J. Liu, P. Green, J.J. Mann, S.I. Rapoport, M.E. Sublette, Pathways of Polyunsaturated Fatty Acid Utilization: Implications for Brain Function in Neuropsychiatric Health and Disease, *Brain Res.* 0 (2015) 220–246. doi:10.1016/j.brainres.2014.11.059.

Q.L. Ma, F. Yang, E.R. Rosario, O.J. Ubeda, W. Beech, D.J. Gant, P.P. Chen, B. Hudspeth, C. Chen, Y. Zhao, H. V Vinters, S.A. Frautschy, G.M. Cole, Beta-amyloid oligomers induce phosphorylation of tau and inactivation of insulin receptor substrate via c-Jun N-terminal kinase signaling: suppression by omega-3 fatty acids and curcumin, *J. Neurosci.* 29 (2009) 9078–9089. doi:10.1523/jneurosci.1071-09.2009.

T. Oster, T. Pillot, Docosahexaenoic acid and synaptic protection in Alzheimer's disease mice, *Biochim Biophys Acta.* 1801 (2010) 791–798. doi:10.1016/j.bba-lip.2010.02.011.

T. Mita, T. Mayanagi, H. Ichijo, K. Fukumoto, K. Otsuka, A. Sakai, K. Sobue, Docosahexaenoic Acid Promotes Axon Outgrowth by Translational Regulation of Tau and Collapsin Response Mediator Protein 2 Expression, *J Biol Chem.* 291 (2016) 4955–4965. doi:10.1074/jbc.M115.693499.

D. Arsenault, C. Julien, C. Tremblay, F. Calon, DHA improves cognition and prevents dysfunction of entorhinal cortex neurons in 3xTg-AD mice, *PLoS One.* 6 (2011) e17397. doi:10.1371/journal.pone.0017397.

K.N. Green, H. Martinez-Coria, H. Khashwji, E.B. Hall, K.A. Yurko-Mauro, L. Ellis, F.M. LaFerla, Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid-beta and tau pathology via a mechanism involving presenilin 1 levels, *J. Neurosci.* 27 (2007) 4385–4395. doi:10.1523/jneurosci.0055-07.2007.

A.A. Farooqui, L.A. Horrocks, T. Farooqui, Modulation of inflammation in brain: a matter of fat, *J. Neurochem.* 101 (2007) 577–599. doi:10.1111/j.1471-4159.2006.04371.x.

A. Zarrouk, M. Debbabi, M. Bezine, E.M. Karym, A. Badreddine, O. Rouaud, T. Moreau, M. Cherkaoui-Malki, M. El Ayeb, B. Nasser, M. Hammami, G. Lizard, Lipid Biomarkers in Alzheimer's Disease, *Curr Alzheimer Res.* (2017). doi:10.2174/1567205014666170505101426.

G.P. Lim, F. Yang, T. Chu, P. Chen, W. Beech, B. Teter, T. Tran, O. Ubeda, K.H. Ashe, S.A. Frautschy, G.M. Cole, Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease, *J. Neurosci.* 20 (2000) 5709–5714. http://dx.doi.org/.

P. Wang, P.P. Guan, T. Wang, X. Yu, J.J. Guo, Z.Y. Wang, Aggravation of Alzheimer's disease due to the COX-2-mediated reciprocal regulation of IL-1β and Abeta between glial and neuron cells, *Aging Cell.* 13 (2014) 605–615. doi:10.1111/acer.12209.

C.H. Chen, W. Zhou, S. Liu, Y. Deng, F. Cai, M. Tone, Y. Tone, Y. Tong, W. Song, Increased NF-κappaB signalling up-regulates BACE1 expression and its therapeutic potential in Alzheimer's disease, *Int J Neuropsychopharmacol.* 15 (2012) 77–90. doi:10.1017/s1461145711000149.

S.Y. Park, H.Y. Kim, H.J. Park, H.K. Shin, K.W. Hong, C.D. Kim, Concurrent Treatment with Taxifolin and Cilostazol on the Lowering of beta-Amyloid Accumulation and Neurotoxicity via the Suppression of P-JAK2/P-STAT3/NF-κappaB/BACE1 Signaling Pathways, *PLoS One.* 11 (2016) e0168286. doi:10.1371/journal.pone.0168286.

CHAPTER 5

Dimethyl Sulfoxide reduces LPS-induced inflammatory gene expression in primary microglia and BV-2 cells

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Abstract

Dimethyl sulfoxide (DMSO) is a widely solvent for a range of both polar and nonpolar compounds and can cross cell membranes. In addition, DMSO has been studied in pharmacological studies as an anti-inflammatory compound. Based on these anti-inflammatory properties of DMSO, the objective of this study was to evaluate the effects of DMSO (0.1-10% v/v) on the viability and the LPS-induced inflammatory response of microglia, the innate immune cells of central nervous system. Effects on cell viability, proinflammatory mRNA and protein production were assessed in primary mouse microglia and BV-2 cells. Here we report that DMSO at high concentrations (10% v/v) reduced the viability of both primary microglia and BV-2 cells. As expected, LPS (100ng/mL) induced an inflammatory response through increased production of mRNA levels of IL-1 β , IL6 and COX2 in both cell cultures. Treatment with DMSO significantly reduced the LPS-induced production of IL-1 β , IL6, nuclear factor kappa B1 (NF- κ B1), cyclooxygenase (COX2) and induced nitric oxide synthase (iNOS) in BV-2 cells in a dose-dependent manner. In primary microglia, this effect was also observed although less pronounced. In addition, at low concentrations (0.2 to 1% v/v), DMSO reduced IL-1 β and IL-6 protein levels. Taken together, at low concentrations, DMSO reduced the LPS-induced inflammatory response of primary microglia and BV-2 cells.

Introduction

Dimethyl sulfoxide (DMSO), a highly polar organic solvent, is an amphiphilic molecule with one hydrophilic sulfoxide group and two hydrophobic methyl groups (Brayton 1986). This dipolar nature facilitates its ability to interact with biological membranes since DMSO is able to solubilize polar and nonpolar substances and cross hydrophobic barriers such as cell membranes (Fernández and Reigada 2014). Due to these properties, DMSO serves as a cryoprotectant and vehicle for intracellular pharmacotherapies (Yoganantharajah et al. 2018). Consequently, a wide number of studies have reported on other biological activities of DMSO, addressing the potential use of DMSO in a variety of medical conditions such as arthritis, scleroderma, musculoskeletal disorders, cancer and CNS disorders (Brayton 1986; Jacob and de la Torre 2009). DMSO is considered a low cytotoxicity molecule at low concentrations (Brayton 1986; Gad and Sullivan 2014), and it can be used up to 10% v/v in cell cultures without changing culture viability, however high DMSO concentrations can change cellular activity and are cytotoxic for glial cells (Kloverpris et al. 2010). In vitro experiments on rat liver cells treated with DMSO (25% v/v) showed increased activity of lysosomal enzymes (Sumida et al. 2011), while DMSO (40% v/v) increased the expression of selectins and the phosphorylation of proteins involved in intracellular activation pathways (de Abreu Costa et al. 2017). DMSO can reduce the inflammatory response of C-28/12 human chondrocytes (Kloesch et al. 2011) as well as Caco-2 cells treated with proinflammatory stimuli, resulting in reduced cytokine levels (Hollebeeck et al. 2011a). DMSO can also affect cell viability, mouse macrophages treated with 2% DMSO displayed increased apoptotic cell death (Hollebeeck et al. 2011b).

Different studies addressed the influence of DMSO on the expression of proteins in cells to understand their role on different biological pathways. Interestingly, the anti-inflammatory effect of DMSO is the most consistent among several studies, and low concentrations of DMSO (0.5 to 2% v/v) can reduce the proinflammatory cytokines/chemokines and prostaglandin E2 expression during stress challenging conditions in human. The same effect was shown in an arthritis C57BL/6 mouse model, where topical exposure to DMSO reduced the expression of pro-inflammatory cytokines (Elisia et al. 2016). Despite these studies, little is known about the effect of DMSO on microglia, the tissue resident macrophages of the central nervous system. Microglia form the primary immune defense in the CNS and continuously surveil the brain parenchyma (Salter and Beggs 2014). Microglia are increasingly implicated in neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Perry and Holmes 2014; Salter and Beggs 2014). Microglia activation is observed in neurodegenerative disorders, with increased activity of intracellular enzymes as Cox2, iNOS, and transcription factors like NF- κ B. Although the anti-inflammatory effect of DMSO has been demonstrated in peripheral immune cells, little information is available concerning the effects of DMSO primary microglia and BV-2 cells which was investigated in this study.

Materials and Methods

Glia cell cultures

C57BL/6J mouse pups were obtained from the animal facility (Centrale Dienst Proefdieren, CDP) of the University Medical Center Groningen. All animal work was approved by the Animal Ethics Committee of University of Groningen, and adhered to the European Directive (2010/63/EU) on the protection of animals used for scientific purposes.

Microglia were derived from primary mixed glia cell cultures originating from 1- or 2-day old mice-pup brains. After isolation, the brains were kept in cold medium A (HBSS with 0.6% glucose [D-(+)-glucose solution, Sigma, G8769], 15 mM HEPES buffer (Lonza BE17-737E), 1% penicillin/streptomycin). The meninges, cerebellum and olfactory bulb were removed and the remaining brain was minced into small pieces and suspended in medium A. Trypsin medium (0.25% trypsin; 0.1 mg/mL DNase I in medium A) was used to get a single-cell solution. This reaction was stopped with trypsin inhibition medium (20% fetal bovine serum (FBS); 0.1 mg/mL DNase I; 0.1 mg/mL trypsin inhibitor in medium A) and the cells were rinsed with wash medium (10% FBS; 0.1 mg/mL DNase I in medium A). The cell suspension was carefully titrated with glass pipettes of decreasing diameter and centrifuged in order to get a pellet of cells. The homogenized cells were supplied with microglia medium (10% FBS; 1 mM sodium pyruvate; 100 U/mL penicillin; 100 mg/mL streptomycin in DMEM) and were seeded at a density of 1.5 brains per T75cm² flask, which were kept in the incubator 37°C and 5% CO₂. When there was a confluent layer of astrocytes present in the flasks, the cultures were enriched with L929 fibroblast-conditioned medium to increase microglial proliferation. Consequently, primary microglia were de-attached from the culture by one or two shake-offs. The primary microglia were seeded in well plates for experiments. The cultures were regularly checked under the microscope and enriched with fresh microglia medium if necessary, to remove debris and supply the cells with new nutrients.

BV-2 cells were cultured in Dubelcco's Modified Eagle Medium (DMEM) (Lonza BE12;604F) supplemented with 10 % FBS (GIBCO, Cat# 10500064); 100 U/mL penicillin; 100 mg/mL streptomycin at 37°C and 5% CO₂. DMSO was obtained from Merck Millipore, to Avoid batch variations in experiments of different LPS lot numbers, one batch of lipopolysaccharide (LPS) was used in all experiments (E. coli 0111:B4, Sigma-Aldrich, cat# L4391).

Cell viability assays

Mitochondrial activity was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Riss et al. 2004). Primary microglia and BV-2 cells were plated in 96-well plates (20,000 cells/well), treated with DMSO (0-10% v/v) and/or

LPS (100ng/mL) for 3 h after which they were incubated with MTT solution (0.5 mg/mL in DMEM) for 2 h at 37°C. MTT conversion was determined by absorption at 570 nm.

Lactate dehydrogenase (LDH) release was measured to determine the cytotoxic effects of the DMSO and LPS treatments. Supernatant of primary microglia or BV-2 cells was mixed 1:1 with LDH-assay reagent mix (5% assay buffer). After incubation for 30 min, the absorption was measured at 490 nm. As a positive control, cell lysates were used as a reference for maximal LDH release.

Quantitative real-time PCR

Primary microglia and BV-2 cells were seeded in 24-well plates (50.000 cells/well). The cells were treated with PBS, DMSO (0-1% final concentration) and/or LPS (100ng/mL final concentration) for 3 h. Total RNA was isolated from primary microglia and BV-2 cells using TRIzol (ambion). 0.3-0.5µg of total RNA was used for cDNA synthesis by reverse transcriptase using random hexamers (Fermentas), M-MuLV Reverse Transcriptase and Ribolock RNase inhibitor (Fermentas) and dNTPs. Quantitative PCR reactions were carried out using 2xiTAQ SYBR supermix (BIORAD) and a real-time thermal cycler (ABI7900HT, Applied Biosystems) with different set of primers (table 1). For each gene, measurements were performed in triplicate in a minimum of three independent experiments. Primers specific for the evaluated genes were designed in PRIMER EXPRESS 2.0 and tested for efficiency. The housekeeping genes Hmbs or Hprt1 were used as an internal standard to calculate relative gene expression levels with the 2- $\Delta\Delta$ CT method (Livak and Schmittgen 2001) which is depicted as fold-induction of control samples. Data were extracted from QuantStudio™ Real-Time PCR Software v1.3 (Thermofisher).

ELISA

Microglia supernatants were collected and used in ELISA analyses. IL-6 and IL1 β protein levels were determined using the mouse IL-6 ELISA MAX™ Deluxe kit, Biolegend, cat# 431304, and mouse IL1 β ELISA MAX deluxe kit, Biolegend cat# 432606 according to the manufacturer's instructions. For IL1 β Elisa, ATP (1mM) was added during the last 15 min of the last LPS treatment to induce secretion.

Table 1. qPCR primer information

Gene	Accession #	Forward primer (5'-3')	Reverse primer (5'-3')
Il1 β	NM_001037859.2	CCCAAAAGATGAAG-GGCTGC	TGATACTGCCTGCCT-GAAGC
Il6	NM_031168	GAGGATACCACTCCAA-CAGACC	AAGTGCATCATCGTTGT-TCATACA
Cox2	NM_011198.4	TCTACGGAGAGAGT-TCATCCCT	ATTTAAGTCCACTCCAT-GGCCC
Hmbs	BC-003861	CCGAGCCAAGGACCAG-GATA	CTCCTTCCAGGTGCCTCA-GA
NFkB1-p65	NM_013556.2	ATACAGGCCAGACT-TTGTGGA	TGCGCTCATCTTAGGCT-TTGTGA

Statistics

All data are presented as means $\pm$ SEM. Data were analyzed using an unpaired Student's t-test when 2 groups were compared or by one-way ANOVA followed by a Tukey's multiple comparison test post hoc to compare several groups. Asterisks indicate * $p \leq 0.05$, ** $p \leq 0.005$ or *** $p \leq 0.001$, respectively.

Results

High concentrations of DMSO are cytotoxic for microglia

DMSO can be cytotoxic for cells at concentrations higher than 1% v/v (Yi et al. 2017)(León-García et al. 2017). However, little is known about the cytotoxic and anti-inflammatory effects of DMSO on microglia. First, we determined the cytotoxic effects of DMSO on BV-2 cells and primary microglia using cell viability assays. The cytotoxic effects were determined by MTT and LDH assays to measure mitochondrial activity and cellular integrity, respectively. Our results showed that treatment of BV-2 cells for 3 h with DMSO concentrations of 5 and 10% significantly decreased the conversion of MTT ($p \leq 0.001$) compared with cells treated with 0, 0.1, and 1% DMSO (Figure 1 A-B). LDH activity was significantly increased in BV-2 cells treated with 10% DMSO ($p \leq 0.001$) but not at DMSO concentrations of 5% and lower (Figure 1B). Similar results were obtained in primary microglia, where 10% DMSO significantly decreased MTT conversion ($p \leq 0.001$) significant increased LDH activity ($p \leq 0.001$) (Figure 1C and 1D). These data show that at 5% DMSO, mitochondrial activity was compromised (MTT) in BV-2 cells and that cellular integrity (LDH release) was affected at concentrations of 10% DMSO in both BV-2 cells and primary microglia.

DMSO modulates pro-inflammatory gene expression by microglia cells.

Stimulation with an inflammatory cocktail and treatment with low concentrations of DMSO (0.1 to 0.5% v/v) reduced the expression of inflammatory cytokines in peripheral blood mononuclear cells (Elisia et al. 2016). However, it is unclear whether DMSO also has anti-inflammatory effects on microglia. LPS was used to induce pro-inflammatory gene expression in mouse microglia cells (Schaafsma et al. 2015). To determine if DMSO affects LPS-induced pro-inflammatory gene expression by microglia, RT-qPCRs were performed. LPS (100ng/mL) significantly induced proinflammatory gene expression (Il1 β , Il6, and Cox2) in BV-2 cells compared to PBS-treated cells. In contrast, treatment with DMSO significantly attenuated the response of microglia to LPS: Il1 β , Il6 and, Cox2 were all significantly less induced (Figure 2). This reduced LPS responsiveness was observed after 3 hours of LPS stimulation and DMSO treatment. The relative expression of the genes Il1 β , Cox2 and Il6 decreased after 3-hour DMSO treatment and stimulation with LPS. Importantly, the LPS-induced gene expression progressively decreased with increasing DMSO concentrations (Figure 2.A-C). Sim-

Figure 1: DMSO affects mitochondrial activity and microglia integrity. The graphs depict MTT conversion and LDH activity of BV-2 cells (A-B) and primary microglia (C-D) treated with different concentrations of DMSO. Mitochondrial activity was measured by MTT conversion and cellular integrity by LDH release in the medium. P values were calculated by a two-way ANOVA followed by a Tukey post-hoc test (***: $p < 0.001$). Data is represented in dot-plots with mean $\pm$

ilar, but less pronounced, anti-inflammatory effects of DMSO were observed in primary microglia cells. The induction of *Il1 β* expression by LPS was not modulated by DMSO, even at the high concentrations (1%) (Figure 2.D). However, the LPS-induced expression of *Cox2* and *Il6* were significant decreased when treated with 1% DMSO ($p \leq 0.005$) (Figure 2E-F). These results show that DMSO has anti-inflammatory activity in BV-2 cells and primary microglia.

Low concentration of DMSO has an anti-inflammatory effect on microglia cells

In order to confirm the anti-inflammatory effect of low DMSO concentrations on BV-2 cells and primary microglia, these cells were treated for 3 h with LPS (100 ng/ml) and/or DMSO (0.8 %). Gene expression of pro-inflammatory genes *Il1 β* , *NF-kb1* (p50), *Cox2* and *iNos* was quantified by RT-qPCR assay. LPS increased the expression of all these genes compared with the control group. Interestingly, simultaneous treatment of microglia with LPS and DMSO significantly reduced the induction of all evaluated genes in both cells type (Figure 3A-H) ($p \leq 0.001$) with the exception of *iNos* in BV-2 cells (Figure 3.D). The attenuation of LPS-induced gene expression by DMSO was more also detected in primary microglia (Figure 3 E-H). These results correlated with our previous results suggesting that DMSO has an anti-inflammatory effect on microglia cells by negatively modulating the LPS-induced expression of pro-inflammatory genes.

Figure 2: DMSO reduces LPS-induced pro-inflammatory gene expression in BV-2 cells and primary microglia. Graphs depict the fold induction in gene expression of the *Il1 β* , *Cox2*, and *Il6* genes in BV-2 cells (A-C) and primary microglia (D-F). The data is depicted as a percentage of the fold induction of the LPS-treated group. Data were analyzed with a two-way ANOVA followed by a Tukey post-hoc test (** $P < 0.01$, *** $P < 0.001$). Data is represented in dot-plots with mean $\pm$ SEM.

DMSO suppresses LPS-induced cytokine secretion by microglia

To assess if the effect of DMSO on pro-inflammatory gene expression after inflammatory stimulus also translated into decreased cytokine secretion, protein levels in the supernatant of BV-2 and primary microglia cells treated with PBS, LPS (100ng/mL) or increasing concentrations of DMSO were performed. In both cell types, DMSO decreased the LPS-induced secretion of *Il-6* and *Il1 β* , which was already observed at the lowest DMSO concentration (0.2%) used (Figure 4 A-D). DMSO reduced the abundance of *Il-6* and *Il1 β* protein and mRNA levels in both microglia cell types.

Discussion

DMSO is frequently used as a solvent and as a compound with anti-inflammatory properties for the treatment of various inflammation-associated diseases including osteoarthritis, rheumatoid arthritis, systemic sclerosis, chronic pancreatitis, ulcerative colitis, and neurological disorders (Santos et al. 2003). However, little is known about how DMSO acts on inflammation in the central nervous system, and most notably what its effect is on the principal CNS immune cells, microglia. This study was aimed to assess the anti-inflammatory effect of DMSO on microglia, the resident tissue macrophages of the CNS (Colucci et al. 2008). Using an *in vitro* approach, we show that DMSO has an anti-inflammatory effect on microglia. Primary microglia cultures and BV-2 cells were immune activated with LPS and treated with a different concentration of DMSO (0.2-10% v/v). This concentration range was tested as these were used in studies to solubilize and deliver bioactive compounds to cells (Colucci et al. 2008). According to our results obtained in microglia cultures, DMSO did not induce any cytotoxicity up to 5% v/v. At higher concentrations however, decreased cell integrity (LDH release) and reduced mitochondrial activity (MTT conversion) were observed after 3 hours incubation. Similar observations were reported for astrocytes, where DMSO concentrations of $\geq 5\%$ v/v reduced mitochondrial activity, cell viability and promoted apoptosis. Starting from 1% v/v, DMSO progressively reduced MTT conversion and caused mitochondrial swelling in cultured astrocytes (C. Yuan et al. 2014). DMSO concentrations $\geq 1\%$ v/v induced a progressive loss of viability and NeuN expression in cultured neurons, already after 12h exposure (C. Zhang et al. 2017). Finally, recently a study showed that DMSO starting at 1% v/v induces cytotoxicity in monocytes (Elisia et al. 2016). DMSO-induced cytotoxicity was resulted in decreased cell membrane thickness and the formation of transient water pores at concentrations ranging from 2.5-7.5% and 10-20%, respectively (Gurtovenko and Anwar 2007). In summary, these data indicate that DMSO, has cytotoxic effects on all CNS cells like astrocytes, neurons and microglia as well as other myeloid cells, monocytes.

To address the potential anti-inflammatory effects of DMSO, we performed RT-qPCR for 5 proinflammatory genes (*Il1 β* , *Il-6*, *NF-kB*, *Cox2*, and *iNOS*) on LPS-treated microglia. LPS

Figure 3: DMSO attenuates pro-inflammatory gene expression induced by LPS in BV-2 cells and primary microglia. The graphs depict the fold induction in gene expression of *IL1β*, *Nfκb1*, *Cox2*, and *iNos* in BV-2 cells (A-D) and primary microglia (E-F), respectively. Cells were treated with LPS (100 ng/mL) and/or DMSO (0.8% v/v) as indicated. Fold induction of gene expression was calculated using the delta-delta CT method with Hmba expression as a reference and an untreated sample as controls. Data were analyzed with two-way ANOVA followed by a Tukey post-hoc test (*P < 0.05, **P < 0.01, ***P < 0.001). Data is represented in dot-plots with mean ± SEM.

Figure 4: DMSO reduces LPS-induced secretion of IL-6 and IL1β by BV-2 cells and primary microglia. Concentration of IL-6 and IL1β in the supernatant of primary microglia (A-B) and BV-2 cells (C-D) are depicted. Data were analyzed with two-way ANOVA followed by a Tukey post-hoc test (**p < 0.01, ***p < 0.001). Data is represented in dot-plots with mean ± SEM.

enhanced the mRNA expression of these genes, and DMSO treatment attenuated this induction in primary microglia and BV-2 cells. DMSO already displayed anti-inflammatory properties at concentrations as low as 0.2% up to 1%. Importantly, not cytotoxic effects of DMSO were observed at these concentrations. IL-6 and IL1β are both pro-inflammatory cytokines with important roles in the CNS inflammation response and are released by microglia (Krasemann et al. 2017). Several studies have indicated that DMSO is able to reduce the expression of pro-inflammatory cytokines via inhibition of transcription factors such as NF-κB. LPS interacts with membrane receptors such as toll-like receptor 4 (TLR4) and induces the activation of NF-κB, leading to the production of reactive oxygen species (ROS) and inflammatory gene expression (Ahn et al. 2014). This process is reversed by DMSO via ROS scavenging and NF-κB inhibition, since, the results showed that DMSO reduced the expression of NF-κB, *IL1β*, *IL-6*, and *Pro-IL-1* in bone marrow-derived macrophages stimulated by LPS (Ahn et al. 2014). In a different study, low concentrations of DMSO (0.1 and 0.5% v/v) attenuated the intestinal inflammatory response through reduction of mRNA expression and secretion of IL6 and IL1β by intestinal cells via inhibiting inflammasome activation (Hollebeek et al. 2011b). Our findings in microglia agree with these results, as DMSO reduced LPS-induced secretion of inflammatory cytokines by microglia. The observed DMSO effects on mRNA and protein levels

suggest that DMSO is involved in both transcriptional and post-transcriptional regulation of the inflammatory response in microglia.

NF- κ B plays a pivotal role by modulating expression of several genes related with inflammatory pathways including cytokines, and pro-inflammatory enzymes (COX2 and iNOS), which facilitates the production of pro-inflammatory mediators such as prostaglandins (PGs) and NO, respectively (Shabab et al. 2017). As expected, LPS increased the expression of COX2 and iNOS in microglia, which was reduced by DMSO.

In summary, our data indicate that DMSO, at low concentration 0.1-0.8% v/v, has an anti-inflammatory activity on primary microglia and BV-2 cells, by reducing pro-inflammatory cytokine gene expression and secretion. In addition, the induction of inflammatory mediators such as PGs and NO are likely reduced by DMSO due to attenuated COX2 and iNOS gene expression. The anti-inflammatory effects of DMSO in microglia are likely caused by reduced activation of the transcription factor NF- κ B and as a consequence its downstream target gene by DMSO.

Disclosure statement

The authors have no actual or potential conflicts of interest.

References

1. Abreu Costa, Lucas de, Marcelo Henrique Fernandes Ottoni, Michaelle dos Santos, Agnes Meireles, Valéria Gomes de Almeida, Wagner de Fátima Pereira, Bethânia Alves de Avelar-Freitas, and Gustavo Eustáquio Alvim Brito-Melo. 2017. "Dimethyl Sulfoxide (DMSO) Decreases Cell Proliferation and TNF- α , IFN- γ , and IL-2 Cytokines Production in Cultures of Peripheral Blood Lymphocytes." *Molecules* 22 (11): 1789. <https://doi.org/10.3390/molecules22111789>.
2. Ahn, Huijeong, Jeeyoung Kim, Eui-Bae Jeung, and Geun-Shik Lee. 2014. "Dimethyl Sulfoxide Inhibits NLRP3 Inflammasome Activation." *Immunobiology* 219 (4): 315–22. <https://doi.org/10.1016/j.imbio.2013.11.003>.
3. Brayton, C F. 1986. "Dimethyl Sulfoxide (DMSO): A Review." *The Cornell Veterinarian* 76 (1): 61–90. <http://www.ncbi.nlm.nih.gov/pubmed/3510103>.
4. Colucci, Mariantonella, Francesco Maione, Maria Carmela Bonito, Alessandro Piscopo, Amalia Di Giannuario, and Stefano Pieretti. 2008. "New Insights of Dimethyl Sulphoxide Effects (DMSO) on Experimental in Vivo Models of Nociception and Inflammation." *Pharmacological Research* 57 (6): 419–25. <https://doi.org/10.1016/J.PHRS.2008.04.004>.
5. Elisia, Ingrid, Hisae Nakamura, Vivian Lam, Elyse Hofs, Rachel Cederberg, Jessica Cait, Michael R Hughes, et al. 2016. "DMSO Represses Inflammatory Cytokine Production from Human Blood Cells and Reduces Autoimmune Arthritis." *PloS One* 11 (3): e0152538. <https://doi.org/10.1371/journal.pone.0152538>.
6. Fernández, M. Laura, and Ramon Reigada. 2014. "Effects of Dimethyl Sulfoxide on Lipid Membrane Electroporation." *The Journal of Physical Chemistry B* 118 (31): 9306–12. <https://doi.org/10.1021/jp503502s>.
7. Gad, S.E., and D.W. Sullivan. 2014. "Dimethyl Sulfoxide (DMSO)." In *Encyclopedia of Toxicology*, 166–68. Elsevier. <https://doi.org/10.1016/B978-0-12-386454-3.00839-3>.
8. Gurtovenko, Andrey A, and Jamshed Anwar. 2007. "Modulating the Structure and Properties of Cell Membranes: The Molecular Mechanism of Action of Dimethyl Sulfoxide." *The Journal of Physical Chemistry. B* 111 (35): 10453–60. <https://doi.org/10.1021/jp073113e>.
9. Hollebeeck, Sylvie, Thomas Raas, Neil Piront, Yves-Jacques Schneider, Olivier Toussaint, Yvan Larondelle, and Alexandrine During. 2011a. "Dimethyl Sulfoxide (DMSO) Attenuates the Inflammatory Response in the in Vitro Intestinal Caco-2 Cell Model." *Toxicology Letters* 206 (3): 268–75. <https://doi.org/10.1016/j.toxlet.2011.08.010>.
10. ———. 2011b. "Dimethyl Sulfoxide (DMSO) Attenuates the Inflammatory Response in the in Vitro Intestinal Caco-2 Cell Model." *Toxicology Letters* 206 (3): 268–75. <https://doi.org/10.1016/j.toxlet.2011.08.010>.
11. Jacob, Stanley W, and Jack C de la Torre. 2009. "Pharmacology of Dimethyl Sulfoxide in Cardiac and CNS Damage." *Pharmacological Reports*. [https://doi.org/10.1016/S1734-1140\(09\)70026-X](https://doi.org/10.1016/S1734-1140(09)70026-X).
12. Kloesch, Burkhard, Melissa Liszt, Johann Broell, and Guenter Steiner. 2011. "Dimethyl Sulphoxide and Dimethyl Sulphone Are Potent Inhibitors of IL-6 and IL-8 Expression in the Human Chondrocyte Cell Line C-28/I2." *Life Sciences* 89 (13–14): 473–78. <https://doi.org/10.1016/J.LFS.2011.07.015>.
13. Kloverpris, Henrik, Anders Fomsgaard, Amanda Handley, Jim Ackland, Mark Sullivan, and Philip Goulder. 2010. "Dimethyl Sulfoxide (DMSO) Exposure to Human Peripheral Blood Mononuclear Cells (PBMCs) Abolish T Cell Responses Only in High Concentrations and Following Coincubation for More than Two Hours." *Journal of Immunological Methods* 356 (1–2): 70–78. <https://doi.org/10.1016/j.jim.2010.01.014>.
14. Krasemann, Susanne, Charlotte Madore, Ron Cialic, Caroline Baufeld, Narghes Calcagno, Rachid El Fatimy, Lien Beckers, et al. 2017. "The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases." *Immunity* 47 (3): 566–581.e9. <https://doi.org/10.1016/j.immuni.2017.08.008>.
15. León-García, María Cristina, Emmanuel Ríos-Castro, Everardo López-Romero, and Mayra Cuéllar-Cruz. 2017. "Evaluation of Cell Wall Damage by Dimethyl Sulfoxide in Candida Species." *Research in Microbiology* 168 (8): 732–39. <https://doi.org/10.1016/j.resmic.2017.06.001>.
16. Livak, Kenneth J., and Thomas D. Schmittgen. 2001. "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2- $\Delta\Delta$ CT Method." *Methods* 25 (4): 402–8. <https://doi.org/10.1006/meth.2001.1262>.
17. Perry, V. Hugh, and Clive Holmes. 2014. "Microglial Priming in Neurodegenerative Disease." *Nature Reviews Neurology* 10 (4): 217–24. <https://doi.org/10.1038/nrneurol.2014.38>.
18. Riss, Terry L, Richard A Moravec, Andrew L Niles, Sarah Duellman, Hélène A Benink, Tracy J Worzella, and Lisa Minor. 2004. *Cell Viability Assays. Assay Guidance*

Manual. Eli Lilly & Company and the National Center for Advancing Translational Sciences. <http://www.ncbi.nlm.nih.gov/pubmed/23805433>.

19. Salter, Michael W., and Simon Beggs. 2014. "Sublime Microglia: Expanding Roles for the Guardians of the CNS." *Cell* 158 (1): 15–24. <https://doi.org/10.1016/J.CELL.2014.06.008>.

20. Santos, Nuno C, J Figueira-Coelho, J Martins-Silva, and Carlota Saldanha. 2003. "Multidisciplinary Utilization of Dimethyl Sulfoxide: Pharmacological, Cellular, and Molecular Aspects." *Biochemical Pharmacology* 65 (7): 1035–41. <http://www.ncbi.nlm.nih.gov/pubmed/12663039>.

21. Schaafsma, W., X. Zhang, K.C. van Zomeren, S. Jacobs, P.B. Georgieva, S.A. Wolf, H. Kettenmann, et al. 2015. "Long-Lasting pro-Inflammatory Suppression of Microglia by LPS-Preconditioning Is Mediated by RelB-Dependent Epigenetic Silencing." *Brain, Behavior, and Immunity* 48 (August): 205–21. <https://doi.org/10.1016/j.bbi.2015.03.013>.

22. Shabab, Tara, Ramin Khanabdali, Soheil Zorofchian Moghadamtousi, Habsah Abdul Kadir, and Gokula Mohan. 2017. "Neuroinflammation Pathways: A General Review." *International Journal of Neuroscience* 127 (7): 624–33. <https://doi.org/10.1080/00207454.2016.1212854>.

23. Sumida, Kayo, Yoshinobu Igarashi, Naoki Toritsuka, Tomochika Matsushita, Kaori Abe-Tomizawa, Mikio Aoki, Tetsuro Urushidani, Hiroshi Yamada, and Yasuo Ohno. 2011. "Effects of DMSO on Gene Expression in Human and Rat Hepatocytes." *Human & Ex-*

perimental Toxicology 30 (10): 1701–9. <https://doi.org/10.1177/0960327111399325>.

24. Yi, Xiaoyang, Minxia Liu, Qun Luo, Hailong Zhuo, Hui Cao, Jiexi Wang, and Ying Han. 2017. "Toxic Effects of Dimethyl Sulfoxide on Red Blood Cells, Platelets, and Vascular Endothelial Cells in Vitro." *FEBS Open Bio* 7 (4): 485–94. <https://doi.org/10.1002/2211-5463.12193>.

25. Yoganantharajah, Prusothman, Alexander P. Ray, Daniel J. Eyckens, Luke C. Henderson, and Yann Gibert. 2018. "Comparison of Solvate Ionic Liquids and DMSO as an in Vivo Delivery and Storage Media for Small Molecular Therapeutics." *BMC Biotechnology* 18 (1): 32. <https://doi.org/10.1186/s12896-018-0442-1>.

26. Yuan, Chan, Junying Gao, Jichao Guo, Lei Bai, Charles Marshall, Zhiyou Cai, Linmei Wang, and Ming Xiao. 2014. "Dimethyl Sulfoxide Damages Mitochondrial Integrity and Membrane Potential in Cultured Astrocytes." Edited by Shilpa J. Buch. *PLoS ONE* 9 (9): e107447. <https://doi.org/10.1371/journal.pone.0107447>.

27. Zhang, Chen, Yuanying Deng, Hongmei Dai, Wenjuan Zhou, Jing Tian, Guoying Bing, and Lingling Zhao. 2017. "Effects of Dimethyl Sulfoxide on the Morphology and Viability of Primary Cultured Neurons and Astrocytes." *Brain Research Bulletin* 128 (January): 34–39. <https://doi.org/10.1016/J.BRAINRESBULL.2016.11.004>.

CHAPTER 6

Summary

Samenvatting Nederlands

General discussion

Summary

Alzheimer disease is an irreversible, progressive brain disorder with progressive deterioration of memory and cognitive skills, which affect the simple and functional tasks of the AD patients. In the most of AD cases, the clinical symptoms first appear around 60 years old (The Lancet 2016). Several estimations indicate that AD may rank third, behind heart disease and cancer, as a cause of death in elderly people. AD is characterized by progressive neuron loss and microgliosis and caused by a combination of environmental and genetic factors. (Reitz and Mayeux 2014). In AD many molecular lesions are observed, and according to multiple observations, the accumulation of misfolded proteins in the AD brain cells results in oxidative and inflammatory damage, which induces to synaptic dysfunction, metabolic alterations, energy failure and finally cell death (Cuervo, Wong, and Martinez-Vicente 2010). Misfolded proteins bind to surface receptors from microglia and astrocytes and trigger the innate immune response, characterized by the release of pro-inflammatory mediators which exacerbates these alterations and increases AD severity (Hong 2015b). Genomic studies identified several AS risk genes, and these genes encode enzymes like BACE1 in neurons, APOE4 in astrocytes and transcription factors that increase pro-inflammatory cytokine production.

External high risk factors for AD include hypertension, dyslipidemia and obesity at midlife, diabetes mellitus, smoking, physical inactivity, depression and low levels of education (Uddin et al. 2018). Interestingly most of these external factors are related to systematic inflammation and lipid metabolic alterations which likely affect the immunological processes favoring the AD progress in the CNS.

In **Chapter 2**, we showed that microinjections of RNAi to silencing of BACE1 protein in the CA1 hippocampal area induced cognitive recovery and restored the fatty acid composition of lysophosphatidylethanolamine (LPE) and etherphosphatidylethanolamine (ePE) in aged 3xTg-AD mice after 6 and 12 months of gene therapy treatment. These changes were related with pro-inflammatory pathways, since cPLA2 phosphorylated enzyme levels were reduced, how has been shown that their activity produces arachidonic acid we observed this fatty acid was reduced as well, subsequently we measured the levels of COX2 due AA is their main substrate, interestingly the levels significantly decreased. Together, our results show, for the first time, that BACE1 levels are not only related to amyloid production, but also control of pro-inflammatory response through restoration of phospholipid composition which downregulated specific proinflammatory agents at the hippocampus promoting cellular homeostasis and cognitive function recovery AD model mice. Those results showed that endogenous lipids are important mediators of AD not only because they are involved in all phases of inflammation, but also involved in the regulation and fine-tuning of its course and cessation. However, as little is known about the brain lipid profile in human AD, we

performed a complete phospholipid profile analysis of brain human tissue diagnosed with familial Alzheimer disease (FAD) and sporadic Alzheimer disease (SAD), which were compared with healthy human tissue in **Chapter 3**. We performed a comparative analysis of the phospholipid composition of temporal cortex from FAD, SAD and, healthy human brains. A significant alteration between poly and unsaturated fatty acids was observed for both types of AD, and the phospholipid composition was different. The levels of polyunsaturated fatty acids were increased in both AD types, and two unsaturated lipids (PE-Cer and PG were significantly increased in SAD, which confirmed that AD cortex has an unbalanced proportion of phospholipids associated to mitochondria impairment. These changes are different between FAD and SAD, in addition, these fatty acid changes suggested that neuroinflammation pathways were activated due to altered lipid metabolism.

To understand how lipid metabolism and inflammatory pathways are affected in AD, we performed *in vitro* experiments described in **chapter 4**. We observed membrane lipid alterations triggering the over activity of membrane proteins such as BACE1 suggesting a strong link between the Inflammation and AD pathogenesis through AA fatty acid metabolism (cPLA2/arachidonic acid/COX2) (chapter 2). In this chapter, we analyzed the role of cPLA2 and fatty acid desaturases such as SCD1 and FAD6 in primary cortical neuron cultures with BACE1 knock-down in a glutamate excitotoxicity model. This model induced hyperphosphorylation of tau, increased apoptotic nuclei, LDH release, and cPLA2 expression, which were all reversed by BACE1-KD. In addition, co-silencing of BACE1 and cPLA2 improved the neuroprotective effects. However, co-silencing of BACE1 and SCD1 or FAD6 reduced these pro-survival effects. These results are supported by overexpression of SCD1 and FAD6, which resulted in a recovery of the neuroprotective effect by BACE1 silencing, but not by each desaturase alone. These findings suggested that BACE1 and lipid metabolism both play an important role in AD pathogenesis due to the over activation of BACE1 which can reduce the processing of fatty acids by desaturases exacerbating neuronal excitotoxicity.

In **chapter 5**, we describe the anti-inflammatory effect of dimethyl sulfoxide (DMSO) on primary microglia and BV-2 cells. We observed that DMSO did not affect cell viability at low concentrations (0.1-1% v/v), however, cell viability was altered at high concentrations of DMSO (5-10% v/v). Interestingly, lipopolysaccharide (LPS) -induced activation of microglia was reduced by DMSO at low concentrations. LPS stimulation resulted in increased gene expression of IL-1B, IL6, NF- κ B, iNOS and, COX2, which was significantly attenuated by 0.4-1% v/v DMSO. In agreement with these results, we also observed that DMSO significantly reduced LPS-induced IL-1B and IL6 protein levels. We conclude that DMSO has *in vitro* anti-inflammatory effects, at low concentrations, by the reducing mRNA and protein expression of pro-inflammatory genes in microglial cells, but more mechanistic investigations would be necessary to better understand how this compound inhibits inflammatory signals.

Nederlandse samenvatting

De ziekte van Alzheimer (AD) is een ongeneeslijke hersenaandoening die gekenmerkt wordt door progressieve achteruitgang van het geheugen en cognitieve vaardigheden. Hierdoor kunnen AD-patiënten eenvoudige en functionele taken niet meer uitvoeren. In de meeste gevallen van AD, beginnen de klinische verschijnselen rond de leeftijd van 60 jaar (The Lancet 2016). Onder ouderen is AD een veel voorkomende ziekte en geschat wordt dat AD, na hartfalen en kanker, de derde meest voorkomende oorzaak voor het overlijden van ouderen is. AD wordt gekenmerkt door progressief verlies van zenuwcellen en activatie van glia cellen, de steuncellen in het centrale zenuwstelsel. AD wordt veroorzaakt door een combinatie van erfelijke en omgevingsfactoren (Reitz and Mayeux 2014). In de hersenen van AD-patiënten ontstaan gebieden, seniele plaques, waarin onjuist gevouwen eiwitten zich ophopen. Dit resulteert in een verstoorde functie van zenuwcellen, glia cellen en de communicatie daartussen, veranderingen in de energiehuishouding van zenuwcellen en uiteindelijk celdood (Cuervo, Wong, and Martinez-Vicente 2010). Onjuist gevouwen eiwitten binden aan receptoren op de buitenkant van twee soorten glia cellen: microglia en astrocyten die daardoor geactiveerd raken. Hierdoor worden ontstekingsmoleculen vrijgelaten die het ziekteproces verergeren (Hong 2015b). DNA is het erfelijk materiaal en de erfelijke informatie op ons DNA ligt vast in genen. Genen bevatten informatie voor eiwitten, en deze eiwitten vervullen belangrijke functies in cellen. Veranderingen in genen kunnen leiden tot verstoorde functies in cellen met zelfs ziekten als gevolg. Met behulp van genetische studies zijn een aantal genen (en veranderingen in deze genen) geïdentificeerd die betrokken zijn bij AD. Een van de bekendste genen die gemuteerd is bij mensen met AD is het Amyloid Precursor Protein (APP) gen, en deze mutaties leiden tot foutieve vouwing en ophoping van het APP-eiwit. →→→Een ander gen dat betrokken is bij AD is het BACE1 eiwit. BACE1 is een enzym dat het APP-eiwit klieft. Verstoorde activiteit van het BACE1 enzym leidt tot verhoogd productie van een APP-fragment dat samenklontert in zenuwcellen. Het BACE1 eiwit wordt als een mogelijk therapeutisch aanknopingspunt beschouwd om AD te behandelen en uitgebreid onderzocht in dit proefschrift.

Externe, hoge risicofactoren voor AD zijn onder andere hoge bloeddruk, verstoringen in de vethuishouding, suikerziekte op middelbare leeftijd, roken, gebrek aan lichamelijke activiteit, depressie en een laag opleidingsniveau (Uddin et al. 2018). Opvallend is dat de meeste van deze externe factoren gerelateerd zijn aan systemische ontsteking en veranderingen in het vet-metabolisme, wat waarschijnlijk leidt tot ontstekingsreacties die AD bevorderen.

In **Hoofdstuk 2** hebben wij laten zien dat het verlagen van de hoeveelheid BACE1 eiwit in een deel van de hippocampus in een muismodel voor AD, een hersengebied dat wordt aangetaast in AD, beschermend werkt. Een herstel van cognitieve functies en in de samenstelling van fosfolipiden lysofosfatidylethanolamine (LPE) en etherfosfatidylethanolamine (ePE)

werd waargenomen in oude AD muizen, 6 en 12 maanden na gentherapie. Deze veranderingen in fosfolipiden hielden verband met ontsteking stimulerende signaaltransductie routes. Onze resultaten laten voor het eerst zien dat BACE1 niveaus niet alleen samenhangen met amyloïde productie, maar ook de productie van ontsteking stimulerende processen controleren. Mogelijk gebeurt dit door herstel van de samenstelling van fosfolipiden wat leidt tot vermindering van bepaalde ontsteking stimulerende moleculen in de hippocampus en herstel van cognitieve functies in dit AD-muis model.

De resultaten in hoofdstuk 2 lieten zien dat fosfolipiden belangrijke factoren zijn in AD, en dat ze betrokken zijn bij de productie van ontstekingsmoleculen.

Momenteel is er weinig bekend over de (fosfo)lipiden samenstelling van het brein van humane AD-patiënten. Daarom hebben we een complete fosfolipide profielanalyse gedaan van humaan hersenweefsel dat gediagnosticeerd is met familiale Alzheimer (FAD) en sporadische Alzheimer (SAD) en dat vergeleken met gezond humaan hersenweefsel (**Hoofdstuk 3**). Deze analyse is uitgevoerd op weefsel van de temporale cortex. Een significant verschil tussen meervoudig onverzadigde vetzuren werd waargenomen bij beide vormen van AD en ook de fosfolipiden samenstelling was anders. Het niveau van de meervoudig onverzadigde vetzuren was verhoogd in beide vormen van AD en twee onverzadigde lipiden fosfatidylethanolamine-ceramide (PE-Cer) and fosfoglycerol (PG) waren significant verhoogd in SAD. De disbalans in fosfolipiden is geassocieerd met verstoorde mitochondriale functie en speelt dus mogelijk een rol in de pathologie van AD in de cortex. Daarnaast wijst de veranderde lipidensamenstelling op een potentiële rol van lipiden-metabolisme in de activatie van neurale ontsteking bij AD.

Om te begrijpen hoe lipiden-metabolisme en ontstekingsroutes aangedaan zijn in AD, hebben we in vitro experimenten gedaan die in **Hoofdstuk 4** beschreven staan. We hebben onderzocht of veranderingen in de samenstelling van lipiden in het membraan tot een verhoogde activiteit van membraaneiwitten zoals BACE1 leidt. De resultaten suggereerden dat er een sterk verband is tussen ontsteking en arachidonzuur metabolisme in AD-pathogenese. In dit hoofdstuk analyseerden wij de rol van cPLA2 en vetzuur desaturases (zoals SCD1 en FAD6) in gekweekte primaire corticale neuronen door het remmen van BACE1 eiwitproductie. In dit model leidde een overdadige hoeveelheid glutamaat tot hyperfosforylatie van tau en een toename van apoptotische cellen, lactaat dehydrogenase uitscheiding, en cPLA2 expressie. Verlaging van de hoeveelheid BACE1 eiwit herstelde deze effecten. Daarnaast zorgde de gelijktijdige verlaging van BACE1 en cPLA2 voor nog grotere neuron-beschermende effecten. Daarentegen, de gelijktijdige uitschakeling van BACE1 met SCD1 of FAD6, leidde voor verminderde neuron-beschermende effecten van BACE1 uitschakeling. Deze observaties werden bevestigd in experimenten waarin de expressie van SCD1 en FAD6 verhoogd werd, wat resulteerde in nog meer bescherming tegen glutamaat dan bij BACE1 uitschakeling alleen. De neuron-beschermende effecten van SCD1 en FAD6 treden alleen

op als beide eiwitten aanwezig zijn. Deze resultaten suggereren dat BACE1 en lipiden-metabolisme alle twee een belangrijke rol spelen in AD-pathogenese. Overactiviteit van BACE1 kan leiden tot een verminderde omzetting van vetzuren door desaturasen wat neuronale excitotoxiciteit versterkt.

In **Hoofdstuk 5** beschrijven we het ontstekingsremmende effect van dimethyl sulfoxide (DMSO) op primaire microglia en BV-2 cellen. We hebben waargenomen dat DMSO geen effect had op cellulaire levensvatbaarheid bij lage concentraties DMSO (0.1-1% v/v), maar wel bij hoge concentraties (5-10% v/v). Opvallend is dat activatie van microglia door lipopolysaccharide (LPS) werd verminderd door DMSO bij lage concentraties. Stimulering door LPS resulteerde in toegenomen expressie van *IL-1B*, *IL6*, *NFkB*, *iNOS* en *COX2*, wat significant minder was bij concentraties van 0.4-1% v/v DMSO. DMSO verlaagde ook de door LPS verhoogde eiwitniveaus van IL-1B en IL6 significant. We concludeerden dat lage concentraties DMSO in vitro een ontstekingsremmend effect hebben wat zorgt voor verlaagde productie van ontstekingsfactoren microglia. Er zijn echter meer mechanistische studies nodig om beter te begrijpen hoe DMSO deze ontstekingssignalen remt.

Conclusies en perspectieven

De resultaten beschreven in dit proefschrift laten zien dat het remmen van BACE1 in de hippocampus, de AD-geassocieerde degeneratie in AD-model muizen verbetert. Dit werk geeft aan dat BACE1 betrokken is bij de vetzuursamenstelling van fosfatidylethanol-derivaten, aangezien BACE1-remming bij AD-muizen de niveaus van deze lipide-derivaten herstelt, wat geassocieerd kan zijn met leer- en geheugenverbetering. Deze verandering in lipiden samenstelling werd ook waargenomen in de hersen van mensen met erfelijke en sporadische AD. De positieve effecten van BACE1 remming hingen af van de activiteit van desaturases, enzymen die pro-inflammatoire signalen verminderen, wat suggereert dat lipide metabolisme een belangrijke regulator is van het neurodegeneratieve proces. De progressie van AD is afhankelijk van de productie van bèta-amyloïd maar tegelijkertijd speelt het veranderde lipide metabolisme en de inflammatoire respons in de hersenen van AD-patiënten een rol. Deze observaties illustreren dat er een grote behoefte is aan nieuw goed opgezet onderzoek naar combinatietherapieën tegen AD.

De mogelijkheid om de TAU- en Aβ-pathologie te verbeteren, biedt potentieel enorme voordelen voor mensen met AD, hopelijk leidt het tot een omkering van het pathologische proces. Niettemin, gezien het functionele belang van BACE1 in het volwassen brein, moet aanvullend onderzoek worden uitgevoerd die de mogelijke gevolgen van de lang termijn reductie in BACE1 hoeveelheden op de functie van zenuwcellen en glia cellen vast te stellen. Evenzo moeten de implicaties van BACE1 reductie in termen van hersenfunctie bij AD muizen worden bepaald door een nauwkeurigere evaluatie van meer cognitieve vaardigheden die

zijn aangedaan in AD. Tenslotte is er nog steeds een grote beperking in de beschikbaarheid van een betrouwbaar afleveringssysteem dat in staat is om de bloed-hersenbarrière te doorkruisen om een systemische behandeling te bewerkstelligen en daarmee een effectieve therapie aan de patiënt te kunnen leveren.

General discussion

BACE1 gene silencing reduces tauopathy by regulating the inflammatory response.

Neurodegeneration is considered the main public health problem for the XXI century, and among neurodegenerative diseases, Alzheimer's disease (AD) is the most abundant and best studied. However, despite intense scientific research, at the moment there is no effective and definitive therapeutic strategy for its treatment (Mandler et al. 2014; Heneka et al. 2015c). In this thesis, we show a novel approach, employing RNA interference (RNAi) as a tool for the treatment of neurodegeneration. As a therapeutic target, we selected the β -site amyloid precursor protein cleaving enzyme 1 (BACE1), a family member of transmembrane aspartic proteases. BACE1 exhibits all the functional properties of beta-secretase, and as it is the key enzyme that initiates the formation of A β , it is an attractive drug target for AD.

BACE1 is a potential drug target for AD. There were and are several clinical trials with multiple BACE1 inhibitors focused on small molecules or anti-A β immunotherapy with the main objective to inhibit BACE1 activity to clean the brain of aggregated A β , respectively. However, these trials have been challenging and several have failed during clinical implementation (Vassar 2014; Koelsch 2017a). Last year, three clinical trials with BACE1 inhibitors were stopped due to collateral adverse events due to a specific effect of these inhibitors. Merck ended phase III studies of Verubecestat and APECS, as well as AstraZeneca/Eli Lilly with phase III trial of Lanabecestat, mainly because all of those studies failed to reduce cognitive decline in AD patients. On the other side BACE1 knockout studies in mice failed as well because of adverse side effects, such as demyelination and reduced muscle spindles, which is related with the physiological function of BACE1. Together these results showed that effective therapies require an exquisitely safe and specific therapeutic agent (Vassar 2014; Koelsch 2017a). Problems that can potentially be overcome with the specific BACE1 knockdown by shRNA-BACE1 (BACE1miR). In chapter 2, our data showed, for the first time, that BACE1 targeting-induced neuroprotection, cognitive recovery, reduces soluble hyperphosphorylated tau, modulating proinflammatory pathways through lipid metabolism modification in the hippocampi of aged 3xTg-AD mice.

Another possible problem to create successful therapies is the time factor, since, the failure to be efficient in stopping or preventing cognitive decline might be related with the starting time of treatment, if the AD stage is too advanced, the A β and tau accumulation and neurodegeneration might be irreversible. However, our results showed that BACE1 knockdown by shBACE1miR is effective at different stages of AD in mice. The long-term therapy started in young mice (6 months) when proteinopathies and cognitive decline are in an early stage and these mice were evaluated 12 months later, and on the other hand, we included a short-term therapy group where BACE12 was knocked down in 12 months old mice, when the proteinopathies and cognitive decline are in a more advanced stage, and this group was

evaluated 6 months later. In both groups, a neuroprotective effect including cognitive recovery and decrease of tau aggregation by BACE1-RNAi was observed.

Previously, Piedrahita et al, validated that silencing of BACE1 in 3xTg-AD mice reduced the CTF (carboxy-terminal fragment) and 1–42 A β and hyperphosphorylated tau levels in the hippocampus by autophagy. However, this effect was stopped by the autophagy inhibitor 3MA, which blocks PE lipidation, avoiding autophagosome formation (Piedrahita et al. 2016). This result opened new questions about the role of lipids in AD, therefore we performed a deep lipidomic analysis of hippocampi from AD model mice to evaluate if BACE1 silencing had an effect on brain lipid composition. Interestingly our results showed different lipid compositions between control and AD model mice. AD mice had significantly higher concentrations of unsaturated lipids which is widely described as a risk factor on AD, since, these lipids reduce the cell membrane fluidity, increasing activity of membrane proteins such as BACE1 favoring the processing of APP and subsequently A β production (Hirsch-Reinshagen, Burgess, and Wellington 2009).

Interestingly, the short- and long-term treatment with shBACE1miR changed the profiles of mainly phospholipid (PL) levels, by regulation of PL levels and specific subspecies composition of PE plasmalogens as LPE and ePE, which were mainly composed of unsaturated fatty acid chains in AD brains. BACE1 silencing balanced their levels to control levels, in agreement with the findings of Piedrahita et al, where PE played an important role to decrease the protein accumulation by the silencing of BACE1 (D Piedrahita, Castro-Alvarez, Boudreau, Villegas-Lanau, Kosik, Gallego-Gomez, and Cardona-Gomez 2015). In addition, those findings were confirmed by the lipid profile evaluation of two types of the human AD (SAD and FAD), where the levels of phospholipids in subspecies PE and PG, both mainly composed by unsaturated fatty acids, increased. In addition, we detected that PE-Cer increased in SAD group, suggesting PE-Cer as a marker of chronic impairment in SAD patients. Different studies reported alterations of PL brain levels in neurodegenerative diseases, PL species such as PI (Whiley et al. 2014), PC (Araki and Wurtman 1997; Whiley et al. 2014) PE and its derivatives, such as LPE and ePE (Nesic et al. 2012), PS (Shea 1997); and, PE-Cer (Chan et al. 2012) seeming to have multifactorial origins, including the hyperactivation of phospholipases, mitochondrial stress, peroxisomal dysfunction and irregular FA composition of PLs. Accordingly, it has been shown that phospholipids participate in numerous cellular functions such as inflammatory processes, intracellular signaling, trafficking, cell growth, survival and, pro-apoptotic pathways. Recently, a specific receptor for phosphatidyl serine was reported which is related to the pathologic over activation of microglia (Krasemann et al. 2017).

The role of PL in chronic inflammation has been extensively investigating and is mainly associated with immune-dependent and vascular-related chronic inflammatory diseases, including diabetes, obesity and neurodegenerative disorders such as AD (Dorninger et al. 2015). This link is mediated by the fatty acid composition of PL as PE, since it has the ω -6 PUFA arachidonic acid (AA) as their common biosynthetic precursor which is released from mem-

brane lipids by PLA2 and used as a substrate for three different enzymes: COX 1 and 2, Lipoxigenases and epoxygenases. In our study, we reported increased levels of PLA2 and COX2 in AD brains which is tightly linked with the production of prostaglandins (PG)(Abdullah et al. 2017a). Indeed, PGs are associated to the inflammation process, mostly with the transition from acute to chronic inflammation; in general, through activation of the cytokine cascade, amplification of innate immune response, exacerbated recruitment of immune cells (microglia) and an increase of pro-inflammatory genes induced by cytokines (Zárate et al. 2017b). Our findings showed that silencing of BACE1 decreased the concentration of AA together with a reduction of PLA2 and COX2 levels supporting these associations between specific PLs, inflammatory processes and neurodegenerative diseases as AD.

We also observed modifications in essential fatty acids such as Docosahexaenoic acid (DHA), an omega-3 fatty acid essential for normal brain growth and cognitive function, which acts as a neurotrophic factor and modulates synaptic activity (Kuratko et al. 2013). DHA is highly enriched in brain PLs, which is consistent with its importance in brain function (Sherratt et al. 2016). The present work indicates that a misbalanced fatty acid composition in AD also includes DHA. The DHA levels increased, in the form of PE and LPE, while in the form of ePE, DHA levels decreased, those changes were observed in AD brains. Interestingly, DHA levels were restored to basal levels by BACE1 gene therapy in AD model mice. Pro-survival properties of DHA by down-regulation of the chronic inflammatory response under neurotoxic conditions was demonstrated in cellular and animal models (Zárate et al. 2017a; Heras-Sandoval, Pedraza-Chaverri, and Pérez-Rojas 2016b). This effect is associated with two physiological properties in animal and in vitro models: DHA increases membrane lipid fluidity due to its chemical structure (Sherratt et al. 2016), which reduces the overactivity of membrane enzymes such as BACE1, and on the other hand, DHA decreases the production of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6, inhibiting the activity of mitogen-activated protein kinases (MAPK), which promotes the activation of enzymes (desaturases) to regulate fatty acid storage and glucose metabolism in neurons and glia cells (Heras-Sandoval, Pedraza-Chaverri, and Pérez-Rojas 2016a). These results together suggest that PL play an important role in AD progress, highlighting that specific lipid species as PE could be promising molecular targets either as a biomarker or therapy in neurodegenerative processes. (Figure 1).

Saturation balance of fatty acids play key role in the control of AD inflammation

Phospholipids are the major component of cell membranes, source of energy and, key pathophysiological mediators of several cell signaling pathways. In fact, they are “bioactive lipids” with a pivotal role in immune regulation, inflammation, and cell homeostasis. Lipids can be classified according to the number of double bonds presents in their fatty acid carbon chain, into three lipid categories: unsaturated, monounsaturated and polyunsaturated, which determines their specific role in cell signaling (Fahy et al. 2005). The bioactive lipids

mostly are composed of mono and polyunsaturated fatty acids which are processed by catalytic enzymes such as stearoyl-CoA desaturase (SCD) and fatty acid desaturases (FADS) respectively (Tosi et al. 2014).

The diverse roles of both enzymes have an impact on cell physiology, and their unbalanced activity leads to pro-inflammatory disorders such as neurodegenerative diseases. However, little is known about the interaction between those lipid enzymes and other proteins such as cPLA2 and BACE1 involved in the neuro-inflammation process in AD. Therefore, in chapter 4, considering cPLA2 is the main enzyme involved in the production of plasmalogens (Makide et al. 2009) and that SCD1 and FADS6 are responsible for fatty acid unsaturation; we analyzed the role of cPLA2, COX2 and desaturases (SCD1, FADS6) in BACE1-KD-induced in neuron cultures. We observed protective action and a reduction in tau hyperphosphorylation in a model of glutamate excitotoxicity. Moreover, a synergic neuroprotective effect of BACE1 silencing was observed when simultaneously SCD1 and FADS6 were overexpressed through downregulation of pro-inflammatory cascade (AA, cPLA2, and COX2). Which was reverted when SCD1 and FADS6 were silencing together with BACE1.

SCD1 is the rate-limiting enzyme catalyzing the monounsaturated fatty acids (MUFA), mainly oleate (18:1) and Palmitoleate (16:1), which are the major FA of membrane conforming PL and TG (Lavandera et al. 2017). The conversion-ratio of saturated to MUFA determine the PL composition, consequently, the alteration of this ratio affects cell homeostasis triggering a variety of diseases including cardiovascular diseases, obesity, diabetes, cancer, and neurological disorders (Ntambi and Miyazaki 2004). Our results showed, that SCD1 expression improved the neuroprotective effect of BACE1 silencing, which is supported by studies where SCD1 significantly inhibited caspase activation, ceramide synthesis, apoptotic cell death and mitochondrial reactive oxygen species (ROS) generation by cardiac myocytes (Matsui et al. 2012). In addition, oleic acid synthesized by SCD1 mediates neuronal growth, migration, and arrangement of synapses (Polo-Hernandez et al. 2014). Although, in our experiments, overexpression of SCD1 alone did restore cellular homeostasis under glutamate excitotoxicity. However, its synergistic effect with the BACE1-KD-mediated neuroprotection clearly suggested an important role of SCD1 in neurons. These data suggest that the activity of SCD1 increased MUFA levels and reduced the high concentration of saturated FA and their adverse catabolism (pro-inflammatory cascade), and eventually preventing glutamate excitotoxicity.

Polyunsaturated fatty acids (PUFAs) are considered to be critical molecules to regulate cell homeostasis, brain development, and cognition, as well as several neurological diseases. Their metabolism is regulated by numerous fatty acid elongases and desaturases play key roles in synthesizing PUFAs. They are precursors of eicosanoids which modulate immune responses by pro and anti-inflammatory pathways; DHA (22:6) is the most described precursor of anti-inflammatory FA (Lavandera et al. 2017). Thereby, it has been established that DHA and AA levels are critical for a normal structure and function of the brain. DHA

is synthesized by FADS6, which introduce a double bond at carbon position 6 and work cooperatively with elongases to extend the carbon chain. In our study the role of FADS6 was assessed on primary neuron cultures, we observed that the silencing of FADS6 diminished the neuroprotective effect of BACE1 silencing, which was reverted by FADS6 overexpression through downregulation of pro-inflammatory proteins such as cPLA2 and COX2.

Deletion of FADS6 gene expression abolishes the initial step in the enzymatic cascade of PUFA synthesis in mouse (Stoffel et al. 2008), resulting in low concentrations of DHA, and high concentrations of saturated FA (palmitic and stearic acids) and an increase in AA, which together are risk factors for AD. In contrast, the expression and activity of FADS6 is essential to maintain tissue homeostasis through the DHA synthesis, promoting pro-survival molecular pathways, such as increased expression of extracellular signal-regulated protein kinase 1 and 2 (ERK1/2), brain-derived neurotrophic factor (BDNF), and anti-apoptotic Bcl-2, reduced expression of cPLA and COX2 proteins in cultured cortical neurons (Figuerola et al. 2012).

FADS6 activity promotes neuronal activity and prevents memory deficits (Bazinet and Laye 2014). Levels of neuronal activity indicators such as c-Fos are decreased in neurodegenerative diseases, which alters the action potentials and the synaptic connections. Interestingly, the synthesis of DHA restored c-Fos expression and enhanced neuronal activity in the hippocampus leading to the recovery of memory deficits in mice (Hussain et al. 2013). FADS6 is also important for the regulation of proinflammatory proteins, such as cPLA and COX2. These results are supported by several gene expression studies performed on mouse brain deficient for DHA that revealed a loss of synaptic proteins. In AD, the proteinopathies induce apoptosis through multiples phosphorylation of kinases such as c-Jun N-terminal kinase (JNK) and cyclin depend on kinase 5 (CDK5) and, consequently, triggering the phosphorylation of tau protein (Yarza et al. 2016; Bhounsule et al. 2017). The induced expression of FADS6 in 3xTg-AD mice prevents the hyperphosphorylation of kinases and ultimately protected neurons from apoptosis. Since lower levels of hyperphosphorylated tau and improved brain function were observed in in vitro and in vivo experiments due to the enhanced endogenous production of DHA (Lebbadi et al. 2011), which is consistent with our results. Finally, it is noteworthy that the beneficial actions of BACE1 silencing and FADS6 overexpression on cortical neurons cultures might be associated with an increase in the expression of transcription factors, which are involved on differentiation and survival pathways of neurons. (Figure 1).

DMSO inhibit the inflammatory response on microglia cells

Microglia has been widely described as a potential source and target of important neuroprotective as well as neurotoxic factors, since their activation may keep CNS homeostasis. However, their uncontrolled activation contributes to neurotoxicity in brain disorders (Hickman,

Allison, and El Khoury 2008c). The over-activation of microglia triggered the production of inflammatory mediators such as cytokines (IL-1 β and IL-6), prostaglandins (PGE), and nitric oxide (NO) through enzymatic activity of COX2 and iNOS respectively. Furthermore, nuclear factors such as NF- κ B is also overexpressed (Shabab et al. 2017). All these molecules are involved with neuroinflammation in several neurological diseases. Thus, intervention with microglia over activation could be a therapeutic target against multiple neuroinflammatory diseases.

IL-1 β and IL-6 are two main pro-inflammatory cytokines overproduced by activated microglia during the course of neuroinflammation (Zheng, Zhou, and Wang 2016a). In the brain, several stimuli, such as bacterial infections and LPS, β -amyloid and acute traumatic injuries have been shown to increase IL-1 β and IL-6 levels (W. Y. Wang et al. 2015). Which is tightly related to detrimental effects such as mitochondrial dysfunction, oxidative stress and apoptosis pathways in neuronal cells. This study investigates whether DMSO inhibits LPS-induced production of inflammatory cytokines in primary microglia and BV2 cells, a microglia cell line. Our data indicate that DMSO inhibited LPS-induced mRNA expression and protein secretion of IL-1 β and IL-6 in activated primary microglia and BV-2 cells in a dose-dependent manner. These results indicate that DMSO is able to modulate the expression of inflammatory cytokines in over activated microglia. After LPS challenge, microglia increased COX-2 levels, which is the main enzyme for the production of PGE during inflammation, contributing to the development of chronic neurological disorders such as stroke, multiple sclerosis, Alzheimer's disease and Parkinson's disease (Ferrer et al. 2018; Phillis, Horrocks, and Farooqui 2006). In this study, DMSO inhibited LPS-induced PGE2 production by reducing the COX-2 mRNA expression in activated primary microglia and BV-2 cells in a concentration-dependent manner. This result indicates that the inhibition of PGE2 production by DMSO might be due to the suppression of COX2 up-regulation in activated microglia.

Another important pro-inflammatory molecule released by activated microglia is NO, which is known (Hickman, Allison, and El Khoury 2008a; Jay, von Saucken, and Landreth 2017) to be involved in the neuroinflammation processes (Gu, Nakamura, and Lipton 2010). NO is synthesized by the iNOS enzyme, and their levels and activity are significantly induced in activated microglia after the exposure to various stimulators such as LPS (Gu, Nakamura, and Lipton 2010; Saini and Singh 2018). For that reason, therapeutic agents targeted to inhibit the iNOS expression are potentially beneficial in the treatment of inflammatory diseases, since, down-regulation of NO production reduced the over activated microglia phenotype. Our results suggest DMSO decreased LPS-induced NO production by down-regulation of iNOS mRNA expression in microglia. That result indicates DMSO has anti-inflammatory properties through reducing NO production by microglial cells.

Finally, we evaluated the mRNA expression of NF- κ B which is a key regulator of inflammatory response in microglia cells, since, it is a pleiotropic regulator of several genes involved in the synthesis of inflammatory cytokines (IL-1 β and IL-6) and enzymes related to the inflammatory process (COX2 and iNOS) during microglia responses to activating LPS stimuli (Kopitar-Jerala 2015). Hence, this transcription factor plays an important role in several pathologic conditions, and it makes NF- κ B a potential target in multiples diseases (Saini and Singh 2018). The molecular activation of NF- κ B starts at the cytoplasm with the activation of I κ B kinase (IKK) and followed by I- κ B α phosphorylation allowing NF- κ B translocation and activation into the nucleus (Sarnico et al. 2009). Thus, activation is crucial to triggering the expression of pro-inflammatory genes in microglia cells. We showed that DMSO at 0.8% may prevented LPS-induced activation of NF- κ B. Therefore, inhibition of NF- κ B signaling pathways in microglia by DMSO results in the down-regulation of inflammatory mediators, which result in a strong anti-inflammatory effect on stimuli-LPS microglia.

In this, study, we observed that an in vitro LPS challenge on microglia cells DMSO exert an anti-inflammatory effect at low concentrations (0,8% v/v). DMSO down-regulated the mRNA and protein secretion of proinflammatory cytokines such as IL-6 and IL-1 β . In addition, our data showed that DMSO decreased the mRNA expression of COX2 and iNOS enzymes, which have been associated with inflammatory pathways in microglia. Furthermore, DMSO treatment was able to reduce the mRNA expression of NF- κ B a central regulator of microglial responses to activating stimuli as LPS. This results together indicate for the first time the fact that DMSO has anti-inflammatory properties on microglia and could provide a beneficial effect against inflammatory brain disorders.

Conclusions and perspectives

The data presented in this thesis show that the silencing of hippocampal BACE1 by RNAi ameliorates AD degeneration in 3xTg-AD mice. This work indicates that BACE1 is involved in the FA composition of PE-derivates, since BACE1 silencing in 3xTg-AD mice restores the levels of lipid subspecies that are altered in AD mice, which might be associated with learning and memory improvement. This alteration in PE composition was also observed in human EOAD and LOAD CNS tissue. The positive effects of BACE1 silencing depended on the activity of desaturases enzymes to reduce the proinflammatory signals, suggesting lipid metabolism is a key regulator of the neurodegenerative process. Showing that the progression of AD depends on beta-amyloid production but at the same time altered lipid metabolism and inflammatory response from CNS. These observations illustrate that there is a great need for new well-designed research on combination therapies against AD.

The possibility of ameliorating tau and A β pathology poses potentially enormous benefits for people affected with AD, hopefully offering a reversion of the pathological process

Figure 1. BACE1 silencing has an anti-inflammatory effect through FA metabolism regulation. Following the red arrow: In AD the levels of BACE1 enzyme are elevated which increases the processing of APP by this enzyme. Furthermore, the levels of SFA at the cell membrane system also are increased, which favors the activity and stability of BACE1. The increased levels of SFA change the rate balance between saturated and unsaturated fatty acids (FA), due to both low activity levels of desaturated FA enzymes such as SCD1 and FADS6, and high levels and activity of cytoplasmic enzymes such as cPLA2 and COX2, which synthesize AA and pro-inflammatory mediators, respectively. Finally, all those events together trigger aggregation of Tau protein, diminished beta oxidation, and excitotoxicity in AD brains. Following the blue arrow: in the nucleus, shBACE1miR is processed by RNAi maturation enzymes to induce the silencing of BACE1 gene expression. Consequently, the levels of BACE1 enzyme and the A β and tau aggregation process decreased, as well as the levels of SFA, due the activity of the desaturases SCD1 and FADS6, which led to up-regulation of unsaturated FA levels and plasmalogens derivatives from the FA phosphatidylcholine (ePE and LPE). These plasmalogens are mainly composed by DHA, which is not a substrate of COX2 protein, suggesting the production of pro-inflammatory mediators from COX2 activity are reduced as a consequence of BACE1 silencing. All these findings together suggest that BACE1 silencing restore the cell homeostasis by lipid metabolism and inflammatory response down-regulation. Source Graphic templates (CREATIVE COMMONS LICENCE and Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License n.d.).

(Figure 1). Nonetheless, given the functional importance of BACE1 in the adult brain, additional studies should be carried out analyzing the possible consequences of its long-term down-regulation on neuronal and glia function and eventually compare these findings with the already demonstrated benefits of pharmacological inhibitors. Likewise, the implications of silencing in terms of brain function in wild type and 3xTg-AD mice should be determined by evaluation of more cognitive skills implied in AD. Finally, there is still great limitation in the availability of a reliable delivery system that is able to cross the blood brain barrier in order to achieve a systemic treatment of neurotropic action and, therefore, deliver an effective therapy to the patient with neurodegeneration.

References

- Abdullah, Laila, James E Evans, Tanja Emmerich, Gogce Crynen, Ben Shackleton, Andrew P Keegan, Cheryl Luis, et al. 2017a. "APOE E4 Specific Imbalance of Arachidonic Acid and Docosahexaenoic Acid in Serum Phospholipids Identifies Individuals with Preclinical Mild Cognitive Impairment/Alzheimer's Disease." *Aging* 9 (3): 964–85. <https://doi.org/10.18632/aging.101203>.
- . 2017b. "APOE E4 Specific Imbalance of Arachidonic Acid and Docosahexaenoic Acid in Serum Phospholipids Identifies Individuals with Preclinical Mild Cognitive Impairment/Alzheimer's Disease." *Aging* 9 (3): 964–85. <https://doi.org/10.18632/aging.101203>.
- Abreu Costa, Lucas de, Marcelo Henrique Fernandes Ottoni, Michaelle dos Santos, Agnes Meireles, Valéria Gomes de Almeida, Wagner de Fátima Pereira, Bethânia Alves de Avelar-Freitas, and Gustavo Eustáquio Alvim Brito-Melo. 2017. "Dimethyl Sulfoxide (DMSO) Decreases Cell Proliferation and TNF- α , IFN- γ , and IL-2 Cytokines Production in Cultures of Peripheral Blood Lymphocytes." *Molecules* 22 (11): 1789. <https://doi.org/10.3390/molecules22111789>.
- Ahn, Huijeong, Jeeyoung Kim, Eui-Bae Jeung, and Geun-Shik Lee. 2014. "Dimethyl Sulfoxide Inhibits NLRP3 Inflammasome Activation." *Immunobiology* 219 (4): 315–22. <https://doi.org/10.1016/j.imbio.2013.11.003>.
- Ajith, Thekkuttuparambil Ananthanarayanan. 2018. "A Recent Update on the Effects of Omega-3 Fatty Acids in Alzheimer's Disease." *Current Clinical Pharmacology* 13 (August). <https://doi.org/10.2174/1574884713666180807145648>.
- Akiyama, H, S Barger, S Barnum, B Bradt, J Bauer, G M Cole, N R Cooper, et al. 2000. "Inflammation and Alzheimer's Disease." *Neurobiol Aging* 21 (3): 383–421. <http://dx.doi.org/>.
- Alonso, R, N Mata, and P Mata. 2005. "Benefits and Risks Assessment of Simvastatin in Familial Hypercholesterolaemia." *Expert Opin Drug Saf* 4 (2): 171–81. <http://www.ncbi.nlm.nih.gov/pubmed/15794711>.
- Araki, W, and R J Wurtman. 1997. "Control of Membrane Phosphatidylcholine Biosynthesis by Diacylglycerol Levels in Neuronal Cells Undergoing Neurite Outgrowth." *Proc Natl Acad Sci U S A* 94 (22): 11946–50. <http://www.ncbi.nlm.nih.gov/pubmed/9342342>.
- Arsenault, D, C Julien, C Tremblay, and F Calon. 2011. "DHA Improves Cognition and Prevents Dysfunction of Entorhinal Cortex Neurons in 3xTg-AD Mice." *PLoS One* 6 (2): e17397. <https://doi.org/10.1371/journal.pone.0017397>.
- Astarita, G, K M Jung, V Vasilevko, N V Dipatrizio, S K Martin, D H Cribbs, E Head, C W Cotman, and D Piomelli. 2011. "Elevated Stearoyl-CoA Desaturase in Brains of Patients with Alzheimer's Disease." *PLoS One* 6 (10): e24777. <https://doi.org/10.1371/journal.pone.0024777>.
- Atagi, Yuka, Chia-Chen Liu, Meghan M. Painter, Xiao-Fen Chen, Christophe Verbeeck, Honghua Zheng, Xia Li, et al. 2015. "Apolipoprotein E Is a Ligand for Triggering Receptor Expressed on Myeloid Cells 2 (TREM2)." *Journal of Biological Chemistry* 290 (43): 26043–50. <https://doi.org/10.1074/jbc.M115.679043>.
- Bamberger, Maria E, Meera E Harris, Douglas R McDonald, Jens Husemann, and Gary E Landreth. 2003. "A Cell Surface Receptor Complex for Fibrillar Beta-Amyloid Mediates Microglial Activation." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 23 (7): 2665–74. <http://www.ncbi.nlm.nih.gov/pubmed/12684452>.
- Barage, Sagar H., and Kailas D. Sonawane. 2015. "Amyloid Cascade Hypothesis: Pathogenesis and Therapeutic Strategies in Alzheimer's Disease." *Neuropeptides* 52 (August): 1–18. <https://doi.org/10.1016/j.npep.2015.06.008>.
- Barao, S, D Moechars, S F Lichtenthaler, and B De Strooper. 2016. "BACE1 Physiological Functions May Limit Its Use as Therapeutic Target for Alzheimer's Disease." *Trends Neurosci* 39 (3): 158–69. <https://doi.org/10.1016/j.tins.2016.01.003>.
- Bate, Clive. 2017. "Breaking the Cycle, Cholesterol Cycling, and Synapse Damage in Response to Amyloid- β ." *Journal of Experimental Neuroscience* 11. <https://doi.org/10.1177/1179069517733096>.
- Bazinet, R P, and S Laye. 2014. "Polyunsaturated Fatty Acids and Their Metabolites in Brain Function and Disease." *Nat Rev Neurosci* 15 (12): 771–85. <https://doi.org/10.1038/nrn3820>.
- Ben Halima, Saoussen, Sabyashachi Mishra, K. Muruga Poopathi Raja, Michael Willem, Antonio Baici, Kai Simons, Oliver Brüstle, et al. 2016. "Specific Inhibition of β -Secretase Processing of the Alzheimer Disease Amyloid Precursor Protein." *Cell Reports* 14 (9): 2127–41. <https://doi.org/10.1016/j.celrep.2016.01.076>.
- Bharadwaj, Prashant, Tanya Solomon, Chris J. Malajczuk, Ricardo L. Mancera, Mark Howard, Damien W.M. Arrigan, Philip Newsholme, and Ralph N. Martins. 2018. "Role of the Cell Membrane Interface in Modulating Production and Uptake of Alzheimer's Beta Amyloid Protein." *Biochimica et Biophysica Acta (BBA) - Biomembranes*, March. <https://doi.org/10.1016/j.bbame.2018.03.015>.
- Bhounsule, Anisha S., Lokesh Kumar Bhatt, Kedar S. Prabhavalkar, and Manisha Oza. 2017. "Cyclin Dependent Kinase 5: A Novel Avenue for Alzheimer's Disease." *Brain Research Bulletin* 132 (June): 28–38. <https://doi.org/10.1016/j.brainresbull.2017.05.006>.
- Bignante, E A, F Heredia, G Morfini, and A Lorenzo. 2013. "Amyloid β Precursor Protein as a Molecular Target for Amyloid β -Induced Neuronal Degeneration in Alzheimer's Disease." *Neurobiol Aging* 34 (11): 2525–37. <https://doi.org/10.1016/j.neurobiolaging.2013.04.021>.
- Bloom, George S. 2014a. "Amyloid- β and Tau." *JAMA Neurology* 71 (4): 505. <https://doi.org/10.1001/jama-neurol.2013.5847>.
- . 2014b. "Amyloid- β and Tau." *JAMA Neurology* 71 (4): 505. <https://doi.org/10.1001/jama-neurol.2013.5847>.
- Botteri, Gaia, Laia Salvadó, Anna Gumà, D. Lee Hamilton, Paul J. Meakin, Gemma Montagut, Michael L.J. Ashford, et al. 2018. "The BACE1 Product SAPP β Induces ER Stress and Inflammation and Impairs Insulin Signaling." *Metabolism*, March. <https://doi.org/10.1016/j.metabol.2018.03.005>.
- Braak, H, and E Braak. 1997. "Staging of Alzheimer-Related Cortical Destruction." *Int Psychogeriatr* 9 Suppl 1: 257–72. <http://dx.doi.org/>.
- Brayton, C F. 1986. "Dimethyl Sulfoxide (DMSO): A Review." *The Cornell Veterinarian* 76 (1): 61–90. <http://www.ncbi.nlm.nih.gov/pubmed/3510103>.
- Bryleva, E Y, M A Rogers, C C Chang, F Buen, B T Harris, E Rousselet, N G Seidah, et al. 2010. "ACAT1 Gene Ablation Increases 24(S)-Hydroxycholesterol Content in the Brain and Ameliorates Amyloid Pathology in Mice with AD." *Proc Natl Acad Sci U S A* 107 (7): 3081–86. <https://doi.org/10.1073/pnas.0913828107>.
- Bu, G. 2009. "Apolipoprotein E and Its Receptors in Alzheimer's Disease: Pathways, Pathogenesis and Therapy." *Nat Rev Neurosci* 10 (5): 333–44. <https://doi.org/10.1038/nrn2620>.
- Buckley, Christopher D., Derek W. Gilroy, and Charles N. Serhan. 2014. "Proresolving Lipid Mediators and Mechanisms in the Resolution of Acute Inflammation." *Immunity* 40 (3): 315–27. <https://doi.org/10.1016/j.immuni.2014.02.009>.
- Butovsky, Oleg, and Howard L. Weiner. 2018. "Microglial Signatures and Their Role in Health and Disease." *Nature Reviews Neuroscience* 19 (10): 622–35. <https://doi.org/10.1038/s41583-018-0057-5>.
- Cai, D, W J Netzer, M Zhong, Y Lin, G Du, M Frohman, D A Foster, et al. 2006. "Presenilin-1 Uses Phospholipase D1 as a Negative Regulator of Beta-Amyloid Formation." *Proc Natl Acad Sci U S A* 103 (6): 1941–46. <https://doi.org/10.1073/pnas.0510708103>.
- Calder, P C. 2012. "Long-Chain Fatty Acids and Inflammation." *Proc Nutr Soc* 71 (2): 284–89. <https://doi.org/10.1017/S0029665112000067>.
- Calignon, A de, L M Fox, R Pitstick, G A Carlson, B J Bacskai, T L Spires-Jones, and B T Hyman. 2010. "Caspase Activation Precedes and Leads to Tangles." *Nature* 464 (7292): 1201–4. <https://doi.org/10.1038/nature08890>.
- Castellano, J. M., J. Kim, F. R. Stewart, H. Jiang, R. B. DeMattos, B. W. Patterson, A. M. Fagan, et al. 2011. "Human ApoE Isoforms Differentially Regulate Brain Amyloid-Peptide Clearance." *Science Translational Medicine* 3 (89): 89ra57–89ra57. <https://doi.org/10.1126/scitranslmed.3002156>.
- Cebers, Gvido, Robert C. Alexander, Samantha Budd Haeberlein, David Han, Ronald Goldwater, Larry Ereshefsky, Tina Olsson, et al. 2016. "AZD3293: Pharmacokinetic and Pharmacodynamic Effects in Healthy Subjects and Patients with Alzheimer's Disease." Edited by M. Paul Murphy. *Journal of Alzheimer's Disease* 55 (3): 1039–53. <https://doi.org/10.3233/JAD-160701>.
- Chakraborty, Chiranjib, Ashish Ranjan Sharma, Garima Sharma, C. George Priya Doss, and Sang-Soo Lee. 2017. "Therapeutic miRNA and siRNA: Moving from Bench to Clinic as Next Generation Medicine." *Molecular Therapy - Nucleic Acids* 8 (September): 132–43. <https://doi.org/10.1016/j.omtn.2017.06.005>.
- Chan, R B, T G Oliveira, E P Cortes, L S Honig, K E Duff, S A Small, M R Wenk, G Shui, and G Di Paolo. 2012. "Comparative Lipidomic Analysis of Mouse and Human Brain with Alzheimer Disease." *J Biol Chem* 287 (4): 2678–88. <https://doi.org/10.1074/jbc.M111.274142>.
- Chang, T Y, C C Chang, N Ohgami, and Y Yamauchi. 2006. "Cholesterol Sensing, Trafficking, and Esterification." *Annu Rev Cell Dev Biol* 22: 129–57. <https://doi.org/10.1146/annurev.cellbio.22.010305.104656>.
- Chen, C H, W Zhou, S Liu, Y Deng, F Cai, M Tone, Y Tone, Y Tong, and W Song. 2012. "Increased NF-KappaB Sig-

nalling up-Regulates BACE1 Expression and Its Therapeutic Potential in Alzheimer's Disease." *Int J Neuropsychopharmacol* 15 (1): 77–90. <https://doi.org/10.1017/s1461145711000149>.

Chen, Chuck T., Joshua T. Green, Sarah K. Orr, and Richard P. Bazinet. 2008. "Regulation of Brain Polyunsaturated Fatty Acid Uptake and Turnover." *Prostaglandins, Leukotrienes and Essential Fatty Acids* 79 (3–5): 85–91. <https://doi.org/10.1016/j.plefa.2008.09.003>.

Choi, Gee Euhn, Sei-Jung Lee, Hyun Jik Lee, So Hee Ko, Chang Woo Chae, and Ho Jae Han. 2017. "Membrane-Associated Effects of Glucocorticoid on BACE1 Upregulation and A β Generation: Involvement of Lipid Raft-Mediated CREB Activation." *The Journal of Neuroscience* 37 (35): 0074–17. <https://doi.org/10.1523/JNEUROSCI.0074-17.2017>.

Colucci, Mariantonella, Francesco Maione, Maria Carmela Bonito, Alessandro Piscopo, Amalia Di Giannuario, and Stefano Pieretti. 2008. "New Insights of Dimethyl Sulphoxide Effects (DMSO) on Experimental in Vivo Models of Nociception and Inflammation." *Pharmacological Research* 57 (6): 419–25. <https://doi.org/10.1016/J.PHRS.2008.04.004>.

Congdon, Erin E., and Einar M. Sigurdsson. 2018a. "Tau-Targeting Therapies for Alzheimer Disease." *Nature Reviews Neurology*, June. <https://doi.org/10.1038/s41582-018-0013-z>.

———. 2018b. "Tau-Targeting Therapies for Alzheimer Disease." *Nature Reviews Neurology*, June. <https://doi.org/10.1038/s41582-018-0013-z>.

CREATIVE COMMONS LICENCE, and Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License. n.d. "SMART - Servier Medical ART - 3000 Free Medical Images." Accessed October 1, 2018. <https://smart.servier.com/>.

Cuervo, A M, E S Wong, and M Martinez-Vicente. 2010. "Protein Degradation, Aggregation, and Misfolding." *Mov Disord* 25 Suppl 1: S49–54. <https://doi.org/10.1002/mds.22718>.

Cunnane, S C, R Chouinard-Watkins, C A Castellano, and P Barberger-Gateau. 2013. "Docosahexaenoic Acid Homeostasis, Brain Aging and Alzheimer's Disease: Can We Reconcile the Evidence?" *Prostaglandins Leukot Essent Fatty Acids* 88 (1): 61–70. <https://doi.org/10.1016/j.plefa.2012.04.006>.

Desbène, C, C Malaplate-Armand, I Youssef, P Garcia, C Stenger, M Sauvée, N Fischer, et al. 2012. "Critical Role of CPLA2 in A β Oligomer-Induced Neurodegen-

eration and Memory Deficit." *Neurobiol Aging* 33 (6): 1123.e17–29. <https://doi.org/10.1016/j.neurobiolaging.2011.11.008>.

Díaz, Mario, Noemí Fabelo, Virginia Martín, Isidre Ferrer, Tomás Gómez, and Raquel Marín. 2014. "Biophysical Alterations in Lipid Rafts from Human Cerebral Cortex Associate with Increased BACE1/A β PP Interaction in Early Stages of Alzheimer's Disease." *Journal of Alzheimer's Disease* 43 (4): 1185–98. <https://doi.org/10.3233/JAD-141146>.

Dinkins, Michael B., Guanghu Wang, and Erhard Bieberich. 2017. "Sphingolipid-Enriched Extracellular Vesicles and Alzheimer's Disease: A Decade of Research." Edited by Michelle Mielke and Pilar Martinez. *Journal of Alzheimer's Disease* 60 (3): 757–68. <https://doi.org/10.3233/JAD-160567>.

Dorninger, F, A Brodde, N E Braverman, A B Moser, W W Just, S Forss-Petter, B Brügger, and J Berger. 2015. "Homeostasis of Phospholipids - The Level of Phosphatidylethanolamine Tightly Adapts to Changes in Ethanolamine Plasmalogens." *Biochim Biophys Acta* 1851 (2): 117–28. <https://doi.org/10.1016/j.bbailp.2014.11.005>.

Doroudgar, M, and M Lafleur. 2017. "Ceramide-C16 Is a Versatile Modulator of Phosphatidylethanolamine Polymorphism." *Biophys J* 112 (11): 2357–66. <https://doi.org/10.1016/j.bpj.2017.04.047>.

Duthey, Béatrice. 2013. "Priority Medicines for Europe and the World "A Public Health Approach to Innovation Alzheimer Disease and Other Dementias." http://www.who.int/medicines/areas/priority_medicines/BP6_11Alzheimer.pdf.

Egan, Michael F., James Kost, Pierre N. Tariot, Paul S. Aisen, Jeffrey L. Cummings, Bruno Vellas, Cyrille Sur, et al. 2018. "Randomized Trial of Verubecestat for Mild-to-Moderate Alzheimer's Disease." *New England Journal of Medicine* 378 (18): 1691–1703. <https://doi.org/10.1056/NEJMoa1706441>.

Elisia, Ingrid, Hisae Nakamura, Vivian Lam, Elyse Hofs, Rachel Cederberg, Jessica Cait, Michael R Hughes, et al. 2016. "DMSO Represses Inflammatory Cytokine Production from Human Blood Cells and Reduces Autoimmune Arthritis." *PLoS One* 11 (3): e0152538. <https://doi.org/10.1371/journal.pone.0152538>.

Epperly, Ted, Megan A Dunay, and Jack L Boice. 2017. "Alzheimer Disease: Pharmacologic and Nonpharmacologic Therapies for Cognitive and Functional Symptoms." *American Family Physician* 95 (12): 771–78. <http://www.ncbi.nlm.nih.gov/pubmed/28671413>.

Evin, G, and C Hince. 2013. "BACE1 as a Therapeutic

Target in Alzheimer's Disease: Rationale and Current Status." *Drugs Aging*. <https://doi.org/10.1007/s40266-013-0099-3>.

Fahy, Eoin, Shankar Subramaniam, H Alex Brown, Christopher K Glass, Alfred H Merrill, Robert C Murphy, Christian R H Raetz, et al. 2005. "A Comprehensive Classification System for Lipids." *Journal of Lipid Research* 46 (5): 839–61. <https://doi.org/10.1194/jlr.E400004-JLR200>.

Farooqui, A A, L A Horrocks, and T Farooqui. 2007. "Modulation of Inflammation in Brain: A Matter of Fat." *J Neurochem* 101 (3): 577–99. <https://doi.org/10.1111/j.1471-4159.2006.04371.x>.

Fassbender, K, M Simons, C Bergmann, M Stroick, D Lutjohann, P Keller, H Runz, et al. 2001. "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* 98 (10): 5856–61. <https://doi.org/10.1073/pnas.081620098>.

Fernández, M. Laura, and Ramon Reigada. 2014. "Effects of Dimethyl Sulfoxide on Lipid Membrane Electroporation." *The Journal of Physical Chemistry B* 118 (31): 9306–12. <https://doi.org/10.1021/jp503502s>.

Ferrer, Miguel D., Carla Busquets-Cortes, Xavier Capó, Silvia Tejada, Josep A. Tur, Antoni Pons, and Antoni Sureda. 2018. "Cyclooxygenase-2 Inhibitors as a Therapeutic Target in Inflammatory Diseases." *Current Medicinal Chemistry* 25 (May). <https://doi.org/10.2174/0929867325666180514112124>.

Figuerola, Johnny D., Kathia Cordero, Keisha Baldeosingh, Aranza I. Torrado, Robert L. Walker, Jorge D. Miranda, and Marino De Leon. 2012. "Docosahexaenoic Acid Pretreatment Confers Protection and Functional Improvements after Acute Spinal Cord Injury in Adult Rats." *Journal of Neurotrauma* 29 (3): 551–66. <https://doi.org/10.1089/neu.2011.2141>.

Fillenbaum, G G, G van Belle, J C Morris, R C Mohs, S S Mirra, P C Davis, P N Tariot, et al. 2008. "CERAD (Consortium to Establish a Registry for Alzheimer's Disease) The First 20 Years." *Alzheimers Dement* 4 (2): 96–109. <https://doi.org/10.1016/j.jalz.2007.08.005>.

Fonseca, Ana Catarina R.G., Rosa Resende, Catarina R. Oliveira, and Cláudia M.F. Pereira. 2010. "Cholesterol and Statins in Alzheimer's Disease: Current Controversies." *Experimental Neurology* 223 (2): 282–93. <https://doi.org/10.1016/j.expneurol.2009.09.013>.

Fonteh, Alfred N., Cora Ormseth, Jiarong Chiang, Matthew Cipolla, Xianghong Arakaki, and Michael G. Harrington. 2015. "Sphingolipid Metabolism Correlates with Cerebrospinal Fluid Beta Amyloid Levels in

Alzheimer's Disease." Edited by Stephan Neil Witt. *PLOS ONE* 10 (5): e0125597. <https://doi.org/10.1371/journal.pone.0125597>.

Fonteh, Alfred N, Matthew Cipolla, Jiarong Chiang, Xianghong Arakaki, and Michael Harrington. 2016. "Indices of Fatty Acyl-CoA Desaturase Activity Are Associated with Cognitive Function and Preclinical Changes in Cerebrospinal Fluid Beta-Amyloid and Tau Levels in Alzheimer's Disease." http://www.fasebj.org/content/30/1_Supplement/625.3.

Fraser, T, H Tayler, and S Love. 2010. "Fatty Acid Composition of Frontal, Temporal and Parietal Neocortex in the Normal Human Brain and in Alzheimer's Disease." *Neurochem Res* 35 (3): 503–13. <https://doi.org/10.1007/s11064-009-0087-5>.

Freude, K K, M Penjwini, J L Davis, F M LaFerla, and M Blurton-Jones. 2011. "Soluble Amyloid Precursor Protein Induces Rapid Neural Differentiation of Human Embryonic Stem Cells." *J Biol Chem* 286 (27): 24264–74. <https://doi.org/10.1074/jbc.M111.227421>.

Freund-Levi, Y, M Eriksdotter-Jönhagen, T Cederholm, H Basun, G Faxén-Irving, A Garlind, I Vedin, B Vessby, L O Wahlund, and J Palmblad. 2006. "Omega-3 Fatty Acid Treatment in 174 Patients with Mild to Moderate Alzheimer Disease: OmegaAD Study: A Randomized Double-Blind Trial." *Arch Neurol* 63 (10): 1402–8. <https://doi.org/10.1001/archneur.63.10.1402>.

Frohnman, Michael A. 2015. "The Phospholipase D Superfamily as Therapeutic Targets." *Trends in Pharmacological Sciences* 36 (3): 137–44. <https://doi.org/10.1016/j.tips.2015.01.001>.

Fukami, Yuki, Toshio Ariga, Mitsunori Yamada, and Nobuhiro Yuki. 2017a. "Brain Gangliosides in Alzheimer's Disease: Increased Expression of Cholinergic Neuron-Specific Gangliosides." *Current Alzheimer Research* 14 (6): 586–91. <https://doi.org/10.2174/156720501466617017094038>.

———. 2017b. "Brain Gangliosides in Alzheimer's Disease: Increased Expression of Cholinergic Neuron-Specific Gangliosides." *Current Alzheimer Research* 14 (6): 586–91. <https://doi.org/10.2174/156720501466617017094038>.

Gad, S.E., and D.W. Sullivan. 2014. "Dimethyl Sulfoxide (DMSO)." In *Encyclopedia of Toxicology*, 166–68. Elsevier. <https://doi.org/10.1016/B978-0-12-386454-3.00839-3>.

Geddes, J W, T L Tekirian, N S Soultanian, J W Ashford, D G Davis, and W R Markesbery. 1997. "Comparison of

Neuropathologic Criteria for the Diagnosis of Alzheimer's Disease." *Neurobiol Aging* 18 (4 Suppl): S99-105. <https://www.ncbi.nlm.nih.gov/pubmed/9330997>.

Giudetti, Anna Maria, Adele Romano, Angelo Michele Lavecchia, and Silvana Gaetani. 2016. "The Role of Brain Cholesterol and Its Oxidized Products in Alzheimer's Disease." *Current Alzheimer Research* 13 (2): 198–205. <http://www.ncbi.nlm.nih.gov/pubmed/26391039>.

Glass, Christopher K., Kaoru Saijo, Beate Winner, Maria Carolina Marchetto, and Fred H. Gage. 2010a. "Mechanisms Underlying Inflammation in Neurodegeneration." *Cell* 140 (6): 918–34. <https://doi.org/10.1016/j.cell.2010.02.016>.

———. 2010b. "Mechanisms Underlying Inflammation in Neurodegeneration." *Cell* 140 (6): 918–34. <https://doi.org/10.1016/j.cell.2010.02.016>.

Green, K N, H Martinez-Coria, H Khashwji, E B Hall, K A Yurko-Mauro, L Ellis, and F M LaFerla. 2007. "Dietary Docosahexaenoic Acid and Docosapentaenoic Acid Ameliorate Amyloid-Beta and Tau Pathology via a Mechanism Involving Presenilin 1 Levels." *J Neurosci* 27 (16): 4385–95. <https://doi.org/10.1523/jneurosci.0055-07.2007>.

Grimm, Marcus O W, Sven Grösgen, Tatjana L Rothhaar, Verena K Burg, Benjamin Hundsdörfer, Viola J Haupenthal, Petra Friess, et al. 2011. "Intracellular APP Domain Regulates Serine-Palmitoyl-CoA Transferase Expression and Is Affected in Alzheimer's Disease." *International Journal of Alzheimer's Disease* 2011: 695413. <https://doi.org/10.4061/2011/695413>.

Gu, Zezong, Tomohiro Nakamura, and Stuart A. Lipton. 2010. "Redox Reactions Induced by Nitrosative Stress Mediate Protein Misfolding and Mitochondrial Dysfunction in Neurodegenerative Diseases." *Molecular Neurobiology* 41 (2–3): 55–72. <https://doi.org/10.1007/s12035-010-8113-9>.

Gurtovenko, Andrey A, and Jamshed Anwar. 2007. "Modulating the Structure and Properties of Cell Membranes: The Molecular Mechanism of Action of Dimethyl Sulfoxide." *The Journal of Physical Chemistry. B* 111 (35): 10453–60. <https://doi.org/10.1021/jp073113e>.

Hammond, Timothy R., Connor Dufort, Lasse Dissing-Olesen, Stefanie Giera, Adam Young, Alec Wysoker, Alec J. Walker, et al. 2018. "Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes." *Immunity*, November. <https://doi.org/10.1016/j.immuni.2018.11.004>.

Hannun, Y A, and L M Obeid. 2008. "Principles of Bioactive Lipid Signalling: Lessons from Sphingolipids." *Nat Rev Mol Cell Biol* 9 (2): 139–50. <https://doi.org/10.1038/nrm2329>.

Hardy, J. 2002. "The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics." *Science* 297 (5580): 353–56. <https://doi.org/10.1126/science.1072994>.

Hartmann, T, J Kuchenbecker, and M O Grimm. 2007. "Alzheimer's Disease: The Lipid Connection." *J Neurochem* 103 Suppl: 159–70. <https://doi.org/10.1111/j.1471-4159.2007.04715.x>.

Hassan, Mubashir, Saba Shahzadi, Sung Y. Seo, Hany Alashwal, Nazar Zaki, and Ahmed A. Moustafa. 2018. "Molecular Docking and Dynamic Simulation of AZD3293 and Solanezumab Effects Against BACE1 to Treat Alzheimer's Disease." *Frontiers in Computational Neuroscience* 12 (June): 34. <https://doi.org/10.3389/fncom.2018.00034>.

Hattori, Chinatsu, Masashi Asai, Hayato Onishi, Noboru Sasagawa, Yasuhiro Hashimoto, Takaomi C. Saïdo, Kei Maruyama, Shigehiko Mizutani, and Shoichi Ishiura. 2006. "BACE1 Interacts with Lipid Raft Proteins." *Journal of Neuroscience Research* 84 (4): 912–17. <https://doi.org/10.1002/jnr.20981>.

He, X, Y Huang, B Li, C X Gong, and E H Schuchman. 2010. "Deregulation of Sphingolipid Metabolism in Alzheimer's Disease." *Neurobiol Aging* 31 (3): 398–408. <https://doi.org/10.1016/j.neurobiolaging.2008.05.010>.

Heneka, Michael T, Monica J Carson, Joseph El Khoury, Gary E Landreth, Frederic Brosse, Douglas L Feinstein, Andreas H Jacobs, et al. 2015a. "Neuroinflammation in Alzheimer's Disease." *The Lancet Neurology* 14 (4): 388–405. [https://doi.org/10.1016/S1474-4422\(15\)70016-5](https://doi.org/10.1016/S1474-4422(15)70016-5).

———. 2015b. "Neuroinflammation in Alzheimer's Disease." *The Lancet Neurology* 14 (4): 388–405. [https://doi.org/10.1016/S1474-4422\(15\)70016-5](https://doi.org/10.1016/S1474-4422(15)70016-5).

———. 2015c. "Neuroinflammation in Alzheimer's Disease." *The Lancet Neurology* 14 (4): 388–405. [https://doi.org/10.1016/S1474-4422\(15\)70016-5](https://doi.org/10.1016/S1474-4422(15)70016-5).

Heppner, Frank L., Richard M. Ransohoff, and Burkhard Becher. 2015a. "Immune Attack: The Role of Inflammation in Alzheimer Disease." *Nature Reviews Neuroscience* 16 (6): 358–72. <https://doi.org/10.1038/nrn3880>.

———. 2015b. "Immune Attack: The Role of Inflammation in Alzheimer Disease." *Nature Reviews Neuroscience* 16 (6): 358–72. <https://doi.org/10.1038/nrn3880>.

Heras-Sandoval, David, José Pedraza-Chaverri, and Jazmin M. Pérez-Rojas. 2016a. "Role of Docosahexaenoic Acid in the Modulation of Glial Cells in Alzheimer's Disease." *Journal of Neuroinflammation* 13 (1): 61. <https://doi.org/10.1186/s12974-016-0525-7>.

Heras-Sandoval, David, José Pedraza-Chaverri, and Jazmin M. Pérez-Rojas. 2016b. "Role of Docosahexaenoic Acid in the Modulation of Glial Cells in Alzheimer's Disease." *Journal of Neuroinflammation* 13 (1): 61. <https://doi.org/10.1186/s12974-016-0525-7>.

Hickman, Suzanne E, Elizabeth K Allison, and Joseph El Khoury. 2008a. "Microglial Dysfunction and Defective Beta-Amyloid Clearance Pathways in Aging Alzheimer's Disease Mice." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 28 (33): 8354–60. <https://doi.org/10.1523/JNEUROSCI.0616-08.2008>.

———. 2008b. "Microglial Dysfunction and Defective Beta-Amyloid Clearance Pathways in Aging Alzheimer's Disease Mice." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 28 (33): 8354–60. <https://doi.org/10.1523/JNEUROSCI.0616-08.2008>.

———. 2008c. "Microglial Dysfunction and Defective Beta-Amyloid Clearance Pathways in Aging Alzheimer's Disease Mice." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 28 (33): 8354–60. <https://doi.org/10.1523/JNEUROSCI.0616-08.2008>.

Hildenbrandt, G R, T Abraham, and L L Bieber. 1971. "Metabolism of Ceramide Phosphorylethanolamine, Phosphatidylinositol, Phosphatidylserine and Phosphatidylglycerol by Housefly Larvae." *Lipids* 6 (7): 508–16. <https://www.ncbi.nlm.nih.gov/pubmed/4330548>.

Hirsch-Reinshagen, V, B L Burgess, and C L Wellington. 2009. "Why Lipids Are Important for Alzheimer Disease?" *Mol Cell Biochem* 326 (1–2): 121–29. <https://doi.org/10.1007/s11010-008-0012-2>.

Hofacer, R, R Jandacek, T Rider, P Tso, I J Magrisso, S C Benoit, and R K McNamara. 2011. "Omega-3 Fatty Acid Deficiency Selectively up-Regulates Delta6-Desaturase Expression and Activity Indices in Rat Liver: Prevention by Normalization of Omega-3 Fatty Acid Status." *Nutr Res* 31 (9): 715–22. <https://doi.org/10.1016/j.nutres.2011.08.007>.

Hollebeeck, Sylvie, Thomas Raas, Neil Piront, Yves-Jacques Schneider, Olivier Toussaint, Yvan Larondelle, and Alexandrine During. 2011a. "Dimethyl Sulfoxide

(DMSO) Attenuates the Inflammatory Response in the in Vitro Intestinal Caco-2 Cell Model." *Toxicology Letters* 206 (3): 268–75. <https://doi.org/10.1016/j.toxlet.2011.08.010>.

———. 2011b. "Dimethyl Sulfoxide (DMSO) Attenuates the Inflammatory Response in the in Vitro Intestinal Caco-2 Cell Model." *Toxicology Letters* 206 (3): 268–75. <https://doi.org/10.1016/j.toxlet.2011.08.010>.

Hong, H. 2015a. "Role of Lipids in Folding, Misfolding and Function of Integral Membrane Proteins." *Adv Exp Med Biol* 855: 1–31. https://doi.org/10.1007/978-3-319-17344-3_1.

———. 2015b. "Role of Lipids in Folding, Misfolding and Function of Integral Membrane Proteins." *Adv Exp Med Biol* 855: 1–31. https://doi.org/10.1007/978-3-319-17344-3_1.

Hu, X, W He, C Diaconu, X Tang, G J Kidd, W B Macklin, B D Trapp, and R Yan. 2008. "Genetic Deletion of BACE1 in Mice Affects Remyelination of Sciatic Nerves." *FASEB J* 22 (8): 2970–80. <https://doi.org/10.1096/fj.08-106666>.

Hussain, Ghulam, Florent Schmitt, Jean-Philippe Loeffler, and Jose-Luis Gonzalez de Aguilar. 2013. "Fattening the Brain: A Brief of Recent Research." *Frontiers in Cellular Neuroscience* 7 (September): 144. <https://doi.org/10.3389/fncel.2013.00144>.

Huttunen, Henri J, Daniel Havas, Camilla Peach, Cory Barren, Stephan Duller, Weiming Xia, Matthew P Frosch, Birgit Hutter-Paier, Manfred Windisch, and Dora M Kovacs. 2010. "The Acyl-Coenzyme A: Cholesterol Acyltransferase Inhibitor CI-1011 Reverses Diffuse Brain Amyloid Pathology in Aged Amyloid Precursor Protein Transgenic Mice." *Journal of Neuropathology and Experimental Neurology* 69 (8): 777–88. <https://doi.org/10.1097/NEN.0b013e3181e77ed9>.

Iacono, Diego, Susan M. Resnick, Richard O'Brien, Alan B. Zonderman, Yang An, Olga Pletnikova, Gay Rudow, Barbara Crain, and Juan C. Troncoso. 2014. "Mild Cognitive Impairment and Asymptomatic Alzheimer Disease Subjects." *Journal of Neuropathology & Experimental Neurology* 73 (4): 295–304. <https://doi.org/10.1097/NEN.0000000000000052>.

Jacob, Stanley W, and Jack C de la Torre. 2009. "Pharmacology of Dimethyl Sulfoxide in Cardiac and CNS Damage." *Pharmacological Reports*. [https://doi.org/10.1016/S1734-1140\(09\)70026-X](https://doi.org/10.1016/S1734-1140(09)70026-X).

Jang, H J, Y R Yang, J K Kim, J H Choi, Y K Seo, Y H Lee, J E Lee, S H Ryu, and P G Suh. 2013. "Phospholipase C- γ 1 Involved in Brain Disorders." *Adv Biol Regul* 53 (1): 51–62. <https://doi.org/10.1016/j.jbior.2012.09.008>.

Janssen, C I, and A J Kiliaan. 2014. "Long-Chain Polyunsaturated Fatty Acids (LCPUFA) from Genesis to Senescence: The Influence of LCPUFA on Neural Development, Aging, and Neurodegeneration." *Prog Lipid Res* 53: 1–17. <https://doi.org/10.1016/j.plipres.2013.10.002>.

Jay, Taylor R, Victoria E von Saucken, and Gary E Landreth. 2017. "TREM2 in Neurodegenerative Diseases." *Molecular Neurodegeneration* 12 (1): 56. <https://doi.org/10.1186/s13024-017-0197-5>.

Jimenez, Sebastian, Manuel Torres, Marisa Vizuete, Raquel Sanchez-Varo, Elisabeth Sanchez-Mejias, Laura Trujillo-Estrada, Irene Carmona-Cuenca, et al. 2011. "Age-Dependent Accumulation of Soluble Amyloid β (A β) Oligomers Reverses the Neuroprotective Effect of Soluble Amyloid Precursor Protein- α (SAPP α) by Modulating Phosphatidylinositol 3-Kinase (PI3K)/Akt-GSK-3 β Pathway in Alzheimer Mouse Model." *Journal of Biological Chemistry* 286 (21): 18414–25. <https://doi.org/10.1074/jbc.M110.209718>.

Kalvodova, L, N Kahya, P Schwiller, R Ehehalt, P Verkade, D Drechsel, and K Simons. 2005. "Lipids as Modulators of Proteolytic Activity of BACE: Involvement of Cholesterol, Glycosphingolipids, and Anionic Phospholipids in Vitro." *J Biol Chem* 280 (44): 36815–23. <https://doi.org/10.1074/jbc.M504484200>.

Kamal, A, A Almenar-Queralt, J F LeBlanc, E A Roberts, and L S Goldstein. 2001. "Kinesin-Mediated Axonal Transport of a Membrane Compartment Containing Beta-Secretase and Presenilin-1 Requires APP." *Nature* 414 (6864): 643–48. <https://doi.org/10.1038/414643a>.

Karran, E, M Mercken, and B De Strooper. 2011. "The Amyloid Cascade Hypothesis for Alzheimer's Disease: An Appraisal for the Development of Therapeutics." *Nat Rev Drug Discov* 10 (9): 698–712. <https://doi.org/10.1038/nrd3505>.

Kennedy, M E, A W Stamford, X Chen, K Cox, J N Cumming, M F Dockendorf, M Egan, et al. 2016. "The BACE1 Inhibitor Verubecestat (MK-8931) Reduces CNS Beta-Amyloid in Animal Models and in Alzheimer's Disease Patients." *Sci Transl Med* 8 (363): 363ra150. <https://doi.org/10.1126/scitranslmed.aad9704>.

Keren-Shaul, Hadas, Amit Spinrad, Assaf Weiner, Orit Matcovitch-Natan, Raz Dvir-Szternfeld, Tyler K. Ulland, Eyal David, et al. 2017. "A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease." *Cell* 169 (7): 1276–1290.e17. <https://doi.org/10.1016/j.cell.2017.05.018>.

Kim, H. W., S. I. Rapoport, and J. S. Rao. 2011. "Altered

Arachidonic Acid Cascade Enzymes in Postmortem Brain from Bipolar Disorder Patients." *Molecular Psychiatry* 16 (4): 419–28. <https://doi.org/10.1038/mp.2009.137>.

Kivipelto, M, E L Helkala, M P Laakso, T Hänninen, M Hallikainen, K Alhainen, H Soininen, J Tuomilehto, and A Nissinen. 2001. "Midlife Vascular Risk Factors and Alzheimer's Disease in Later Life: Longitudinal, Population Based Study." *BMJ* 322 (7300): 1447–51. <http://www.ncbi.nlm.nih.gov/pubmed/11408299>.

Kloesch, Burkhard, Melissa Liszt, Johann Broell, and Guenter Steiner. 2011. "Dimethyl Sulphoxide and Dimethyl Sulphone Are Potent Inhibitors of IL-6 and IL-8 Expression in the Human Chondrocyte Cell Line C-28/I2." *Life Sciences* 89 (13–14): 473–78. <https://doi.org/10.1016/j.lfs.2011.07.015>.

Kloverpris, Henrik, Anders Fomsgaard, Amanda Handley, Jim Ackland, Mark Sullivan, and Philip Goulder. 2010. "Dimethyl Sulfoxide (DMSO) Exposure to Human Peripheral Blood Mononuclear Cells (PBMCs) Abolish T Cell Responses Only in High Concentrations and Following Coincubation for More than Two Hours." *Journal of Immunological Methods* 356 (1–2): 70–78. <https://doi.org/10.1016/j.jim.2010.01.014>.

Koelsch, Gerald. 2017a. "BACE1 Function and Inhibition: Implications of Intervention in the Amyloid Pathway of Alzheimer's Disease Pathology." *Molecules* 22 (10): 1723. <https://doi.org/10.3390/molecules22101723>.

———. 2017b. "BACE1 Function and Inhibition: Implications of Intervention in the Amyloid Pathway of Alzheimer's Disease Pathology." *Molecules* 22 (10): 1723. <https://doi.org/10.3390/molecules22101723>.

Kopitar-Jerala, Nataša. 2015. "Innate Immune Response in Brain, NF-Kappa B Signaling and Cystatins." *Frontiers in Molecular Neuroscience* 8 (December): 73. <https://doi.org/10.3389/fnmol.2015.00073>.

Krasemann, Susanne, Charlotte Madore, Ron Cialic, Caroline Baufeld, Narghes Calcagno, Rachid El Fatimy, Lien Beckers, et al. 2017. "The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases." *Immunity* 47 (3): 566–581.e9. <https://doi.org/10.1016/j.immuni.2017.08.008>.

Kuratko, C N, E C Barrett, E B Nelson, and N Salem. 2013. "The Relationship of Docosahexaenoic Acid (DHA) with Learning and Behavior in Healthy Children: A Review." *Nutrients* 5 (7): 2777–2810. <https://doi.org/10.3390/nu5072777>.

Lai, Mitchell K P, Shirley W Y Tsang, Monica Garcia-Alloza, Stephen L Minger, James A R Nicoll, Margaret M

Esiri, Peter T-H Wong, Christopher P L-H Chen, María J Ramirez, and Paul T Francis. 2006. "Selective Effects of the APOE Epsilon4 Allele on Presynaptic Cholinergic Markers in the Neocortex of Alzheimer's Disease." *Neurobiology of Disease* 22 (3): 555–61. <https://doi.org/10.1016/j.nbd.2005.12.016>.

Landman, N, S Y Jeong, S Y Shin, S V Voronov, G Serban, M S Kang, M K Park, G Di Paolo, S Chung, and T W Kim. 2006. "Presenilin Mutations Linked to Familial Alzheimer's Disease Cause an Imbalance in Phosphatidylinositol 4,5-Bisphosphate Metabolism." *Proc Natl Acad Sci U S A* 103 (51): 19524–29. <https://doi.org/10.1073/pnas.0604954103>.

Lavandera, Jimena Verónica, Juliana Saín, Ana Clara Fariña, Claudio Adrián Bernal, and Marcela Aída González. 2017. "N-3 Fatty Acids Reduced Trans Fatty Acids Retention and Increased Docosahexaenoic Acid Levels in the Brain." *Nutritional Neuroscience* 20 (7): 424–35. <https://doi.org/10.1080/1028415X.2016.1173343>.

Lebbadi, Meryem, Carl Julien, Alix Phivilay, Cyntia Tremblay, Vincent Emond, Jing X Kang, and Frédéric Calon. 2011. "Endogenous Conversion of Omega-6 into Omega-3 Fatty Acids Improves Neuropathology in an Animal Model of Alzheimer's Disease." *Journal of Alzheimer's Disease* : JAD 27 (4): 853–69. <https://doi.org/10.3233/JAD-2011-111010>.

LeBlanc, A C, M Koutroumanis, and C G Goodyer. 1998. "Protein Kinase C Activation Increases Release of Secreted Amyloid Precursor Protein without Decreasing Abeta Production in Human Primary Neuron Cultures." *J Neurosci* 18 (8): 2907–13. <http://www.ncbi.nlm.nih.gov/pubmed/9526007>.

Lee, J C M, A Simonyi, A Y Sun, and G Y Sun. 2011. "Phospholipases A2 and Neural Membrane Dynamics: Implications for Alzheimer's Disease." *J Neurochem* 116 (5): 813–19. <https://doi.org/10.1111/j.1471-4159.2010.07033.x>.

Lefort, R, J Pozueta, and M Shelanski. 2012. "Cross-Linking of Cell Surface Amyloid Precursor Protein Leads to Increased β -Amyloid Peptide Production in Hippocampal Neurons: Implications for Alzheimer's Disease." *J Neurosci* 32 (31): 10674–85. <https://doi.org/10.1523/JNEUROSCI.6473-11.2012>.

Lei, E, K Vacy, and W C Boon. 2016. "Fatty Acids and Their Therapeutic Potential in Neurological Disorders." *Neurochem Int* 95: 75–84. <https://doi.org/10.1016/j.neuint.2016.02.014>.

León-García, María Cristina, Emmanuel Ríos-Castro,

Everardo López-Romero, and Mayra Cuéllar-Cruz. 2017. "Evaluation of Cell Wall Damage by Dimethyl Sulfoxide in Candida Species." *Research in Microbiology* 168 (8): 732–39. <https://doi.org/10.1016/j.resmic.2017.06.001>.

Leslie, C C. 2015. "Cytosolic Phospholipase A(2): Physiological Function and Role in Disease." *J Lipid Res* 56 (8): 1386–1402. <https://doi.org/10.1194/jlr.R057588>.

Li, Sisi, and Dinshaw J Patel. 2016. "Drosha and Dicer: Slicers Cut from the Same Cloth." *Cell Research* 26 (5): 511–12. <https://doi.org/10.1038/cr.2016.19>.

Lichtenthaler, S F. 2012. "Alpha-Secretase Cleavage of the Amyloid Precursor Protein: Proteolysis Regulated by Signaling Pathways and Protein Trafficking." *Curr Alzheimer Res* 9 (2): 165–77. <http://www.ncbi.nlm.nih.gov/pubmed/21605033>.

Lim, G P, F Yang, T Chu, P Chen, W Beech, B Teter, T Tran, et al. 2000. "Ibuprofen Suppresses Plaque Pathology and Inflammation in a Mouse Model for Alzheimer's Disease." *J Neurosci* 20 (15): 5709–14. <http://dx.doi.org/>.

Lingwood, D., and K. Simons. 2010. "Lipid Rafts As a Membrane-Organizing Principle." *Science* 327 (5961): 46–50. <https://doi.org/10.1126/science.1174621>.

Liu, J J, P Green, J J Mann, S I Rapoport, and M E Sublette. 2015. "Pathways of Polyunsaturated Fatty Acid Utilization: Implications for Brain Function in Neuropsychiatric Health and Disease." *Brain Res* 0: 220–46. <https://doi.org/10.1016/j.brainres.2014.11.059>.

Liu, Y, A Käch, U Ziegler, A C Ong, D P Wallace, A Arcaro, and A L Serra. 2013. "The Role of Phospholipase D in Modulating the MTOR Signaling Pathway in Polycystic Kidney Disease." *PLoS One* 8 (8): e73173. <https://doi.org/10.1371/journal.pone.0073173>.

Livak, Kenneth J., and Thomas D. Schmittgen. 2001. "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2- $\Delta\Delta$ CT Method." *Methods* 25 (4): 402–8. <https://doi.org/10.1006/meth.2001.1262>.

Luo, Dong, Yan Long, and Guo-Jun Chen. 2015. "Cytochrome P-450 2E1 Gene Polymorphisms and Risk of Alzheimer's Disease: A Meta-Analysis." *Journal of the Neurological Sciences* 359 (1–2): 100–105. <https://doi.org/10.1016/j.jns.2015.10.053>.

Ma, H, S Lesné, L Kotilinek, J V Steidl-Nichols, M Sherman, L Younkin, S Younkin, et al. 2007. "Involvement of Beta-Site APP Cleaving Enzyme 1 (BACE1) in Amyloid Precursor Protein-Mediated Enhancement of Mem-

ory and Activity-Dependent Synaptic Plasticity." *Proc Natl Acad Sci U S A* 104 (19): 8167–72. <https://doi.org/10.1073/pnas.0609521104>.

Ma, Q L, F Yang, E R Rosario, O J Ubeda, W Beech, D J Gant, P P Chen, et al. 2009. "Beta-Amyloid Oligomers Induce Phosphorylation of Tau and Inactivation of Insulin Receptor Substrate via c-Jun N-Terminal Kinase Signaling: Suppression by Omega-3 Fatty Acids and Curcumin." *J Neurosci* 29 (28): 9078–89. <https://doi.org/10.1523/jneurosci.1071-09.2009>.

Mahfoud, R, N Garmy, M Maresca, N Yahi, A Puigserver, and J Fantini. 2002. "Identification of a Common Sphingolipid-Binding Domain in Alzheimer, Prion, and HIV-1 Proteins." *J Biol Chem* 277 (13): 11292–96. <https://doi.org/10.1074/jbc.M111679200>.

Makide, K, H Kitamura, Y Sato, M Okutani, and J Aoki. 2009. "Emerging Lysophospholipid Mediators, Lysophosphatidylserine, Lysophosphatidylthreonine, Lysophosphatidylethanolamine and Lysophosphatidylglycerol." *Prostaglandins Other Lipid Mediat* 89 (3–4): 135–39. <https://doi.org/10.1016/j.prostaglandins.2009.04.009>.

Mandler, M, L Walker, R Santic, P Hanson, A R Upadhyaya, S J Colloby, C M Morris, et al. 2014. "Pyroglutamylated Amyloid-Beta Is Associated with Hyperphosphorylated Tau and Severity of Alzheimer's Disease." *Acta Neuropathol* 128 (1): 67–79. <https://doi.org/10.1007/s00401-014-1296-9>.

Marin, Raquel, Noemí Fabelo, Virginia Martín, Paula García-Esparcia, Isidre Ferrer, David Quinto-Aleman, and Mario Díaz. 2017. "Anomalies Occurring in Lipid Profiles and Protein Distribution in Frontal Cortex Lipid Rafts in Dementia with Lewy Bodies Disclose Neurochemical Traits Partially Shared by Alzheimer's and Parkinson's Diseases." *Neurobiology of Aging* 49 (January): 52–59. <https://doi.org/10.1016/j.neurobiolaging.2016.08.027>.

Marquer, C, V Devauges, J C Cossec, G Liot, S Lécart, F Saudou, C Duyckaerts, S Lévêque-Fort, and M C Potier. 2011. "Local Cholesterol Increase Triggers Amyloid Precursor Protein-Bace1 Clustering in Lipid Rafts and Rapid Endocytosis." *FASEB J* 25 (4): 1295–1305. <https://doi.org/10.1096/fj.10-168633>.

Marwarha, Gurdeep, Stephen Rostad, Jaclyn Lilek, Mason Kleinjan, Jared Schommer, and Othman Ghribi. 2017. "Palmitate Increases β -Site A β PP-Cleavage Enzyme 1 Activity and Amyloid- β Genesis by Evoking Endoplasmic Reticulum Stress and Subsequent C/EBP Homologous Protein Activation." Edited by Bernard Schreurs. *Journal of Alzheimer's Disease* 57 (3): 907–25.

<https://doi.org/10.3233/JAD-161130>.

Matsui, Hiroki, Tomoyuki Yokoyama, Kenichi Sekiguchi, Daisuke Iijima, Hiroaki Sunaga, Moeno Maniwa, Manabu Ueno, Tatsuya Iso, Masashi Arai, and Masahiko Kurabayashi. 2012. "Stearoyl-CoA Desaturase-1 (SCD1) Augments Saturated Fatty Acid-Induced Lipid Accumulation and Inhibits Apoptosis in Cardiac Myocytes." Edited by Krisztian Stadler. *PLoS ONE* 7 (3): e33283. <https://doi.org/10.1371/journal.pone.0033283>.

Mattson, M P, Z H Guo, and J D Geiger. 1999. "Secreted Form of Amyloid Precursor Protein Enhances Basal Glucose and Glutamate Transport and Protects against Oxidative Impairment of Glucose and Glutamate Transport in Synaptosomes by a Cyclic GMP-Mediated Mechanism." *J Neurochem* 73 (2): 532–37. <http://www.ncbi.nlm.nih.gov/pubmed/10428048>.

Maurice, A, and M Malgat. 1993. "Phosphatidylethanolamine: Ceramide-Ethanolaminephosphotransferase Activity in Synaptic Plasma Membrane Vesicles. Influence of Some Cations and Phospholipid Environment on Transferase Activity. Further Proof of Its Location." *Int J Biochem* 25 (8): 1183–87. <https://www.ncbi.nlm.nih.gov/pubmed/8405660>.

Mawuenyega, Kwasi G, Wendy Sigurdson, Vitaliy Ovod, Ling Munsell, Tom Kasten, John C Morris, Kevin E Yarasheski, and Randall J Bateman. 2010. "Decreased Clearance of CNS Beta-Amyloid in Alzheimer's Disease." *Science (New York, N.Y.)* 330 (6012): 1774. <https://doi.org/10.1126/science.1197623>.

Meakin, Paul J, Susan M Jalicy, Gemma Montagut, David J P Allsop, Daniella L Cavellini, Stuart W Irvine, Christopher McGinley, et al. 2018. "Bace1-Dependent Amyloid Processing Regulates Hypothalamic Leptin Sensitivity in Obese Mice." *Scientific Reports* 8 (1): 55. <https://doi.org/10.1038/s41598-017-18388-6>.

Mielke, Michelle M., Norman J. Haughey, Veera Venkata Ratnam Bandaru, Danielle D. Weinberg, Eveleen Darby, Noman Zaidi, Valory Pavlik, Rachelle S. Doody, and Constantine G. Lyketsos. 2011. "Plasma Sphingomyelins Are Associated with Cognitive Progression in Alzheimer's Disease." *Journal of Alzheimer's Disease* 27 (2): 259–69. <https://doi.org/10.3233/JAD-2011-110405>.

Mita, T, T Mayanagi, H Ichijo, K Fukumoto, K Otsuka, A Sakai, and K Sobue. 2016. "Docosahexaenoic Acid Promotes Axon Outgrowth by Translational Regulation of Tau and Collapsin Response Mediator Protein 2 Expression." *J Biol Chem* 291 (10): 4955–65. <https://doi.org/10.1074/jbc.M115.693499>.

Mohaibes, Raheem J., María A. Fiol-deRoque, Manuel Torres, Margarita Ordinas, David J. López, José A. Cas-

tro, Pablo V. Escribá, and Xavier Busquets. 2017. "The Hydroxylated Form of Docosahexaenoic Acid (DHA-H) Modifies the Brain Lipid Composition in a Model of Alzheimer's Disease, Improving Behavioral Motor Function and Survival." *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1859 (9): 1596–1603. <https://doi.org/10.1016/j.bbamem.2017.02.020>.

Moussa, Charbel E-H. 2017. "Beta-Secretase Inhibitors in Phase I and Phase II Clinical Trials for Alzheimer's Disease." *Expert Opinion on Investigational Drugs* 26 (10): 1131–36. <https://doi.org/10.1080/13543784.2017.1369527>.

Mrdjen, Dunja, Anto Pavlovic, Felix J Hartmann, Bettina Schreiner, Sebastian G Utz, Brian P Leung, Iva Lelios, et al. 2018. "High-Dimensional Single-Cell Mapping of Central Nervous System Immune Cells Reveals Distinct Myeloid Subsets in Health, Aging, and Disease." *Immunity* 48 (2): 380–395.e6. <https://doi.org/10.1016/j.immuni.2018.01.011>.

Murphy, Stephanie R, Catherine CY Chang, Godwin Dogbevia, Elena Y Bryleva, Zachary Bowen, Mazahir T Hasan, and Ta-Yuan Chang. 2013. "Acat1 Knockdown Gene Therapy Decreases Amyloid- β in a Mouse Model of Alzheimer's Disease." *Molecular Therapy* 21 (8): 1497–1506. <https://doi.org/10.1038/mt.2013.118>.

Nalivaeva, N N, and A J Turner. 2013. "The Amyloid Precursor Protein: A Biochemical Enigma in Brain Development, Function and Disease." *FEBS Lett* 587 (13): 2046–54. <https://doi.org/10.1016/j.febslet.2013.05.010>.

Nesic, I, F X Guix, K Vennekens, V Michaki, P P Van Veldhoven, F Feiguin, B De Strooper, C G Dotti, and T Wahle. 2012. "Alterations in Phosphatidylethanolamine Levels Affect the Generation of A β ." *Aging Cell* 11 (1): 63–72. <https://doi.org/10.1111/j.1474-9726.2011.00760.x>.

Noel, Anastasia, Sabrina Ingrand, and Laurence Barrier. 2017. "Ganglioside and Related-Sphingolipid Profiles Are Altered in a Cellular Model of Alzheimer's Disease." *Biochimie* 137 (June): 158–64. <https://doi.org/10.1016/j.biochi.2017.03.019>.

Ntambi, James M, and Makoto Miyazaki. 2004. "Regulation of Stearoyl-CoA Desaturases and Role in Metabolism." *Progress in Lipid Research* 43 (2): 91–104. [https://doi.org/10.1016/S0163-7827\(03\)00039-0](https://doi.org/10.1016/S0163-7827(03)00039-0).

Obregon, D, H Hou, J Deng, B Giunta, J Tian, D Darlington, M Shahaduzzaman, et al. 2012. "Soluble Amyloid Precursor Protein- α Modulates β -Secretase Activity and Amyloid- β Generation." *Nat Commun* 3: 777. <https://doi.org/10.1038/ncomms1781>.

Oliveira, T G, R B Chan, H Tian, M Laredo, G Shui, A Stanislawski, H Zhang, et al. 2010. "Phospholipase D2 Ablation Ameliorates Alzheimer's Disease-Linked Synaptic Dysfunction and Cognitive Deficits." *J Neurosci* 30 (49): 16419–28. <https://doi.org/10.1523/JNEUROSCI.3317-10.2010>.

Oster, T, and T Pilot. 2010. "Docosahexaenoic Acid and Synaptic Protection in Alzheimer's Disease Mice." *Biochim Biophys Acta* 1801 (8): 791–98. <https://doi.org/10.1016/j.bbalip.2010.02.011>.

Ostrowski, S M, B L Wilkinson, T E Golde, and G Landreth. 2007. "Statins Reduce Amyloid-Beta Production through Inhibition of Protein Isoprenylation." *J Biol Chem* 282 (37): 26832–44. <https://doi.org/10.1074/jbc.M702640200>.

Panchal, Mai, Mathieu Gaudin, Adina N. Lazar, Elisa Salvati, Isabelle Rivals, Sophie Aycirix, Luce Dauphinot, et al. 2014. "Ceramide and Sphingomyelinases in Senile Plaques." *Neurobiology of Disease* 65 (May): 193–201. <https://doi.org/10.1016/j.nbd.2014.01.010>.

Paolo, G Di, and T W Kim. 2011. "Linking Lipids to Alzheimer's Disease: Cholesterol and Beyond." *Nat Rev Neurosci* 12 (5): 284–96. <https://doi.org/10.1038/nrn3012>.

Park, S Y, H Y Kim, H J Park, H K Shin, K W Hong, and C D Kim. 2016. "Concurrent Treatment with Taxifolin and Cilostazol on the Lowering of Beta-Amyloid Accumulation and Neurotoxicity via the Suppression of P-JAK2/P-STAT3/NF-KappaB/BACE1 Signaling Pathways." *PLoS One* 11 (12): e0168286. <https://doi.org/10.1371/journal.pone.0168286>.

Parkhurst, Christopher N., Guang Yang, Ipe Ninan, Jeffrey N. Savas, John R. Yates, Juan J. Lafaille, Barbara L. Hempstead, Dan R. Littman, and Wen-Biao Gan. 2013. "Microglia Promote Learning-Dependent Synapse Formation through Brain-Derived Neurotrophic Factor." *Cell* 155 (7): 1596–1609. <https://doi.org/10.1016/j.cell.2013.11.030>.

Perry, V. Hugh, and Clive Holmes. 2014. "Microglial Priming in Neurodegenerative Disease." *Nature Reviews Neurology* 10 (4): 217–24. <https://doi.org/10.1038/nrneurol.2014.38>.

Pfriege, F W. 2003. "Cholesterol Homeostasis and Function in Neurons of the Central Nervous System." *Cell Mol Life Sci* 60 (6): 1158–71. <https://doi.org/10.1007/s00018-003-3018-7>.

Phillis, John W, Lloyd A Horrocks, and Akhlaq A Farooqui. 2006. "Cyclooxygenases, Lipoxygenases, and Epoxygen-

ases in CNS: Their Role and Involvement in Neurological Disorders." *Brain Research Reviews* 52 (2): 201–43. <https://doi.org/10.1016/j.brainresrev.2006.02.002>.

Piedrahita, D, J F Castro-Alvarez, R L Boudreau, A Villegas-Lanau, K S Kosik, J C Gallego-Gomez, and G P Cardona-Gomez. 2015. "Beta-Secretase 1's Targeting Reduces Hyperphosphorylated Tau, Implying Autophagy Actors in 3xTg-AD Mice." *Front Cell Neurosci* 9: 498. <https://doi.org/10.3389/fncel.2015.00498>.

Piedrahita, D, J F Castro-Alvarez, R L Boudreau, A Villegas-Lanau, K S Kosik, J C Gallego-Gomez, and G P Cardona-Gómez. 2015. "β-Secretase 1's Targeting Reduces Hyperphosphorylated Tau, Implying Autophagy Actors in 3xTg-AD Mice." *Front Cell Neurosci* 9: 498. <https://doi.org/10.3389/fncel.2015.00498>.

Piedrahita, Diego, John Fredy Castro-Alvarez, Ryan L. Boudreau, Andres Villegas-Lanau, Kenneth S. Kosik, Juan Carlos Gallego-Gomez, and Gloria Patricia Cardona-Gómez. 2016. "β-Secretase 1's Targeting Reduces Hyperphosphorylated Tau, Implying Autophagy Actors in 3xTg-AD Mice." *Frontiers in Cellular Neuroscience* 9. <https://doi.org/10.3389/fncel.2015.00498>.

Polo-Hernandez, E, V Tello, A A Arroyo, M Dominguez-Prieto, F de Castro, A Tabernero, and J M Medina. 2014. "Oleic Acid Synthesized by Stearoyl-CoA Desaturase (SCD-1) in the Lateral Periventricular Zone of the Developing Rat Brain Mediates Neuronal Growth, Migration and the Arrangement of Prospective Synapses." *Brain Res* 1570: 13–25. <https://doi.org/10.1016/j.brainres.2014.04.038>.

Poloni, S, H J Blom, and I V Schwartz. 2015. "Stearoyl-CoA Desaturase-1: Is It the Link between Sulfur Amino Acids and Lipid Metabolism?" *Biology (Basel)* 4 (2): 383–96. <https://doi.org/10.3390/biology4020383>.

Posada-Duque, R A, D Velasquez-Carvajal, G P Eckert, and G P Cardona-Gomez. 2013. "Atorvastatin Requires Geranylgeranyl Transferase-I and Rac1 Activation to Exert Neuronal Protection and Induce Plasticity." *Neurochem Int* 62 (4): 433–45. <https://doi.org/10.1016/j.neuint.2013.01.026>.

Qin, W, L Ho, P N Pompl, Y Peng, Z Zhao, Z Xiang, N K Robakis, J Shioi, J Suh, and G M Pasinetti. 2003. "Cytochrome P-450 (CYP) 2 and COX-1 Potentiate Beta-Amyloid Peptide Generation through Mechanisms That Involve Gamma-Secretase Activity." *J Biol Chem* 278 (51): 50970–77. <https://doi.org/10.1074/jbc.M307699200>.

Rao, J S, M Kellom, H W Kim, S I Rapoport, and E A Reese. 2012. "Neuroinflammation and Synaptic Loss." *Neurochem Res* 37 (5): 903–10. <https://doi.org/10.1007/s11064-012-0708-2>.

s11064-012-0708-2.

Rebeck, G William. 2017. "The Role of APOE on Lipid Homeostasis and Inflammation in Normal Brains." *Journal of Lipid Research* 58 (8): 1493–99. <https://doi.org/10.1194/jlr.R075408>.

Reid, Patrick C, Yasuomi Urano, Tatsuhiko Kodama, and Takao Hamakubo. 2007. "Alzheimer's Disease: Cholesterol, Membrane Rafts, Isoprenoids and Statins." *Journal of Cellular and Molecular Medicine* 11 (3): 383–92. <https://doi.org/10.1111/j.1582-4934.2007.00054.x>.

Reitz, C, and R Mayeux. 2014. "Alzheimer Disease: Epidemiology, Diagnostic Criteria, Risk Factors and Biomarkers." *Biochem Pharmacol* 88 (4): 640–51. <https://doi.org/10.1016/j.bcp.2013.12.024>.

Riss, Terry L, Richard A Moravec, Andrew L Niles, Sarah Duellman, Hélène A Benink, Tracy J Worzella, and Lisa Minor. 2004. *Cell Viability Assays*. Assay Guidance Manual. Eli Lilly & Company and the National Center for Advancing Translational Sciences. <http://www.ncbi.nlm.nih.gov/pubmed/23805433>.

Rituper, B, H H Chowdhury, J Jorgačevski, J R Coorssen, M Kreft, and R Zorec. 2013. "Cholesterol-Mediated Membrane Surface Area Dynamics in Neuroendocrine Cells." *Biochim Biophys Acta* 1831 (7): 1228–38. <https://doi.org/10.1016/j.bbali.2013.04.007>.

Saeedi Saravi, Seyed Soheil, Seyed Sobhan Saeedi Saravi, Alireza Arefidoust, and Ahmad Reza Dehpour. 2017. "The Beneficial Effects of HMG-CoA Reductase Inhibitors in the Processes of Neurodegeneration." *Metabolic Brain Disease* 32 (4): 949–65. <https://doi.org/10.1007/s11011-017-0021-5>.

Sagy-Bross, C, K Kasianov, Y Solomonov, A Braiman, A Friedman, N Hadad, and R Levy. 2015. "The Role of Cytosolic Phospholipase A2 Alpha in Amyloid Precursor Protein Induction by Amyloid Beta1-42: Implication for Neurodegeneration." *J Neurochem* 132 (5): 559–71. <https://doi.org/10.1111/jnc.13012>.

Saini, Rashmi, and Sarika Singh. 2018. "Inducible Nitric Oxide Synthase: An Asset to Neutrophils." *Journal of Leukocyte Biology*, October. <https://doi.org/10.1002/JLB.4RU0418-161R>.

Salter, Michael W, and Simon Beggs. 2014. "Sublime Microglia: Expanding Roles for the Guardians of the CNS." *Cell* 158 (1): 15–24. <https://doi.org/10.1016/j.CELL.2014.06.008>.

Sanchez-Mejia, R O, and L Mucke. 2010. "Phospholipase A2 and Arachidonic Acid in Alzheimer's Disease." *Biochim Biophys Acta* 1801 (8): 784–90. <https://doi.org/10.1016/j.bbali.2010.05.013>.

org/10.1016/j.bbali.2010.05.013.

Sanchez-Mejia, R O, J W Newman, S Toh, G Q Yu, Y Zhou, B Halabisky, M Cisse, et al. 2008. "Phospholipase A2 Reduction Ameliorates Cognitive Deficits in a Mouse Model of Alzheimer's Disease." *Nat Neurosci* 11 (11): 1311–18. <https://doi.org/10.1038/nn.2213>.

Santos, Nuno C, J Figueira-Coelho, J Martins-Silva, and Carlota Saldanha. 2003. "Multidisciplinary Utilization of Dimethyl Sulfoxide: Pharmacological, Cellular, and Molecular Aspects." *Biochemical Pharmacology* 65 (7): 1035–41. <http://www.ncbi.nlm.nih.gov/pubmed/12663039>.

Santos Picanco, Leide Caroline dos, Priscilla F. Ozela, Maiara de Fatima de Brito Brito, Abraao A. Pinheiro, Elias C. Padilha, Francinaldo S. Braga, Carlos Henrique Tomich de Paula da Silva, Cleydson Breno Rodrigues dos Santos, Joaquin M.C. Rosa, and Lorane Izabel da Silva Hage-Melim. 2018. "Alzheimer's Disease: A Review from the Pathophysiology to Diagnosis, New Perspectives for Pharmacological Treatment." *Current Medicinal Chemistry* 25 (26): 3141–59. <https://doi.org/10.2174/0929867323666161213101126>.

Sarnico, Ilenia, Annamaria Lanzillotta, Marina Benarese, Manuela Alghisi, Cristina Baiguera, Leontino Battistin, PierFranco Spano, and Marina Pizzi. 2009. "Chapter 24 NF-KappaB Dimers in the Regulation of Neuronal Survival." In *International Review of Neurobiology*, 85:351–62. [https://doi.org/10.1016/S0074-7742\(09\)85024-1](https://doi.org/10.1016/S0074-7742(09)85024-1).

Sato, Naoyuki, and Ryuichi Morishita. 2015. "The Roles of Lipid and Glucose Metabolism in Modulation of β-Amyloid, Tau, and Neurodegeneration in the Pathogenesis of Alzheimer Disease." *Frontiers in Aging Neuroscience* 7: 199. <https://doi.org/10.3389/fnagi.2015.00199>.

Schaafsma, W., X. Zhang, K.C. van Zomeren, S. Jacobs, P.B. Georgieva, S.A. Wolf, H. Kettenmann, et al. 2015. "Long-Lasting pro-Inflammatory Suppression of Microglia by LPS-Preconditioning Is Mediated by RelB-Dependent Epigenetic Silencing." *Brain, Behavior, and Immunity* 48 (August): 205–21. <https://doi.org/10.1016/j.bbi.2015.03.013>.

Schaeffer, Evelin L., Vanessa J. De-Paula, Emanuelle R. da Silva, Barbara de A. Novaes, Heni D. Skaf, Orestes V. Forlenza, and Wagner F. Gattaz. 2011. "Inhibition of Phospholipase A2 in Rat Brain Decreases the Levels of Total Tau Protein." *Journal of Neural Transmission* 118 (9): 1273–79. <https://doi.org/10.1007/s00702-011-0619-4>.

Schafer, Dorothy P., and Beth Stevens. 2015. "Microglia Function in Central Nervous System Development and

Plasticity." *Cold Spring Harbor Perspectives in Biology* 7 (10): a020545. <https://doi.org/10.1101/cshperspect.a020545>.

Schreiber, Stefanie, Frank Schreiber, Samuel N. Lockhart, Andy Horng, Alexandre Bejanin, Susan M. Landau, and William J. Jagust. 2017. "Alzheimer Disease Signature Neurodegeneration and APOE Genotype in Mild Cognitive Impairment With Suspected Non-Alzheimer Disease Pathophysiology." *JAMA Neurology* 74 (6): 650. <https://doi.org/10.1001/jamaneuro.2016.5349>.

Selkoe, D J, and J Hardy. 2016. "The Amyloid Hypothesis of Alzheimer's Disease at 25 Years." *EMBO Mol Med*. <https://doi.org/10.15252/emmm.201606210>.

Selkoe, D, and R Kopan. 2003. "Notch and Presenilin: Regulated Intramembrane Proteolysis Links Development and Degeneration." *Annu Rev Neurosci* 26: 565–97. <https://doi.org/10.1146/annurev.neuro.26.041002.131334>.

Shabab, Tara, Ramin Khanabdali, Soheil Zorofchian Moghadamtousi, Habsah Abdul Kadir, and Gokula Mohan. 2017. "Neuroinflammation Pathways: A General Review." *International Journal of Neuroscience* 127 (7): 624–33. <https://doi.org/10.1080/00207454.2016.1212854>.

Sharpe, L J, and A J Brown. 2013. "Controlling Cholesterol Synthesis beyond 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR)." *J Biol Chem* 288 (26): 18707–15. <https://doi.org/10.1074/jbc.R113.479808>.

Shea, T B. 1997. "Phospholipids Alter Tau Conformation, Phosphorylation, Proteolysis, and Association with Microtubules: Implication for Tau Function under Normal and Degenerative Conditions." *J Neurosci Res* 50 (1): 114–22. <http://www.ncbi.nlm.nih.gov/pubmed/9379487>.

Sherratt, Samuel C.R., Sandeep Shrivastava, Robert F. Jacob, Amitabha Chattopadhyay, and R. Preston Mason. 2016. "Docosahexaenoic Acid (DHA), but Not Eicosapentaenoic Acid (EPA), Increases Both Membrane Fluidity and Cholesterol Crystalline Domain Formation in Lipid Vesicles." *Biophysical Journal* 110 (3): 583a. <https://doi.org/10.1016/j.bpj.2015.11.3115>.

Shi, S, D Liang, Y Chen, Y Xie, Y Wang, L Wang, Z Wang, and Z Qiao. 2016. "Gx-50 Reduces Beta-Amyloid-Induced TNF-Alpha, IL-1beta, NO, and PGE2 Expression and Inhibits NF-KappaB Signaling in a Mouse Model of Alzheimer's Disease." *Eur J Immunol* 46 (3): 665–76. <https://doi.org/10.1002/eji.201545855>.

Sierant, M, K Kubiak, J Kazmierczak-Baranska, M War-

- ashina, T Kuwabara, and B Nawrot. 2009. "Evaluation of BACE1 Silencing in Cellular Models." *Int J Alzheimers Dis* 2009. <https://doi.org/10.4061/2009/257403>.
- Stertz, L., V. Contreras-Shannon, N. Monroy-Jaramillo, J. Sun, and C. Walss-Bass. 2018. "BACE1-Deficient Mice Exhibit Alterations in Immune System Pathways." *Molecular Neurobiology* 55 (1): 709–17. <https://doi.org/10.1007/s12035-016-0341-1>.
- Stoffel, Wilhelm, Barbara Holz, Britta Jenke, Erika Binczek, Robert Heinz Günter, Christine Kiss, Iakowos Karakesisoglou, et al. 2008. "Δ6-Desaturase (FADS2) Deficiency Unveils the Role of Ω3- and Ω6-Polyunsaturated Fatty Acids." *The EMBO Journal* 27 (17): 2281–92. <https://doi.org/10.1038/emboj.2008.156>.
- Stoothoff, W H, and G V Johnson. 2005. "Tau Phosphorylation: Physiological and Pathological Consequences." *Biochimica et Biophysica Acta* 1739 (2–3): 280–97. <https://doi.org/10.1016/j.bbdis.2004.06.017>.
- Sumida, Kayo, Yoshinobu Igarashi, Naoki Toritsuka, Tomochika Matsushita, Kaori Abe-Tomizawa, Mikio Aoki, Tetsuro Urushidani, Hiroshi Yamada, and Yasuo Ohno. 2011. "Effects of DMSO on Gene Expression in Human and Rat Hepatocytes." *Human & Experimental Toxicology* 30 (10): 1701–9. <https://doi.org/10.1177/0960327111399325>.
- Sundaram, J R, E S Chan, C P Poore, T K Pareek, W F Cheong, G Shui, N Tang, C M Low, M R Wenk, and S Kesavapany. 2012. "Cdk5/P25-Induced Cytosolic PLA2-Mediated Lysophosphatidylcholine Production Regulates Neuroinflammation and Triggers Neurodegeneration." *J Neurosci* 32 (3): 1020–34. <https://doi.org/10.1523/JNEUROSCI.5177-11.2012>.
- Szczepanik, A M, S Funes, W Petko, and G E Ringheim. 2001. "IL-4, IL-10 and IL-13 Modulate A Beta(1–42)-Induced Cytokine and Chemokine Production in Primary Murine Microglia and a Human Monocyte Cell Line." *Journal of Neuroimmunology* 113 (1): 49–62. [https://doi.org/10.1016/S0165-5728\(00\)00404-5](https://doi.org/10.1016/S0165-5728(00)00404-5).
- Taberner, A, E M Lavado, B Granda, A Velasco, and J M Medina. 2001. "Neuronal Differentiation Is Triggered by Oleic Acid Synthesized and Released by Astrocytes." *J Neurochem* 79 (3): 606–16. <http://dx.doi.org/>.
- Takayama, K, S Tsutsumi, T Suzuki, K Horie-Inoue, K Ikeda, K Kaneshiro, T Fujimura, et al. 2009. "Amyloid Precursor Protein Is a Primary Androgen Target Gene That Promotes Prostate Cancer Growth." *Cancer Res* 69 (1): 137–42. <https://doi.org/10.1158/0008-5472.CAN-08-3633>.
- Tan, Charlene Siew-Hon, Yee-Kong Ng, and Wei-Yi Ong. 2016. "Epigenetic Regulation of Cytosolic Phospholipase A2 in SH-SY5Y Human Neuroblastoma Cells." *Molecular Neurobiology* 53 (6): 3854–72. <https://doi.org/10.1007/s12035-015-9314-z>.
- Tanabe, Fuminori, Tomoko Nakajima, and Masahiko Ito. 2014. "Involvement of Diacylglycerol Produced by Phospholipase D Activation in Aβ-Induced Reduction of SAPPα Secretion in SH-SY5Y Neuroblastoma Cells." *Biochemical and Biophysical Research Communications* 446 (4): 933–39. <https://doi.org/10.1016/j.bbrc.2014.03.038>.
- The Lancet, The. 2016. "Alzheimer's Disease: Expedition into the Unknown." *Lancet* (London, England) 388 (10061): 2713. [https://doi.org/10.1016/S0140-6736\(16\)32457-6](https://doi.org/10.1016/S0140-6736(16)32457-6).
- Tosi, Federica, Filippo Sartori, Patrizia Guarini, Oliviero Olivieri, and Nicola Martinelli. 2014. "Delta-5 and Delta-6 Desaturases: Crucial Enzymes in Polyunsaturated Fatty Acid-Related Pathways with Pleiotropic Influences in Health and Disease." *In* , 61–81. Springer, Cham. https://doi.org/10.1007/978-3-319-07320-0_7.
- Uddin, Md. Sahab, Md. Tanvir Kabir, Abdullah Al Mamun, Mohamed M. Abdel-Daim, George E. Barreto, and Ghulam Md Ashraf. 2018. "APOE and Alzheimer's Disease: Evidence Mounts That Targeting APOE4 May Combat Alzheimer's Pathogenesis." *Molecular Neurobiology*, July. <https://doi.org/10.1007/s12035-018-1237-z>.
- Uranga, Romina María, Natalia Paola Alza, Melisa Ailén Conde, Silvia Susana Antollini, and Gabriela Alejandra Salvador. 2017a. "Phosphoinositides: Two-Path Signaling in Neuronal Response to Oligomeric Amyloid β Peptide." *Molecular Neurobiology* 54 (5): 3236–52. <https://doi.org/10.1007/s12035-016-9885-3>.
- . 2017b. "Phosphoinositides: Two-Path Signaling in Neuronal Response to Oligomeric Amyloid β Peptide." *Molecular Neurobiology* 54 (5): 3236–52. <https://doi.org/10.1007/s12035-016-9885-3>.
- Vacaru, A M, J van den Dikkenberg, P Ternes, and J C Holthuis. 2013. "Ceramide Phosphoethanolamine Biosynthesis in Drosophila Is Mediated by a Unique Ethanolamine Phosphotransferase in the Golgi Lumen." *J Biol Chem* 288 (16): 11520–30. <https://doi.org/10.1074/jbc.M113.460972>.
- Vassar, R. 2014. "BACE1 Inhibitor Drugs in Clinical Trials for Alzheimer's Disease." *In* *Alzheimers Res Ther*. Vol. 6. <https://doi.org/10.1186/s13195-014-0089-7>.
- Venegas, Carmen, Sathish Kumar, Bernardo S. Franklin, Tobias Dierkes, Rebecca Brinkschulte, Dario Tejera, Ana Vieira-Saecker, et al. 2017. "Microglia-Derived ASC Specks Cross-Seed Amyloid-β in Alzheimer's Disease." *Nature* 552 (7685): 355–61. <https://doi.org/10.1038/nature25158>.
- Venkataramani, V, C Rossner, L Iffland, S Schweyer, I Y Tamboli, J Walter, O Wirths, and T A Bayer. 2010. "Histone Deacetylase Inhibitor Valproic Acid Inhibits Cancer Cell Proliferation via Down-Regulation of the Alzheimer Amyloid Precursor Protein." *J Biol Chem* 285 (14): 10678–89. <https://doi.org/10.1074/jbc.M109.057836>.
- Villamil-Ortiz, J G, A Barrera-Ocampo, D Piedrahita, C M Velasquez-Rodriguez, J D Arias-Londono, and G P Cardona-Gomez. 2016. "BACE1 RNAi Restores the Composition of Phosphatidylethanolamine-Derivates Related to Memory Improvement in Aged 3xTg-AD Mice." *Front Cell Neurosci* 10: 260. <https://doi.org/10.3389/fncel.2016.00260>.
- Walter, J. 2012. "γ-Secretase, Apolipoprotein E and Cellular Cholesterol Metabolism." *Curr Alzheimer Res* 9 (2): 189–99. <http://www.ncbi.nlm.nih.gov/pubmed/21605030>.
- Wang, Hua-Long, Yan-Yong Wang, Xin-Gang Liu, Sheng-Han Kuo, Na Liu, Qiao-Yun Song, and Ming-Wei Wang. 2016a. "Cholesterol, 24-Hydroxycholesterol, and 27-Hydroxycholesterol as Surrogate Biomarkers in Cerebrospinal Fluid in Mild Cognitive Impairment and Alzheimer's Disease: A Meta-Analysis." *Journal of Alzheimer's Disease* 51 (1): 45–55. <https://doi.org/10.3233/JAD-150734>.
- . 2016b. "Cholesterol, 24-Hydroxycholesterol, and 27-Hydroxycholesterol as Surrogate Biomarkers in Cerebrospinal Fluid in Mild Cognitive Impairment and Alzheimer's Disease: A Meta-Analysis." *Journal of Alzheimer's Disease* 51 (1): 45–55. <https://doi.org/10.3233/JAD-150734>.
- Wang, P, P P Guan, T Wang, X Yu, J J Guo, and Z Y Wang. 2014. "Aggravation of Alzheimer's Disease Due to the COX-2-Mediated Reciprocal Regulation of IL-1β and Aβeta between Glial and Neuron Cells." *Aging Cell* 13 (4): 605–15. <https://doi.org/10.1111/ace.12209>.
- Wang, W Y, M S Tan, J T Yu, and L Tan. 2015. "Role of Pro-Inflammatory Cytokines Released from Microglia in Alzheimer's Disease." *In* *Ann Transl Med*. Vol. 3. <https://doi.org/10.3978/j.issn.2305-5839.2015.03.49>.
- Whiley, L, A Sen, J Heaton, P Proitsi, D García-Gómez, R Leung, N Smith, et al. 2014. "Evidence of Altered Phosphatidylcholine Metabolism in Alzheimer's Disease." *Neurobiol Aging* 35 (2): 271–78. <https://doi.org/10.1016/j.neurobiolaging.2013.08.001>.
- Wolfe, M S. 2010. "Structure, Mechanism and Inhibition of Gamma-Secretase and Presenilin-like Proteases." *Biol Chem* 391 (8): 839–47. <https://doi.org/10.1515/BC.2010.086>.
- Wu, Pei-Jung, Yun-Fen Hung, Hsin-Yu Liu, and Yi-Ping Hsueh. 2017. "Deletion of the Inflammasome Sensor $Nlrp1$ Mitigates Aβ Deposition and Microglial Activation but Increases Inflammatory Cytokine Expression in an Alzheimer Disease Mouse Model." *Neuroimmunomodulation* 24 (1): 29–39. <https://doi.org/10.1159/000477092>.
- Xiang, Yanhui, Sin Man Lam, and Guanghou Shui. 2015. "What Can Lipidomics Tell Us about the Pathogenesis of Alzheimer Disease?" *Biological Chemistry* 396 (12): 1281–91. <https://doi.org/10.1515/hsz-2015-0207>.
- Yahi, Nouara, and Jacques Fantini. 2014. "Deciphering the Glycolipid Code of Alzheimer's and Parkinson's Amyloid Proteins Allowed the Creation of a Universal Ganglioside-Binding Peptide." Edited by Vladimir N. Uversky. *PLoS ONE* 9 (8): e104751. <https://doi.org/10.1371/journal.pone.0104751>.
- Yanagisawa, K. 2005. "GM1 Ganglioside and the Seeding of Amyloid in Alzheimer's Disease: Endogenous Seed for Alzheimer Amyloid." *Neuroscientist* 11 (3): 250–60. <https://doi.org/10.1177/1073858405275177>.
- Yarza, Ramon, Silvia Vela, Maite Solas, and Maria J. Ramirez. 2016. "C-Jun N-Terminal Kinase (JNK) Signaling as a Therapeutic Target for Alzheimer's Disease." *Frontiers in Pharmacology* 6 (January): 321. <https://doi.org/10.3389/fphar.2015.00321>.
- Yassine, Hussein N., Etienne Croteau, Varun Rawat, Joseph R. Hibbeln, Stanley I. Rapoport, Stephen C. Cunnane, and John C. Umhau. 2017. "DHA Brain Uptake and APOE4 Status: A PET Study with [1-11C]-DHA." *Alzheimer's Research & Therapy* 9 (1): 23. <https://doi.org/10.1186/s13195-017-0250-1>.
- Yi, Xiaoyang, Minxia Liu, Qun Luo, Hailong Zhuo, Hui Cao, Jiexi Wang, and Ying Han. 2017. "Toxic Effects of Dimethyl Sulfoxide on Red Blood Cells, Platelets, and Vascular Endothelial Cells in Vitro." *FEBS Open Bio* 7 (4): 485–94. <https://doi.org/10.1002/2211-5463.12193>.
- Yoganantharajah, Prusothman, Alexander P. Ray, Daniel J. Eyckens, Luke C. Henderson, and Yann Gibert. 2018. "Comparison of Solvate Ionic Liquids and DMSO as an in Vivo Delivery and Storage Media for Small Molecular Therapeutics." *BMC Biotechnology* 18 (1): 32. <https://doi.org/10.1186/s13195-017-0250-1>.

doi.org/10.1186/s12896-018-0442-1.

Yu, C J, W Liu, H Y Chen, L Wang, and Z R Zhang. 2014. "BACE1 RNA Interference Improves Spatial Memory and Attenuates Abeta Burden in a Streptozotocin-Induced Tau Hyperphosphorylated Rat Model." *Cell Biochem Funct* 32 (7): 590–96. <https://doi.org/10.1002/cbf.3055>.

Yuan, Chan, Junying Gao, Jichao Guo, Lei Bai, Charles Marshall, Zhiyou Cai, Linmei Wang, and Ming Xiao. 2014. "Dimethyl Sulfoxide Damages Mitochondrial Integrity and Membrane Potential in Cultured Astrocytes." Edited by Shilpa J. Buch. *PLoS ONE* 9 (9): e107447. <https://doi.org/10.1371/journal.pone.0107447>.

Yuan, Peng, Carlo Condello, C Dirk Keene, Yaming Wang, Thomas D Bird, Steven M Paul, Wenjie Luo, Marco Colonna, David Baddeley, and Jaime Grutzendler. 2016. "TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy." *Neuron* 92 (1): 252–64. <https://doi.org/10.1016/j.neuron.2016.09.016>.

Yuyama, K, and K Yanagisawa. 2010. "Sphingomyelin Accumulation Provides a Favorable Milieu for GM1 Ganglioside-Induced Assembly of Amyloid Beta-Protein." *Neurosci Lett* 481 (3): 168–72. <https://doi.org/10.1016/j.neulet.2010.06.080>.

Zárate, Rafael, Nabil el Jaber-Vazdekis, Noemi Tejera, José A. Pérez, and Covadonga Rodríguez. 2017a. "Significance of Long Chain Polyunsaturated Fatty Acids in Human Health." *Clinical and Translational Medicine* 6 (1): 25. <https://doi.org/10.1186/s40169-017-0153-6>.

———. 2017b. "Significance of Long Chain Polyunsaturated Fatty Acids in Human Health." *Clinical and Translational Medicine* 6 (1): 25. <https://doi.org/10.1186/s40169-017-0153-6>.

Zarrouk, A, M Debbabi, M Bezine, E M Karym, A Badreddine, O Rouaud, T Moreau, et al. 2017. "Lipid Biomarkers in Alzheimer's Disease." *Curr Alzheimer Res*. <https://doi.org/10.2174/1567205014666170505101426>.

Zarrouk, Amira, Meryam Debbabi, Maryem Bezine, El Mostafa Karym, Asmaa Badreddine, Olivier Rouaud, Thibault Moreau, et al. 2018. "Lipid Biomarkers in Alzheimer's Disease." *Current Alzheimer Research* 15 (4): 303–12. <https://doi.org/10.2174/1567205014666170505101426>.

Zhang, Chen, Yuanying Deng, Hongmei Dai, Wenjuan Zhou, Jing Tian, Guoying Bing, and Lingling Zhao. 2017. "Effects of Dimethyl Sulfoxide on the Morphology and Viability of Primary Cultured Neurons and Astrocytes." *Brain Research Bulletin* 128 (January): 34–39. <https://doi.org/10.1016/J.BRAINRESBULL.2016.11.004>.

Zhang, H, Q Ma, Y W Zhang, and H Xu. 2012. "Proteolytic Processing of Alzheimer's β -Amyloid Precursor Protein." *J Neurochem* 120 Suppl: 9–21. <https://doi.org/10.1111/j.1471-4159.2011.07519.x>.

Zhao, Y, F Calon, C Julien, J W Winkler, N A Petasis, W J Lukiw, and N G Bazan. 2011. "Docosahexaenoic Acid-Derived Neuroprotectin D1 Induces Neuronal Survival via Secretase- and PPAR γ -Mediated Mechanisms in Alzheimer's Disease Models." *PLoS One* 6 (1): e15816. <https://doi.org/10.1371/journal.pone.0015816>.

Zhao, Z, N Liu, J Huang, P-H. Lu, and X-M. Xu. 2011. "Inhibition of CPLA2 Activation by Ginkgo Biloba Extract Protects Spinal Cord Neurons from Glutamate Excitotoxicity and Oxidative Stress-Induced Cell Death." *Journal of Neurochemistry* 116 (6): 1057–65. <https://doi.org/10.1111/j.1471-4159.2010.07160.x>.

Zheng, Cong, Xin-Wen Zhou, and Jian-Zhi Wang. 2016a. "The Dual Roles of Cytokines in Alzheimer's Disease: Update on Interleukins, TNF- α , TGF- β and IFN- γ ." *Translational Neurodegeneration* 5 (1): 7. <https://doi.org/10.1186/s40035-016-0054-4>.

———. 2016b. "The Dual Roles of Cytokines in Alzheimer's Disease: Update on Interleukins, TNF- α , TGF- β and IFN- γ ." *Translational Neurodegeneration* 5 (1): 7. <https://doi.org/10.1186/s40035-016-0054-4>.

APPENDIX

Acknowledgements
List of abbreviations
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I have stayed at my current room (man-cave), for more than one year sharing with great friends and incredible funny guys. Dear Leroy you know how special and important you were for me, I really enjoyed every moment together and your good sense of humor. I learned from you to have a balanced life style, also your advice during hard times helped me lot to deal with it. You are great human being thanks a lot, my vriend. Mister Miguel (Michel) such a good time we spend talking about everything especially non-sense conversations, that

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Te AMO con todo mi ser y bien lo sabes cuentas conmigo hoy y siempre.
Tu padre.

List of Abbreviations

AA: Arachidonic acid	LTP: long-term potentiation
AAV: Adeno associate virus	MHC-II: major histocompatibility class 2 complex
ACAT1: Acetyl-Coenzyme A acetyltransferase 1	NFT: neurofibrillary tangles
AChR: Acetylcholine receptor	NF-κB: nuclear factor-κB
AD: Alzheimer Disease	NMDA: N-methyl-D-aspartate
AICD: intracellular APP fragment	NOS: nitrogen oxide synthetase
APOE: apolipoprotein	PA: phosphatidic acid
APP: amyloid precursor protein	PC: phosphatidylcholine
ASC: Apoptosis-associated speck-like protein containing a CARD	PE: phosphatidylethanolamine
ATP: Adenosine triphosphate	PG: phosphatidylglycerol
Aβ: Amyloid Beta	PGE2: prostaglandin E2 receptors
BACE1: Beta secretase 1	PI: phosphatidylinositol
BBB: blood-brain barrier	PI3K: phosphatidylinositol-3 kinase
CDK5: Cyclin dependent of kinase 5	PKC: protein kinase C
Cer: ceramide	PLA: phospholipase A2, C, and D
CNS: Central nervous system	PLD: phospholipase D
COX2: cyclooxygenase 2	PS: phosphatidylserine
CTF: Carboxy-terminal fragment	PSEN: presenilin
DAG: diacylglycerol	PtdIns: Phosphatidylinositol-4, 5-bisphosphate
DHA: Docosahexaenoic acid	RNA: Ribonucleic Acid
DNA: Deoxyribonucleic Acid	RNAi: RNA interference
ePC: etherphosphatidylcholine	ROS: Reactive oxygen species
ePE: etherphosphatidylethanolamine	sAPP: soluble amyloid protein precursor sAPPα
ePS: etherphosphatidylserine	shBACE1miR: short hairpin BACE1 micro RNA
GLs: glycosphingolipids	shSCRmiR: short hairpin scrambles micro RNA
GM: monogangliosides	SM: sphingomyelin
HMG-CoA-R: Hydroxy-Methyl-Glutaryl CoA-Reductase	SMase: sphingomyelinase
IL: interleukin	TLR: toll-like receptors
LDH: Lactate dehydrogenase	TNF-α: Tumoral Necrosis Factor- alpha
LPA: lysophosphatidic acid	TREM2: triggering receptor expressed by myeloid cells 2
LPC: lysophosphatidylcholine	βMCD: β-methyl cyclodextrin
LPE: lysophosphatidylethanolamine	

List of publications

J.G. Villamil Ortiz et al. cPLA2 and desaturases underlie the tau hyperphosphorylation offset induced by BACE knock-down in neuronal primary cultures. *Biochim Biophys Acta Mol Basis Dis.* 2018 Nov;1864(11):3696-3707. doi: 10.1016/j.bbadis.2018.08.028.

J.G. Villamil-Ortiz, et al. Differential Pattern of Phospholipid Profile in the Temporal Cortex from E280A-Familiar and Sporadic Alzheimer's Disease Brains. *J Alzheimers Dis.* 2018;61(1):209-219. doi: 10.3233/JAD-170554.

J.G. Villamil-Ortiz, et al. BACE1 RNAi Restores the Composition of Phosphatidylethanolamine-Derivates Related to Memory Improvement in Aged 3xTg-AD Mice. *Front Cell Neurosci.* 2016 DOI: 10.3389/fncel.2016.00260 Nov 11;10:260. eCollection 2016.

J.G. Villamil Ortiz, et al. Comparative analysis of autophagy and tauopathy related markers in cerebral ischemia and Alzheimer's disease animal models. *Front Aging Neurosci.* 2015 May 19;7:84. doi: 10.3389/fnagi.2015.00084. eCollection 2015.

Angelica Maria Sabogal-Guaqueta, Javier Gustavo Villamil, Julián David Arias-Londoño and Gloria Patricia Cardona Gómez. Inverse Phosphatidylcholine/Phosphatidylinositol Levels as Peripheral Biomarkers and Phosphatidylcholine/Lysophosphatidylethanolamine-Phosphatidylserine as a Central Indicator of Postischemic Cognitive Impairment in Rats. *Frontiers in Neuroscience, section Neurodegeneration.* Accepted December 2018. DOI: 10.3389/fnins.2018.00989

Javier Villamil was born on February 14, 1985, in Bogota, Colombia. He studied in Medellin, Colombia and performed his master's degree in Biomedical sciences in 2013 from the University of Antioquia, during this period he works with Antioquia Neuroscience group (GNA) where he wrote his thesis about the role of Autophagy in Chronic and acute brain injuries, this thesis was qualified as outstanding Thesis and was published in 2015 (doi: 10.3389/fnagi.2015.00084).

Thereafter he started his Ph.D. studies at the Antioquia Neuroscience group, under the supervision of Prof. dr. Patricia Cardona

Gomez. Working on understanding the role of lipid metabolism on Alzheimer disease. After he accomplished most of his research goals in the GNA, he moved to Biomedical Science of cells & Systems in University of Groningen, The Netherlands, in 2016 as part of Ph.D. sandwich program between the Universities of Groningen and Antioquia. Working on development and optimization of laser capture microdissections in combination with transcriptomic studies in human Alzheimer's brain tissue. Under the supervision of Prof. dr. Bart Eggen. Here he developed high interest in understanding the correlation between the immunological system and Neurodegenerative diseases such as Alzheimer and how to use this system as a potential therapeutic application against complex diseases.

After finishing his Ph.D. project in 2019, he moved to Colombia and worked as a postdoc researcher in GNA focusing on validating the role of desaturases enzymes in the development of several neurodegenerative diseases. During this period, he also worked as a Biochemistry teacher in the Agriculture faculty of the University of Antioquia. Later in July of 2019 he moved to Radboud University Nijmegen and joined the group of Prof. r. Roland Brock at the Department of Biochemistry in the Radboud Institute for Molecular Life Sciences as a postdoc researcher where he is developing an immunological therapy using high specific nanocarriers such as Cell-penetrating peptides and Lipid nanoparticles to decrease the tumoral growth rate on in vitro and in vivo models for ovarian cancer.