Universidad de Navarra

Facultad de Farmacia y Nutrición

Targeting JNK for the diagnosis and treatment of Alzheimer's disease

Silvia Vela Lumbreras

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Fdo. Silvia Vela Lumbreras

El presente trabajo ha sido realizado bajo nuestra dirección en el **Departamento de Farmacología y Toxicología** de la Facultad de Farmacia y Nutrición de la Universidad de Navarra y autorizamos su presentación ante el Tribunal que lo ha de juzgar.

VºBº Directora

María Javier Ramírez Gil

VºBº Co-Directora

Maite Solas Zubiaurre

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-***All truths are easy to understand once they are discovered;***

the point is to discover them.

Galileo Galilei

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A

Aβ:	Amyloid beta
ABCA1:	ATP-Binding cassete transporter 1
AD:	Alzheimer's disease
ADRDA:	Alzheimer's disease and related disorders association.
ALA:	Alpha-linoleic acid
AP-1:	Activator protein-1
APP:	β-amyloid precursor protein
ApoE:	Apolipoprotein E
ASK-1:	Apoptosis signal-regulating kinase 1
ATF:	Activator transcription factor
ATP:	Adenosin triphosphate

B

BA10:	Broadmann Area 10
BACE-1:	Beta-site amyloid precursor protein cleaving enzyme 1
BBB:	Blood-brain barrier
Bcl-2:	B-cell lymphoma 2
Bcl-xL:	B-cell lymphoma-extra large
BDR:	Brains for dementia research initiative network

C

CA1:	Cornu ammonis region 1
CD14:	Cluster of differentiation 14
CERAD:	Consortium to establish a registry for Alzheimer's disease
CHME3:	Human microglial cells line
CMS:	Chronic mild stress
CNS	Central nervous system
COX2:	Cyclooxygenase 2
COX:	Cytochrome C oxidase
CPP:	Cell-penetrating peptide
CPPi:	Cell-permeable peptide inhibitor
CSF:	Cerebrospinal fluid

CxF: Frontal cortex

Cyt C: Cytochrome C

D

dH_2O: Distilled water

DHA: Docosahexaenoic acid

D-JNKi1: D-isomer c-Jun N-terminal kinase inhibitor 1

DLK: Dual leucine zipper kinase

DMSO: Dimethyl sulfoxide

DPR: Dipeptide repeat protein

DSM-IV: Diagnostic and statistical manual of mental disorders (4^{th} edition)

E

ELISA: Enzyme-linked immunosorbent assay

ER: Endoplasmic reticulum

ERS: Endoplasmic reticulum's stress

ERK: Extracellular signal-regulated kinase

EPA: Eicosapentaenoic acid

F

FA: Fatty acids

ω-3-FA Omega-3 fatty acids

FTD: Frontotemporal dementia

G

GAP-43: Growth-associated protein 43

GC: Glucocorticoid

GM-CSF: Granulocyte-macrophage colony-stimulating

GR: Glucocorticoid receptor

GROα: Growth related oncogene-alpha

H

HFD: High fat diet

I

ICV: Intracerebroventricular

IL: Interleukin

Ire-1: Endoplasmic reticulum to nucleus signaling 1

IRS-1: Insulin receptor substrate 1

J

JBD: c-Jun N-terminal kinse binding domain

JIP: c-Jun N-terminal kinase interacting protein

JLP: c-Jun N-terminal kinase leucine zipper protein

JNK: c-Jun N-terminal kinase

JNKi: c-Jun N-terminal kinase inhibitors

K

KO: Knock out

L

LBD: Lewy body dementia

LXA_4: Lipoxin A4

LOX: Lipoxygenase

LPS: Lipopolysaccharide

LTP: Long term potentiation

LZK: Leucine zipper-bearing kinase

M

MAPK: Mitogen activated protein kinase

MaR: Maresins

MaR1: Maresin 1 (Macrophage-derived mediator of inflammation resolution 1)

MCI: Mild cognitive impairment

MetS: Metabolic syndrome

miRNA: Micro ribonucleic acid

MIP-1β: Machrophage inflammatory protein-1beta

MKK: Mitogen activated protein kinase kinase

MLK: Mixed-linage kinase

MLKi: Mixed-linage kinase inhibitors

MMSE: Mini-mental state examination

MPTP: 1-metil-4-phenyl-1,2,3,6-terahydropyridine

MWM: Morris water maze

N

NF-κB: Nuclear factor-kappa B

NFT: Neurofibrillary tangles

NGF: Nerve growth factor

NINCDS: National Institute of neurologic, communicative disorders and stroke

NORT: Novel object recognition test

NPD1: Neuroprotectin D1

O

OD: Optical density

P

PBS: Phosphate buffered saline

PBMC: Peripheral blood mononuclear cells

PDX: Protectins

PD1: Protectin D1

PET: Positron emission tomography

PHFs: Paired helical filaments

PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase

PMCI: Progressive mild cognitive impairment

PPAR-γ: Peroxisome proliferator-activated receptor-gamma

PS1: Presenilin-1

PUFA: Polyunsaturated fatty acids

Puma: p53 up-regulated modulator of apoptosis

R

ROI: Reactive oxygen intermediates

ROS: Radical oxygen species

RvD: Resolvin D

RvE: Resolvin E

S

SAMP8: Senescence accelerated mouse prone-8

SAMR1: Senescence accelerated mouse resistant-1

SCI: Subjective cognitive impairment

SNAP-25: Synaptosomal-associated protein 25

SPMs: Specialized pro-resolving mediators

STZ: Streptozotocin

T

TBS: Tris buffered saline

TDP-43: Trans-activator regulatory DNA-binding protein 43

TLR4: Toll like receptor 4

TNF-α: Tumor necrosis factor alpha

TRAF2: TNF receptor-associated factor 2

U

UPR: Unfolded protein response

UV: Ultraviolet

V

VaD: Vascular dementia

VLP-1: Visinin-like protein-1

W

WB: Western blot

WHO: World health organization

WT: Wild type

Y

YLK-40: Chitinase-3 like -1

Z

Zym: Zymosan

INTRODUCTION

1. C-JUN N-TERMINAL KINASE (JNK)

Since its discovery more than 20 years ago, the c-Jun N-terminal kinase family (JNK) has remained a subject of intense research interest with continued efforts to evaluate its biochemistry and regulation, and its contribution to cellular events under physiological and pathophysiological conditions. The JNK family of protein kinases is one of the three identified families of mitogen activated protein kinases (MAPK). Three genes, namely *jnk1* (MAPK8), *jnk2* (MAPK9), and *jnk3* (MAPK10), encode for 10 different splice variants with molecular weights of 46 and 54kDa (Davis, 2000). The 10 different variants are grouped depending on the homologous protein regions in the 3 known isoforms of JNK: JNK1, JNK2 and JNK3 (**Figure 1**). Whereas JNK1 and JNK2 have a broad tissue distribution, JNK3 is mainly localized in neurons and to a lesser extent in the heart and the testis (Coffey, 2014).

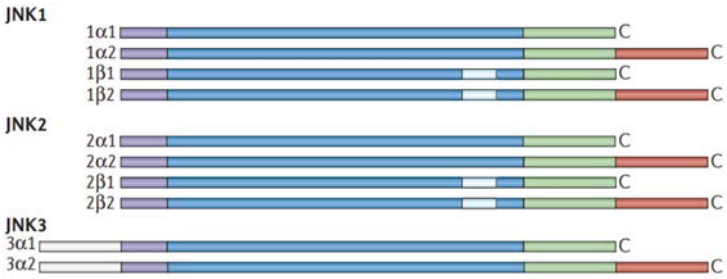

Figure 1. Structural features of JNK isoforms. JNK is a multifunctional enzyme beginning with an amino terminal end (NH_2 – terminal, in purple) and, in the other side, a carboxyl terminal end (COOH-terminal) where the long (54KDa, orange) or short (46KDa, green) JNK variants are determined. The schematic illustration depicts the ten JNK isoforms grouped by their homologous region. All isoforms presents 11 kinase subdomains (I-XI, in blue), but the alternative splicing took place in subdomains IX and X (light blue). Modified from Coffey, 2014.

The discovery of JNK pathway scaffolds such as JNK-interacting protein-1 (JIP1) and related proteins, as well as the identification of JNK inhibitors have contributed to unmask the roles for the JNKs in both normal physiology and disease. JNK signaling process has been studied as an active pathological mechanism in many different diseases, especially in the field of oncology. To mention a few, JNK has been involved in regulation of the natural killer cells cytokine production and secretion (Lee et al., 2014),

oncology models and drug-resistant tumor cells (Chuang et al., 2014; Kim et al., 2014; Okada et al., 2014; Volk et al., 2014) or myeloproliferative disorders (Funakoshi-Tago et al., 2012).

1.1. JNK distribution and functions in CNS

Regarding the central nervous system (CNS), studies in rodents described the presence of *jnk1*, *jnk2* and *jnk3* mRNA in cortex, hippocampus and cerebellum. Specifically, JNK3 is widely expressed throughout the hippocampus, while JNK1 is concentrated around the dentate gyrus. The subcellular distribution of the different isoforms differs, with JNK1 being more abundant in the cytosol, whereas JNK3 is mostly found in the nucleus. JNK2 is distributed in both the cytosol and the nucleus (Coffey, 2014).

Transgenic knockouts of JNK isoforms have provided crucial insights into the roles played in the brain by each JNK isoform. It has been established that JNK1 and JNK2 have important roles in the modulation of immune cell function and in the development of the embryonic nervous system. A study using JNK1 KO mice demonstrated that JNK1 has a regulatory role and maintains physiological functions in the CNS; similarly, JNK2 KO mice established that this isoform may also participate in some physiological functions and, particularly, in the long term potentiation (LTP; Chen et al., 2005). JNK3 is a multifunctional enzyme important in controlling brain functions under both normal and pathological conditions. JNK3 has been implicated in brain development (Kuan et al., 1999), neurite formation and plasticity (Eminel et al., 2008; Waetzig et al., 2006), in addition to memory and learning (Bevilaqua et al., 2003; Brecht et al., 2005). Under pathological conditions, JNK3 has been considered as a degenerative signal transducer and it seems to be the isoform that is over-activated after deleterious stress-stimuli in adult brain, such as ischemia, hypoxia or epilepsies. This principle is supported by the data on the reduced apoptosis of hippocampal neurons and reduced seizures induced by kainic acid in JNK3 KO mice, and by the notion that JNK3 KO mice are also protected against ischemia (Okazawa & Estus, 2002; Sahara et al., 2008; Yang et al., 1997).

1.2. JNK signaling

1.2.1. JNK activation

Activation of the JNK pathway relies on the coordinated interaction of the scaffold proteins belonging to the JNK activation complex. These proteins are able to mediate the biochemical signal amplification and also to ensure substrate-specificity as well as a coordinated cascade signaling (**Figure 2**). The interaction between scaffold proteins leads to the phosphorylation/activation of JNK which, in turn, by phosphorylation of different substrates, enables the activation of different functions (Antoniou et al., 2011).

Different stimuli that have been described as able to trigger the signaling response to JNK include nerve growth factor (NGF) deprivation, trophic support withdrawal, DNA damage, oxidative stress, β-amyloid (Aβ) exposure, low potassium, excitotoxic stress, 6-hydroxydopamine (6-OHDA), UV irradiation, tumor necrosis factor (TNF) or the Wnt cascade (for review see Cui et al., 2007; Mudher et al., 2001). Many are the scaffold proteins that have been described as the signaling proteins that converge in the activation of JNK: JIP1a (JNK interacting protein 1a) and JIP1b (also named IB1), JIP2 and JIP3 (firstly named JSAP1), JNK-interacting leucine zipper protein (JLP) and plenty of SH3 (POSH) (Engstrom et al., 2010). JIPs belong to second-order-activating proteins that are dependent on previous interaction with MAPK activating kinases (MAPKK or MAP2K) and MAPKK activating kinases (MAPKKK or MAP3K; Cui et al., 2007; Engstrom et al., 2010; Wang et al., 2004). Specifically, JNKs are directly activated by phosphorylation by two MAPKK: MKK4 and MKK7; and these are, in turn, activated by mixed-linage kinases (MLK) among other MAP3K (Mehan et al., 2011; Davis, 2000; **Figure 2**). Therefore, JIPs interaction with MLK, MKK4 or MKK7 and JNK is required to JNK activation (Antoniou et al., 2011; Davis, 2000). Thus, the coordination of what is called the “signalosome” that leads to the activation of JNK is complex and requires interaction of first messengers at different cellular levels for further activation of the scaffold-protein-complex and final JNK activation.

Endoplasmic reticulum’s (ER) stress phenomena that induce the unfolded protein response (UPR) signaling is also involved in the control of activation of JNK pathway

(**Figure 2**). As a result of anomalous protein burden, an interaction between Ire1 (endoplasmic reticulum to nucleus signaling 1) and presenilin 1 (PS1) has been proposed to enable the activation of JNK thus leading to pro-apoptotic signaling activation (Shoji et al., 2000). In addition, it has been suggested that ER stress activates apoptosis-signal regulating kinase 1 (ASK1) through IRE1/TRAF2/ASK1 pathway. ASK1 is included in MAP3K group, which subsequently triggers JNK signalling (Viana et al., 2012). Direct modulation of JNK-activation by the cdk5/p35 complex has also been described, although the underlying mechanisms that lead to this molecular phenomenon are unclear (Otth et al., 2003).

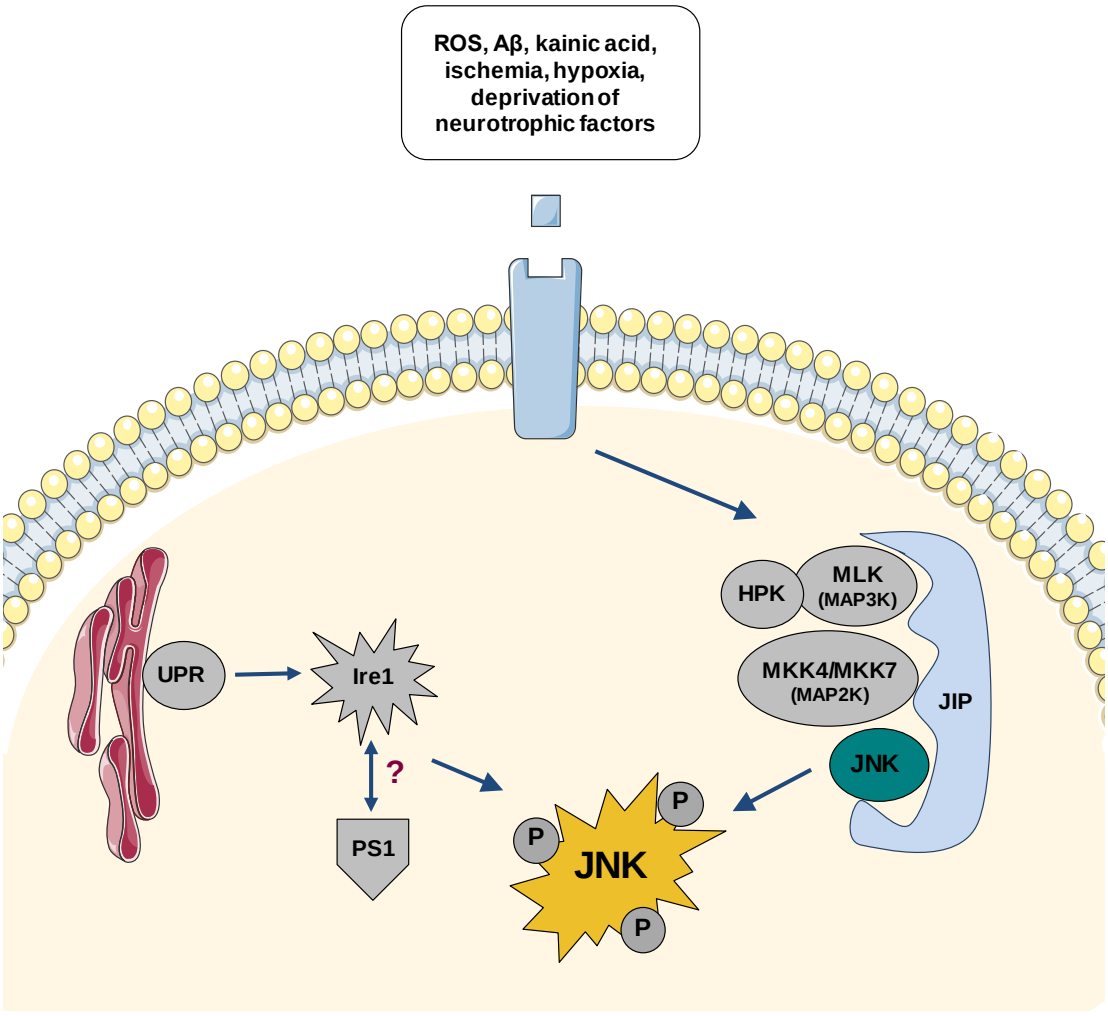

Figure 2. Simplified diagram showing mechanisms involved in activation of the JNK pathway. Different stress conditions might activate JNK signalling via scaffold proteins. UPR and an interaction between IRE1 and PS1 have also been described as potential activators of JNK. Aβ: β-amyloid; HPK: hematopoietic progenitor kinase 1; Ire1: endoplasmic reticulum to nucleus signalling 1; JIP: JNK interacting protein; MKK: mitogen activated protein kinase kinase; MLK: mixed-linage kinase; ROS, radical oxygen species; UPR: unfolded protein response.

1.2.2. JNK substrates

The main cellular substrate activated by JNK mediated phosphorylation is c-Jun (**Figure 3**), which acquires greater stability and increases its transcriptional activity after its phosphorylation. c-Jun is able to interact with JunB, JunD, c-Fos and ATF constituting the AP-1 transcription factor, a nuclear complex that activates the transcription of diverse genes involved in processes related to proliferation, differentiation or apoptosis (Cui et al., 2007; Pearson et al., 2006; **Figure 3**). In addition, c-Jun can act by regulating the maturation of the cellular stress-response or modulating the signals that lead finally to activation of caspases (Nishina et al., 2004; Pearson et al., 2006).

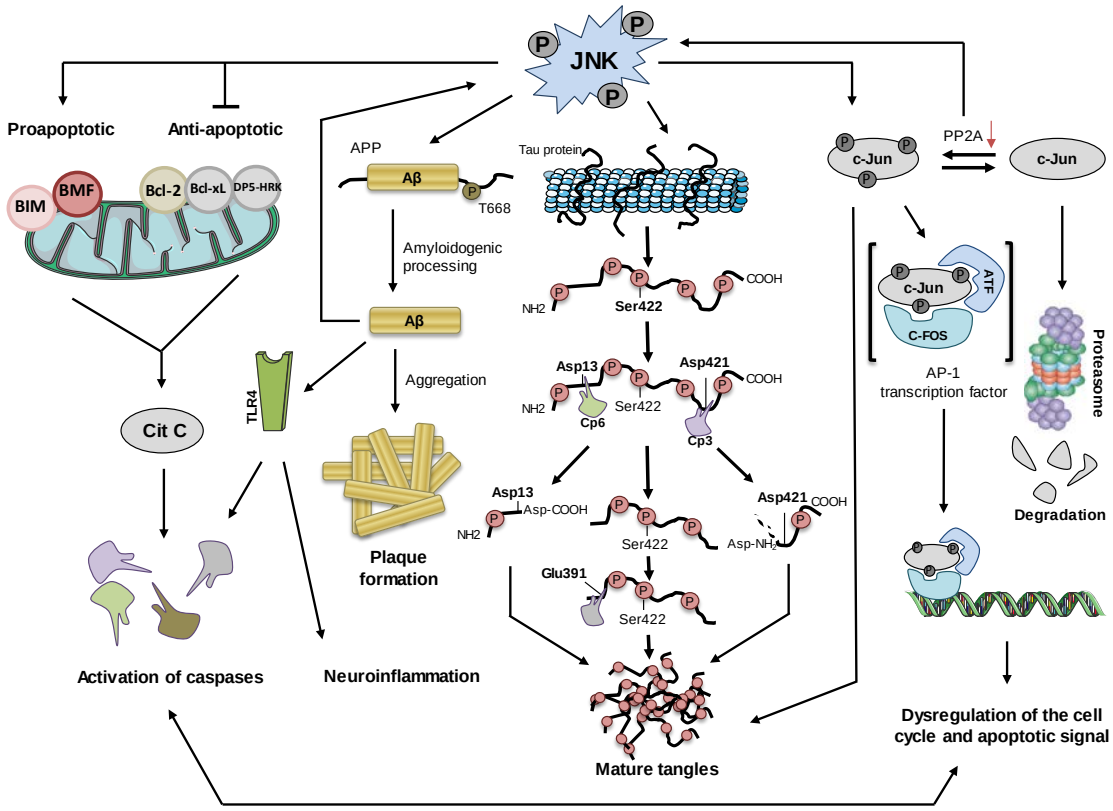

Figure 3. Schematic representation of some cellular mechanisms activated by JNK phosphorylation. Activated JNK leads to phosphorylation of c-Jun, which modulates gene expression as well as tangle maturation. JNK plays also a direct role in the formation of tangles by phosphorylation of Tau and it also contributes to the regulation of paired helical filament (PHF) formation and proteolytic cleavage processing. Moreover, JNK activation is involved in Aβ aggregation and plaque formation. Finally, JNK is responsible for the phosphorylation of the BH3-only proteins BIM and BMF and as consequence, for the activation of caspases leading to cellular apoptosis. BMF: Bcl-2 modifying factor.

Given the cytosolic nature of active JNK, several cytoskeletal components and intracellular transport proteins are found within JNK substrates such as Tau or other proteins associated with microtubules (Zeke et al., 2016). Moreover, JNK is able to phosphorylate and activate directly apoptosis-related proteins such as BIM (homologous to BAX) and BMF, both pro-apoptotic proteins resulting in activation of caspases. JNK also phosphorylates DP5-HRK, Bcl-2 and Bcl-xL (Cui et al., 2007; Okazawa & Estus, 2002), which are anti-apoptotic proteins inhibited by phosphorylation of JNK (**Figure 3**). In summary, JNK activation induces an imbalance between the pro-apoptotic and anti-apoptotic members of the Bcl-2 family that regulate mitochondrial control of apoptosis and, thus, leading to mitochondrial cytochrome C release, caspases activation and, finally, apoptotic death (Coffey, 2014).

Furthermore, it has also been described that JNK might exert its effects via microRNA (miRNA) mechanisms or regulation of histone H3 acetylation, as reviewed by Bogoyevitch et al., (2010).

2. JNK AND ALZHEIMER'S DISEASE

2.1. Alzheimer's disease

Alzheimer's disease (AD) is the main cause of dementia and it is considered a global priority in the field of health-care. According to the World Health Organization (WHO), more than 47 million people worldwide have dementia and there are near 10 million of new cases every year, being AD the major contributor with a prevalence close to 70% of the cases (WHO, 2017).

AD is an age-related neurodegenerative disorder clinically characterized by progressive deterioration of cognitive functions that leads to an irreversible memory loss and executive dysfunction interfering with daily life activities due to a progressive neuronal deterioration. Although the clinical signs and symptoms are widely characterized, AD etiology remains unclear and treatment strategies are only effective in the early disease stages (Thal et al., 2006). It has a variable duration presenting a

deterioration of the cognitive state that leads to death about 10 years after diagnosis (Querfurth & Laferla, 2010).

Within AD, two subtypes are distinguished: familiar AD and sporadic AD. The prevalence of familiar AD is lower (5% of AD cases) than sporadic. In familiar AD, there is a mutated gene that causes AD so manifestations start much earlier than in sporadic AD due to its genetic origin. Several mutations in different genes, such as APP, presenilin 1 or presenilin 2, have been identified. Among them, several autosomal dominant mutations have been described in APP gene that lead to the stimulation of the amyloidogenic processing of the APP such as the Swedish, London, Artic or Indiana mutation.

In sporadic AD (95% of AD cases), the symptoms usually appear after 65 years old. Its etiology is attributed to several risk factors: age, gender, vascular factors, metabolic factors and other risk factors related to lifestyle such as stress, tobacco or alcohol. The age is the most important risk factor to suffer AD; indeed, its incidence and prevalence increase exponentially as patients get older (Prince et al., 2013). Women show a higher probability to suffer from AD (Carter et al., 2012). Furthermore, any disease related to vascular events, such as hypertension, cardiovascular or cerebrovascular disease, increases the risk of suffering from AD (Newman et al., 2005). Moreover, some authors refer to AD as diabetes mellitus type 3 because of the relationship between hyperglycemia, insulin resistance, loss of grey matter and brain atrophy. In this line, it has been probed that there is a decrease of insulin levels in CNS of AD patients (Gil-Bea et al. 2010b). Moreover, hypercholesterolemia and obesity are associated with neurodegeneration and cognitive impairment (Popp et al., 2013). Special attention must be paid to the implication of obesity in AD. Several studies reported a negative correlation between obesity and hippocampus and frontal cortex atrophy in non-demented people (for review see Miller & Spencer, 2014). Given the importance of these brain regions sizes in the cognitive function performance, this fact might contribute to a cognitive decline in obese individuals (Miller & Spencer, 2014). Furthermore, it has been shown that obese population presents worse cognitive state compared with normal weight cases, categorized by body mass index (BMI) (Trakas et al. 2001).

There are different AD clinical stages depending on the disease development. Normally, the diagnosis is made in the later stages when the symptoms are evident; but, fortunately, increasing attention is now paid to early-onset AD, where AD biomarkers can contribute to the early diagnosis (Scheltens et al., 2016).

At cellular level, major neuropathological lesions of AD include extracellular deposits of Aβ peptides leading to formation of senile/neuritic plaques and intracellular neurofibrillary tangles (NFTs) which are paired helical filaments (PHF) of hyper-phosphorylated Tau proteins (Haas, 2012). According to the amyloid hypothesis, Aβ peptides are produced by amyloidogenic processing of amyloid precursor protein (APP) that is a ubiquitous transmembrane protein. β-amyloid peptides originate from proteolysis of the amyloid precursor protein by the sequential enzymatic actions of beta-site amyloid precursor protein–cleaving enzyme 1 (BACE-1), a β-secretase, and γ-secretase, a protein complex with presenilin 1 at its catalytic core. An imbalance between the Aβ peptides production and clearance results in aggregation, accumulation and Aβ oligomerization into insoluble fibrils and subsequent Aβ deposition establishing the primary factor that propitiates AD.

On the other hand, the main component of NFTs is an abnormally hyperphosphorylated and aggregated form of Tau. Under physiological conditions, Tau binds to tubulin, among other proteins, stabilizing the axonal microtubules, which confers a very important role in the maintenance of the neuronal structure and in the transport of proteins through the axons. In physiological conditions Tau is responsible not only for the stabilization of neuronal cytoskeleton by its binding to tubulin monomers but also for many intra and extracellular signaling processes (Kolarova et al., 2012). Hyperphosphorylated Tau lacks affinity for microtubules and becomes to aggregate into PHF, leading to microtubule destabilization (Querfurth & Laferla, 2010).

Synaptic dysfunction and intra- and extracellular accumulation of abnormal proteins, as NFTs and senile plaques, are the main pathological hallmarks of AD. Synaptic neuronal loss has been observed in AD, which is related with the level of cognitive deficit (Terry, 2000). Synapsis degeneration in AD is characterized by progressive terminal loss, lower expression of pre- and post-synaptic proteins, alterations and loss of dendritic spines (Scheff et al., 2007). Aβ peptide accumulation seems to be one of the main mechanisms that produce synaptic dysfunction in AD.

Indeed, correlation between synaptic dysfunction and alterations of learning and memory in different animal models of AD has been observed. For example, it has been demonstrated that Aβ-related synaptic dysfunction can lead to learning deficits in APP transgenic mice (Westerman et al., 2002).

The etiology of AD remains elusive, but the nosogenic basis of AD seems to be related to neuron apoptosis and loss of synaptic terminals within the central nervous system's parenchyma. Thus, the increased concentration of reactive oxygen intermediates (ROI) and superoxide dismutase, both as markers of cellular stress, and increased intracellular calcium in AD are congruent with an underlying activation of apoptotic mechanisms via mitochondrial dysfunction. However, the molecular mechanisms that lead to the activation of apoptotic signals are not fully understood.

2.2. JNK and pathological markers of Alzheimer's disease

It has been shown an increased expression of phosphorylated JNK (pJNK) in human post-mortem brain samples from AD patients and a positive co-localization with Aβ (Killick et al., 2014; Zhu et al., 2001). In particular, JNK3 is highly expressed and activated in brain tissue and cerebrospinal fluid from patients with AD and statistically correlates with the rate of cognitive decline (Gourmaud et al., 2015).

It has been described that Aβ peptides might be able to induce JNK activation, as it has been found *in vitro* that pJNK increases after treatment with Aβ in primary cortical and hippocampal cultures from C57BL/6 mice, in primary cortical cell cultures from Wistar rat and in SH-SY5Y neuroblastoma cells (Morishima et al., 2001; Suwanna et al., 2014; Xu et al., 2015). Some reports confirmed that JNK3-mediated phosphorylation regulated APP cleavage by inducing the amyloidogenic processing of the protein, while JNK inhibition reduced amyloidogenic processing in favour of the non-amyloidogenic route *in vitro* by blocking APP phosphorylation (Colombo et al., 2009; Morishima et al., 2001; Savage et al., 2002). Interestingly, Yoon et al. (2012) demonstrated that JNK3 is the major kinase for APP phosphorylation at T668, a phosphorylation site that favours the amyloidogenic processing. In fact, genetic depletion of JNK3 in transgenic AD mice

resulted in a dramatic reduction in $A\beta_{42}$ peptide levels and overall plaque loads as well as in an increased number of neurons and improved cognition (Yoon et al., 2012).

In experimental models of AD, research using a mouse model of AD that incorporates the Swedish APP mutation and a mutant presenilin-1 (PS1) -Tg2576/PS1- has demonstrated that JNK activation is associated with increased levels of senile plaques and Tau phosphorylation (Savage et al., 2002; **Figure 4**). However, in contrast with these data, no significant differences were found in pJNK levels in the triple transgenic mice (3xTg mice, Feld et al. 2014).

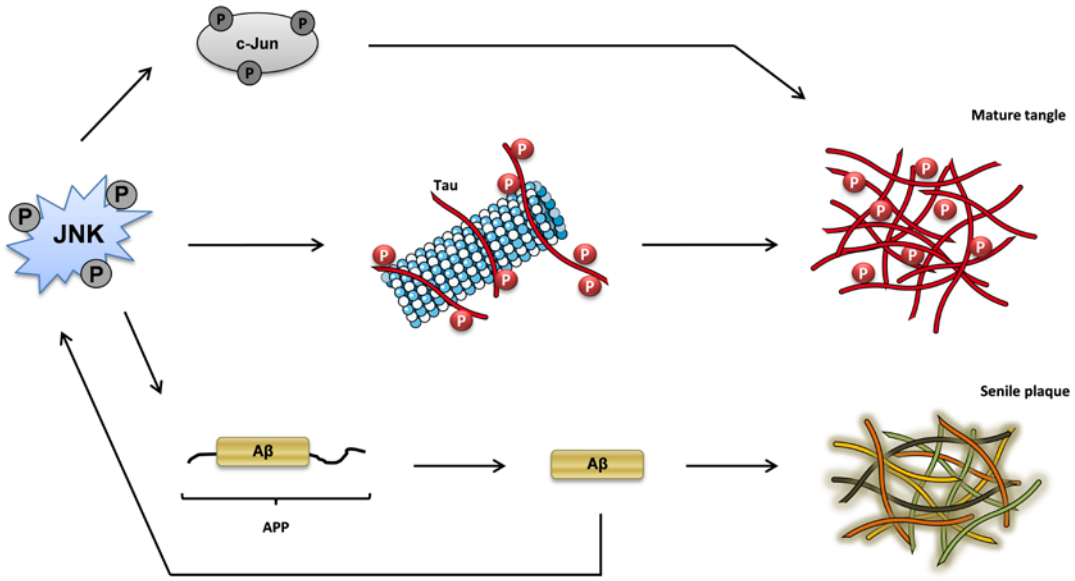

Figure 4. Simplified schematic of cellular mechanisms relevant for AD activated by JNK phosphorylation. Activated JNK (pJNK) leads to tangle maturation by phosphorylation of Tau as well as c-Jun activation. JNK activation contributes to Aβ aggregation and plaque formation. The amyloidogenic processing of APP also induces a positive feedback increasing JNK activation.

Research has also been conducted in experimental models of AD based on well-known risk factors contributing to the development of AD, such as stress or insulin resistance (Dhikav & Anand, 2007; Martisova et al., 2013). In mice subjected to chronic mild stress (CMS) known to increase Tau misprocessing and amyloidogenic processing, JNK phosphorylation is increased (Solas et al. 2013a). The intracerebroventricular (ICV) administration of subdiabetogenic doses of streptozotocin (STZ) induced cognitive and brain cholinergic deficits, oxidative stress, insulin resistant brain state and high levels of pJNK (Giuliani et al. 2013; Salkovic-Petrisic et al. 2013; Xiong et al. 2013). It is to be noted that JNK may also directly induce insulin resistance, as JNK phosphorylates insulin

receptor substrate 1 (IRS1), in an inhibitory site, blocking the transduction signal produced by the insulin receptor (Sabio et al., 2008; Solas et al., 2013b).

c-Jun has been identified to play other possible roles in AD, e.g., phosphorylated c-Jun burdens within the structure of NFTs may play an indirect regulatory role in tangle maturation in AD, mostly regulated by its phosphorylation by JNK. Due to the imbalance established between decreased PPA2 (protein phosphatase 2) expression and JNK mediated phosphorylation of c-Jun, phospho-c-Jun levels are preponderant over the non-phosphorylated form. As a matter of fact, phospho-c-Jun shows a lesser tendency for its degradation via proteasomes, leading to its accumulation within NFTs and, thus, contributing to tangle maturation process (Pearson et al., 2006; **Figure 4**).

JNK also modulates directly the formation of NFTs (**Figure 4**) by direct phosphorylation of Tau (Lagalwar et al., 2006). *In vitro* phosphorylation experiments show that JNK3 isoform can strongly autophosphorylate itself and contribute to Tau hyperphosphorylation (Vogel et al., 2009). JNK was identified to phosphorylate Tau at Ser422, and concretely, JNK3 has the highest affinity towards phosphorylation at Ser422 (Yoshida et al., 2004). In fact, phosphorylation at Ser422 has proved to protect against caspase hydrolysis (Guillozet-Bongaarts et al., 2005; Guillozet-Bongaarts et al., 2006; Kolarova et al., 2012).

2.3. Apoptosis regulation mediated by JNK activation

JNK plays a key role in the balance between cell survival and apoptosis. There are two pathways capable of initiating apoptosis and are classified as extrinsic pathway activated by death receptors such as TNF-α and targeted at the nuclear events, and intrinsic pathway stimulated by mitochondrial dysfunction. Thus, JNK is involved in both processes, as it is able to activate apoptotic signaling by its translocation to the nucleus and upregulation of pro-apoptotic genes. Furthermore, it is able to directly modulate the mitochondrial activity, through phosphorylation of pro-apoptotic proteins (Zeke et al., 2016).

In AD, Aβ accumulation, ROS release and oxidative stress cause JNK activation leading to c-Jun phosphorylation, which allows formation of the AP-1 complex that

induces the transcription of pro-apoptotic genes such as *Tnfα*, *Fas, Puma* or *Bak*. In addition, activated JNK translocates to mitochondria and phosphorylates the Bcl-2 family members in order to antagonize the anti-apoptotic activity of Bcl-2 and Bcl-xL. Ultimately, cytochrome C release and caspases activation is induced leading to neuronal death, as explained above (Akhter et al., 2015; Dhanasekaran & Reddy, 2008).

It has been suggested that neurodegeneration in early age of AD patients could be a result of an increased vulnerability of neurons through activation of different apoptotic pathways as a consequence of elevated levels of oxidative stress, and that these effects could be mediated by JNK activation (Marques et al., 2003; Sahara et al., 2008). Furthermore, JNK has been involved in Aβ triggered down regulation of the anti-apoptotic Bcl-w (Yao et al., 2005) and activation of toll-like receptor 4 (TLR4) signaling (**Figure 3**). Neurons from TLR4 mutant mice exhibit reduced JNK and caspase-3 activation and protection against Aβ induced apoptosis (Tang et al., 2008).

2.4. Role of JNK in neuroinflammation

Inflammation is a protective mechanism in the body, as its main function is to repair and protect against infections, injury or disease. Neuroinflammation is defined as the natural immune system activation at CNS. Initially, neuroinflammation is a protective response in the brain, but a chronic neuroinflammatory response might play an important role in the onset and progression of neurodegenerative disease such as AD, Parkinson's disease or multiple sclerosis (Kempuraj et al., 2016; Zhang & Jiang, 2015).

The regulation of the inflammatory cascade is under the control of intracellular signalling pathways, including MAPK signalling pathway. It has been suggested that Aβ peptide induces neuroinflammation mediated by JNK pathway activation resulting in c-Jun phosphorylation. There is evidence that Aβ induces translational block leading to activation of JNK (Yoon et al., 2012), which in turn, results in neuroinflammation and neurodegeneration. Activated c-Jun leads to stimulation of AP-1 that up-regulated the expression of inflammatory genes. Interestingly, some studies have reported that JNK inhibitor SP600125 attenuates the neuroinflammatory response induced by Aβ (Lin et al., 2013; Vukic et al., 2009; Zhang & Jiang, 2015).

In the elderly and specially in AD patients, there are changes in blood perfusion, blood-brain barrier (BBB) disruption, together with Aβ aggregation and NFT that result in ATP synthesis and electrolytes imbalance, leading to oxidative stress and ROS production which cause overproduction of pro-inflammatory molecules such as interleukin 1 and 6 (IL-1 and IL-6), TNFα, cluster of differentiation-14 (CD14) or toll like receptor 2 and/or 4 (TLR2 or TLR4). Stabilization of this inflammatory cascade induces an irreversible neuronal damage (Bagyinszky et al., 2017).

HPA axis dysregulation in AD leads to glucocorticoids (GC) excess (Gil-Bea et al. 2010a) and decreased insulin sensitivity, trough TLR4, JNK and nuclear factor-kappa B (NF-κB) pathways. Given that hippocampus contains the highest concentrations of glucocorticoids receptors (GR) in the brain, altered GC levels causes hippocampal inflammation and disruption that is likely to lead to cognitive dysfunction. However, inflammation induced hypothalamic outputs are not only targeted to hippocampus, but also to other brain regions, such as cortical tissue and amygdala (Miller & Spencer, 2014; Williams, 2012).

3. NOVEL COMPOUNDS TARGETING JNK INHIBITION

Inhibition of JNKs is an attractive therapeutic strategy that has been investigated with considerable recent effort from both the pharmaceutical industry and academia. The development of JNK inhibitors prior to 2010 has been extensively reviewed (Siddiqui & Reddy, 2010). Within the last years, several patents claiming inhibitors of all JNK isoforms have been published, but few JNK inhibitors have entered clinical trials for different indications such as cancer, lupus erythematosus or stroke (Gehringer et al., 2015), but none for the treatment of AD (Bogoyevitch & Arthur, 2008).

Current compounds under evaluation are: bentamapimod for the treatment of inflammatory endometriosis, CC-930 (tanzisertib) for the treatment of idiopathic pulmonary fibrosis and discoid lupus erythematous and D-JNKi1 for the treatment of inflammation and stroke (as reviewed by Koch et al. 2015). In the following sections, and shown in **Tables 1** and **2** and **Figure 5**, current knowledge of the JNK inhibitors will be described.

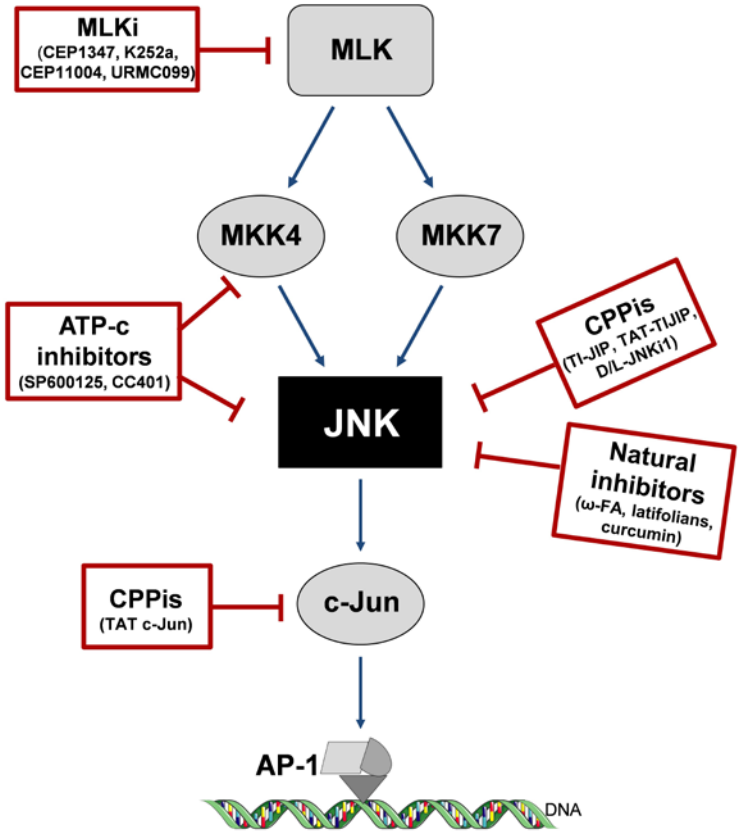

Figure 5. Pharmacology of JNK inhibitors, targets and mechanism of action. ATP-c: direct ATP-competitive inhibitors; CPPi: cell-permeable peptide inhibitors; MKK; mitogen activated protein kinase kinase; MLKi: mixed linage kinase inhibitors.

3.1. Direct ATP-competitive inhibitors: SP600125

Since JNK need to transfer a phosphate from ATP in order to catalyze their specific substrate phosphorylation, ATP-competitive inhibitors were synthesized operating by competitive interaction at the ATP-binding site of JNK (Bogoyevitch et al., 2004). ATP-competitive inhibitors group is typified by SP600125 (Anthra[1,9-cd]pyrazol-6-(2H)-one) which acted by reversible interaction to JNK. The use of SP600125 has been increasingly used in order to understand and elucidate the role of JNK in pathological conditions and a purported therapeutic role of this compound (Bogoyevitch et al., 2004; **Table 1**). However, it is to note that SP600125 has shown a limited specificity toward JNK, as it also inhibits not only MKK4 and MKK7, but also other protein kinases unrelated to JNK,

such as SGK, p70 ribosomal protein S6 kinase (S6K1), AMpk, CDk2, CK1d, and DYRK1A (Bain et al., 2003).

Table 1. Use of SP600125 as a possible therapeutic strategy in Alzheimer's disease**.**

	EXPERIMENTAL MODEL	FINDINGS	REFERENCES
In vitro	F11 cells	Blockade of βAPP dimerization and ASK1 (MAP3K5) mediated neuronal cell death	Hashimoto et al. 2003
	Hippocampal cell culture from Wistar rats	Increased synaptic transmission in CA1 region	Costello & Herron 2004
	Murine L929 fibroblasts	Block of Tau phosphorylation induced by WOX1 knock-down in cell culture	Sze et al. 2004
	Primary rat microglia culture	Reduced nitrite accumulation and prevention of iNOS's activation in glial cells	Bodles & Barger 2005
	Primary cortical cell culture from Sprague Dawley rat	Inhibition of Bcl-w and Bcl-xL down-regulation	Yao et al. 2005
	Neuroglioma U251 cells	Inhibition of IL-1β induced sAPPα release	Ma et al. 2005
	PC12 cells	Attenuation of 4-hydroxynonenal induced apoptosis	Cho et al. 2009
	Primary glial culture from Swiss-Webster mice	Increase of ApoE/ABCA1 expression	Pocivavsek & Rebeck 2009
	Cultured human brain endothelial cells	Inhibition of Aβ induced AP-1 activation and MCP1	Vukic et al. 2009
	Primary rat hippocampal culture	Inhibition of both hetero- and autophosphorylation of JNK.	Vogel et al. 2009
	Human Neuroglioma H4 cells expressing Swedish APP695 or intracellular APP C99	Inhibition of staurosporine-induced Aβ	Chae et al. 2010
	SK-N-SH cell line	Reduction of passive calcium leak in endoplasmic reticulum	Das et al. 2012
	Primary cortical cell culture from Sprague Dawley rat	Reduction of morphine induced Tau phosphorylation	Cao et al. 2013
	CMEC/D3 cells	Reduction of Aβ induced cytokine expression	Bamji-Mirza et al. 2014
In vivo	Male Swiss-Webster mice	Increase of ApoE/ABCA1 expression	Pocivavsek & Rebeck 2009
	Male albino Wistar rats	Improvement of escape latency on MWM	Ramin et al. 2011
	Drosophila spp fly strains	Rescue of Aβ42 induced apoptosis	Hong et al. 2012
	Male C57BL/6 mice	Reduction of PS1 expression	Rahman et al. 2012
	Sprague Dawley neonatal rats	Attenuate isoflurane-induced hippocampal apoptosis mediated by JNK	Li et al. 2013b
	APPswe/PS1dE9 mice	Reversion of synaptic loss, decrease of IL-1β, IL-6 and TNF-α expression, decrease of phosphorylated Tau, increase of αAPP, decrease of βAPP and Aβ oligomers and improvement of spatial learning	Zhou et al. 2015

ABCA-1: ATP-Binding Cassete transporter 1; ApoE: Apolipoprotein E; APP: Amyloid precursor protein; ASK-1: Apoptosis signal-regulating kinase-1; CA1: Cornu Ammonis region 1; IL: Interleukin; MWM: Morris Water Maze; PS1: Presenilin-1.

In both *in vitro* and *in vivo* models of AD, SP600125 has demonstrated to prevent the pathological mechanisms triggered by the up-regulation of pJNK.

In vitro, SP600125 has demonstrated that this inhibitor prevents βAPP induced neuronal cell death as well as down-regulation of ASK1 in F11 cell-lines (Hashimoto et al., 2003). Interestingly, this study did not find neuroprotection against βAPP induced neuroapoptosis when exposing cultures to a p38 inhibitor, highlighting the importance of JNK within this process. Other *in vitro* experiments showed decrease of Aβ-induced cytokine expression (IL6, IL8, MIP1β, TNF-α, Gro-α, GM-CSF) (Bamji-Mirza et al., 2014). *In vivo* studies have shown that ICV administration of SP600125 improved escape latency in the Morris water maze (MWM) (Ramin et al., 2011). In this line, in an AD transgenic mouse model (APPxPS1), administration of SP600125 improved spatial learning impairment in the MWM, and reduced pTau and Aβ oligomeric burden (Zhou et al., 2015).

3.2. Mixed linage kinase inhibitors

As already mentioned, MLKs have been thought to be a plausible target for JNK inhibition. Their inhibition could lead to down-regulation of the JNK signaling pathway resulting in anti-apoptotic and neuroprotective outcomes within neuropathological models. As a result of this, different MLK inhibitors have been developed in order to assess their potential role as a possible therapeutic agent in different pathologies including AD (**Table 2**).

Table 2. Use of different mixed linage kinase inhibitors as a possible therapeutic strategy in AD.

JNK INHIBITOR	EXPERIMENTAL MODEL	FINDINGS	REFERENCES
K252a	Primary cell culture	Conferred neuroprotection to Aβ-exposition	Goodman & Mattson 1994
	Primary cell culture	Prevention against Aβ-induced neuroapoptosis	Xu et al. 2009
CEP1347	Primary cell culture	Prevention against Aβ-induced neuronal cell	Bozyczko-Coyne et al. 2001
	PC12 cell cultures	Prevention against Aβ-induced neuronal cell death	Troy et al. 2001

The indolocarbazole K252a was the first MLK inhibitor found in *Nocadiopsis* sp. and it has been frequently used in experimental models implicating JNK signaling. In cell culture studies, it has been shown that K252a conferred neuroprotection to Aβ-exposed

hippocampal cells (Goodman & Mattson, 1994) and prevented Aβ-induced neuroapoptosis (Xu et al., 2009) which could be of potential benefit in AD.

The compound CEP1347 derives from K252a by the addition of two ethylthiomethyl groups (Saporito et al., 2002) and acts over MLK1, MLK2, MLK3, DLK (dual leucine zipper kinase) and leucine zipper-bearing kinase (LZK, Bogoyevitch et al. 2004). CEP1347 reached clinical phase studies (Parkinson Study Group, 2004; Parkinson Study Group PRECEPT Investigators, 2007; Schwid et al., 2010) for the treatment of Parkinson's disease (Wang et al., 2004). Unfortunately, the results were disappointing. Regarding AD, CEP1347 has been shown to prevent Aβ-induced neuronal cell death and it reduced caspase-3 activity (Bozyczko-Coyne et al., 2001; Troy et al., 2001).

CEP11004 is another carbazole-derived MLK inhibitor that has proved to be useful in PD models, as this compound prevented 6-hydroxydopamine-induced neuroapoptosis in neurons of the *substantia nigra* (Ganguly et al., 2004) and it also appeared as a good inhibitor of the JNK cascade in a MPTP-induced cellular stress model (De Girolamo & Billett, 2006). Further studies will be needed to evaluate the effects of CEP11004 in AD experimental models for its possible relation towards the AD-related pathophysiologic mechanisms explained above.

URMC099 is a novel MLK inhibitor with good BBB penetrating properties which has already been proved to be useful in reducing inflammatory response both *in vivo* and *in vitro* models (Goodfellow et al., 2013; Marker et al., 2013). However, no studies have been performed up to date to evaluate the effects of URMC099 in neurodegenerative models.

In summary, although the use of MLK inhibitors has been limited in the AD field, further studies are expected to come.

3.3. Cell-permeable peptide inhibitors

Cell-permeable peptide inhibitors of JNK (CPPis) are peptide sequences that specifically bind to the JNK binding domain (JBD) leading to its inhibition (Bogoyevitch et al., 2004; Borsello & Bonny, 2004). Their characterization came primarily from studies that confirm the interaction of highly expressed JIP1 with JNK, showing that high

concentrations of JIP1 are able to induce inhibition of JNK and down-regulation of JNK substrates (Dickens et al., 1997). A conserved 21 amino acid long sequence was firstly identified within JNK's primary protein conformation at position 143-163 (Barr et al., 2002; Bogoyevitch et al., 2004). This region is widely known to be the JNK binding domain where JIP1 mediates down-modulation over JNK, leading to its inhibition. Purification of the 143-163 region and synthesis of the polypeptide out of this sequence, named I-JIP, showed the capacity of triggering inhibition of JNK. Moreover, a shorter polypeptide obtained from the sequence specified in-between 153-163 demonstrated to exert the minimal inhibitory effect on JNK. This compound receives the name of TI-JIP (Barr et al., 2002; Bogoyevitch & Arthur, 2008).

However, the disadvantages that result from the relative non-permeability of TI-JIP need to be solved. In order to achieve this aim, it has been proposed to couple TI-JIP to a cell-penetrating peptide (CPP, Bogoyevitch et al., 2004). CPPs are small peptides (typically 5-25 amino acids) which are used to facilitate the delivery of normally non-permeable cargos such as other peptides, proteins, nucleic acids, or drugs into cells (Meloni et al., 2014). Hence Borsello & Bonny, (2004) observed that attaching CPPs, such as TAT 48-57 or antannapedia, to TI-JIP facilitates the diffusion of peptides through membranes in order to exert their action over the desired targets.

JNK-interacting protein derived compounds have been studied for their possible role in preventing neurodegenerative pathways in which JNK has been proved to be implicated. TAT-TIJIP was obtained linking TI-JIP (truncated form of I-JIP) to TAT 48-57 (CPP) which is a transporter sequence of 10 amino acids derived from the human immunodeficiency virus TAT protein (Borsello et al., 2003). TAT-TIJIP has been demonstrated to be able to prevent neuronal apoptosis via JNK inhibition and effectively prevented cell death by interfering with several processes that have been identified as leading to cell death by necrosis. In particular, reactive oxygen species production was reduced and the increase in cytosolic calcium following the excitotoxic insult was attenuated. These neuroprotective properties of JNK peptide inhibitors likely reflect their abilities to prevent cell death by necrosis as well as apoptosis (Arthur et al., 2007). Interestingly, a peptide inhibitor of c-Jun has also been synthesized, the TAT-c-Jun peptide (Antoniou et al., 2011; Holzberg et al., 2003).

It is noteworthy that CPPs show themselves as promising molecules for targeting JNK, as these compounds have the advantage of specificity, showing little activity over other kinases (Barr et al., 2002). In fact, one of the most important disadvantages shown by other synthetic JNK inhibitors, such as SP600125 or MLK inhibitor, is their lack of specificity towards their target (Bogoyevitch et al., 2004). Moreover, a study showed the neuroprotective efficacy of four CPPs, namely TAT, penetratin, Ar-9 and Pep-1 in a glutamic acid, kainic acid and *in vitro* ischemia injury model (Meloni et al., 2014). AP-1 inhibitory peptides (both full-length and truncated) have also shown neuroprotective efficacy in kainic acid and glutamate neuronal excitotoxicity models (Meade et al., 2010). However it is to note that TAT-like peptides and other non-related CPPs possess intrinsic neuroprotective properties (Meloni et al., 2014) and pose the question of the contribution of the CPP versus cargo in the neuroprotective effect.

In this way, different post-modifications of TAT-TIJIP were performed that led to the synthesis of JNK inhibitors (JNKi). Furthermore, the *in vitro* synthesis of JNKi using pure D-isomers with the intention of preserving protein functionality and avoiding proteolytic instability, led to the obtaining of D-JNKi1 and its L-isomer (L-JNKi1, Borsello & Bonny 2004). D-JNKi1 is the most frequently used inhibitor in experimental neurodegenerative models. It has been proved useful utility to reverse ischemia-induced neuronal damage (Borsello et al., 2003). It has been demonstrated that D-JNKi1 is able to decrease levels of APP in human neuroglioma H4 cell lines with the consequent reduction of βAPP levels and Aβ burdens, and it also shifted APP processing toward the non-amyloidogenic pathway (Colombo et al., 2009). Again, these events are of high interest as they are directly related to the central pathogenesis of AD.

Regarding AD models (**Table 3**), different studies have confirmed the potential therapeutic benefit of these inhibitors for their capacity to interact within a wide variety of molecular signaling processes implicated in this pathology.

Sclip et al. pointed out the efficacy of D-JNKi1 in a murine AD model, TgCRND8, in which D-JNKi1 demonstrated to prevented JNK action leading to decreased APP phosphorylation at Thr-668 and reduced amyloidogenic cleavage of APP and Aβ oligomers (Sclip et al., 2011). Tran et al. demonstrated that D-JNKi1 mediated down-regulation of JNK induced Tau phosphorylation in an AD transgenic model (PS1xAPPxTau) (Tran et al., 2012). D-JNKi1 has also proved beneficial effects rescuing

synaptic loss and potentiating LTP in TgCRND8 (Sclip et al., 2014). The increase in pTau levels and neuronal cell death shown in a stress model of AD was also reversed by the administration of D-JNKi1 (Solas et al. 2013b). In this scenario, peptide inhibitors could represent a good therapeutic option for the continuously widening therapeutic armamentarium in AD.

Table 3. Use of different peptide inhibitors as a possible therapeutic strategy in AD.

JNK INHIBITOR	EXPERIMENTAL MODEL	FINDINGS	REFERENCES
TAT-TIJIP	Primary cell culture	Prevention against neuronal apoptosis	Meade et al. 2010
	Primary cell culture	Decrease of neuronal degenerarion and dendrite loss	Meloni et al. 2014
D-JNKi	TgCRND8 mice	Decrease of APP phosphorylation. Improvement of memory	Sclip et al. 2011
	3xTg-AD mice with traumatic brain injury	Prevention of Tau phosphorylation	Tran et al. 2012
	SAMP8 mice	Decrease of Tau phosphorylation. Improvement of memory in MWM task	Orejana et al., 2013
	C57BL/6J mice + corticosterone regimen	Reversion of insulin resistance. Improvement of cognitive function in NORT task	Solas et al., 2013b
	TgCRND8 mice	Decrease of synaptic loss and preventing synaptic dysfunction	Sclip et al. 2014

APP: amyloid precursor protein; MWM: Morris water maze; NORT: novel object recognition test.

3.4. Natural inhibitors

Three different types of compounds can be mentioned in this section: latifolians, curcumin and ω-fatty acids (ω-FAs).

Latifolians A and B are natural compounds isolated from the stem bark of the Papua New Guinean vine *Gnetum latifolium* that have been identified as inhibitors of JNK3 (Rochfort et al., 2005). However, no studies have been performed concerning the possible use of latifolians as neuroprotective agents in neurodegenerative models.

Curcumin is a natural compound that resides in the Zingiberaceae spp. family. Aside for its implications as an anti-inflammatory and antioxidant agent, curcumin has also proved to play a direct role in the modulation of the JNK pathway (Chen & Tan, 1998). Indeed, it has been proposed an underlying role of curcumin toward the inhibition of JNK, demonstrating its capacity to ameliorate MPTP (1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine) and MPP^+ (1-methyl-4-phenylpyridnium) induced neuronal loss models both *in vivo* and *in vitro* (Yu et al., 2010). It also promotes an increase in the expression of HSPs (heat shock proteins) that are centrally implicated in preserving the functionality of the proteasome-mediated degradation of abnormally misfolded proteins (Maiti et al., 2014).

Regarding AD models (**Table 4**), curcumin showed to mediate a significant reduction in $A\beta_{40}$ and $A\beta_{42}$ levels within the hippocampal structures in APPswe/PS1 mice after 6-month follow up (Feng et al., 2014) as well as a reduction in Aβ levels and senile plaques histopathology in Tg2576 mouse model (Yang et al., 2005). In addition to this, it has also been demonstrated a significant improvement of the spatial learning and memory ability after a 3-month based dosage regimen, as well as a reduced expression of presenilin 2, and an increased activity of Aβ degrading enzymes such as neprilysin (Wang et al., 2014).

Table 4. Use of curcumin as a possible therapeutic strategy in AD.

JNK INHIBITOR	EXPERIMENTAL MODEL	FINDINGS	REFERENCES
Curcumin	Tg2576 mice	Reduced amyloid levels and plaque burden. Direct Aβ-binding prevention of fibril formation and aggregation	Yang et al. 2005
	3xTg-AD-mice	Reduced Aβ, plaque deposition and cytoquines levels	Ma et al. 2009
	APPswe/PS1dE9 mice	Reduced hippocampal Aβ40/42 levels	Feng et al. 2014
	APPswe/PS1dE9 mice	Spatial learning and memory improvements. Reduced hippocampal Aβ levels	Wang et al. 2014

APP: amyloid precursor protein; PS1: presenilin-1.

In fact, the combination of docosahexanoic acid (ω-3 FA) and curcumin showed reduced phosphorylation of JNK and Tau as well as a decreased degradation of insulin receptor substrate-1 (IRS1) in 3xTg AD mice, leading to a Y-maze performance improvement due to a possible role of curcumin in an insulin-sensitization process that directly supports and preserves the insulin trophism within cerebral tissue (Ma et al., 2009). In this way, curcumin could be considered an encouraging compound in order to obtain a potential therapeutic drug for AD.

Based on the importance of ω-3 FAs in the present Doctoral Thesis, these compounds will be described in detail in the following section.

4. ω-3 DERIVATIVES AND ALZHEIMER'S DISEASE

The implication of polyunsaturated fatty acids (PUFAs) in neurodegenerative diseases is currently widely accepted. Albeit, some controversy regarding the benefit of ω-3 FAs in mild to moderate AD patients have been reported and the effects on people with other type of dementia remain unclear (Burckhardt et al., 2016). Therefore, all above mentioned suggest the importance of further studies that could confirm the beneficial outcomes of the use of ω-FAs in the onset and progression of AD.

4.1. ω-3 polyunsaturated fatty acid

The organism can receive two different types of fat through diet: saturated and unsaturated fat. Saturated fats can be found naturally in food, mainly from animal sources, such as meat and dairy products, and have been related with higher risk of heart disease (de Souza et al., 2015). On the other hand, unsaturated fats are predominantly found in food derived from fish or plants, such as olive oil, nuts and seeds. These unsaturated fats can be differentiated in monounsaturated and polyunsaturated fats.

In this sense, the long chain omega-3 polyunsaturated fatty acids (ω-3 PUFAs) are derived from marine or vegetal sources (**Figure 6**). Among marine ω-3 PUFAs, the most significant ones are Eicosapentaenoic acid (EPA, ω-3, 20:5) and Docosahexaenoic acid (DHA, ω-3, 22:6). EPA and DHA can be found mainly in oily fish including salmon, tuna, mackerel and anchovy among others (for review see Lorente-Cebrián et al., 2013; Lorente-Cebrián et al., 2015). On the other hand, the most relevant ω-3 PUFA derived from plants is the α-linolenic acid (ALA, 18:3). Nevertheless, mammals can obtain ALA through EPA and DHA processing into organism (Burdge et al., 2002; Burdge & Wootton, 2002).

Marine ω-3 PUFAs have been widely recognised to exert favourable anti-inflammatory actions on inflammatory-related diseases such as cardiovascular diseases, atherosclerosis, AD, asthma, arthritis, colitis, obesity or metabolic syndrome (MetS) (for review see Calder, 2015).

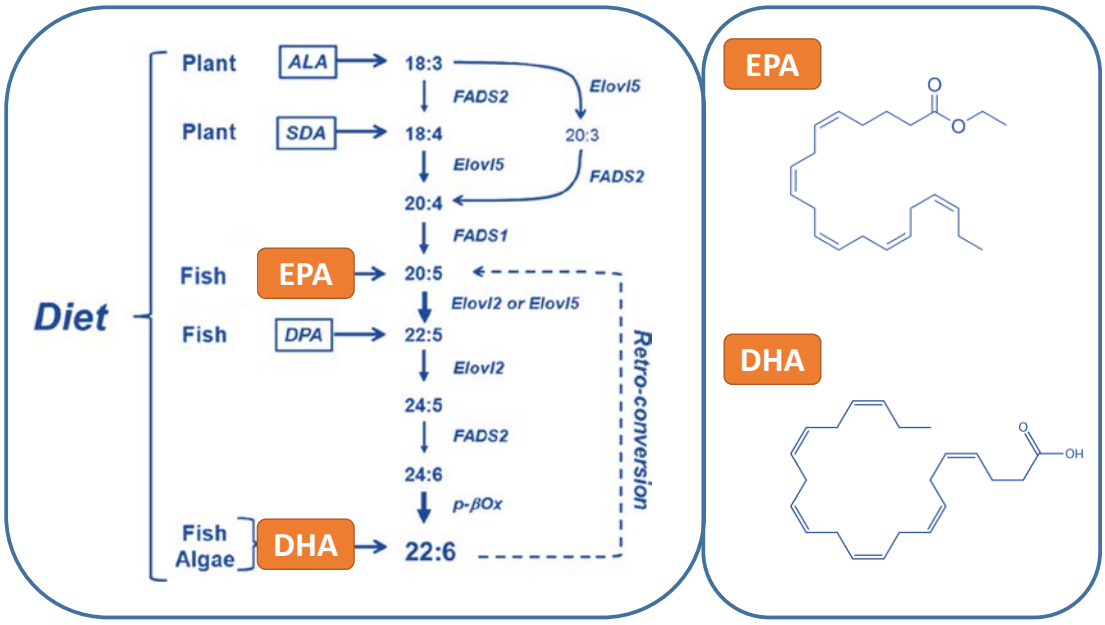

Figure 6. ω-3 PUFAs biosynthesis and chemical structure. Modified from (Jump, et al., 2012). ALA: α-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; SDA: stearidonic acid.

It has been suggested that increased intake of ω−3 PUFAs might reduce cardiovascular disease risk and hypertriglyceridemia, improve some MetS features and have potential anti-obesity properties (Abete et al., 2011; Lorente-Cebrián et al., 2013). Obesity is considered as a chronic low-grade inflammatory disease, which increases the risk of both cardiovascular diseases and insulin resistance (Gregor & Hotamisligil, 2011). In this context, murine models of obesity and clinical trials strongly proposed ω-3 PUFAs supplementation as a potential therapeutic strategy to protect from diet-induced inflammatory state in obesity (Martínez-Fernández et al., 2015).

4.1.1. ω-3 polyunsaturated fatty acid and Alzheimer's disease

Brain composition is highly enriched in fatty acids observing an imbalance of them in elderly. Specially, a ω-3 fatty acids decrease has been reported in AD brains (Cederholm et al., 2013). Moreover, several researches indicate that malnutrition is a common problem of people with dementia, which is associated with cognitive decline. In contrast, it is also true that obesity and high fat diet feeding induce systemic inflammation that leads to neuroinflammation beginning within hypothalamus with outputs to other brain regions. The result is a cognitive impairment mediated by

inflammation in hippocampal and cortical regions where cognitive processing, learning and memory are performed (Miller & Spencer, 2014).

It's widely accepted that ω-3 PUFAs are involved in neuronal development and neuroinflammation, mechanisms that are directly related to AD pathology. Changes in the lipid-metabolism as a source of ROS production, and the implication of a dys-homeostasis within the regulation of cholesterol-derivatives have been described to play an important role in the development of AD (Corsinovi et al., 2011). Indeed, an adequate ω-FAs/cholesterol ratio plays an important role in the regulation of APP-processing pathway, and it has also been suggested that a low consumption of ω-FAs could lead to a major up-regulation of pro-inflammatory responses (Corsinovi et al., 2011). Considering these facts, ω-3 PUFAs might be a promising treatment option for dementia, particularly AD (Burckhardt et al., 2016). **Table 5** shows the outcomes found when using ω-3 PUFAs as therapeutic strategy in experimental models of AD.

Table 5. Use of different ω-3 PUFAs as a possible therapeutic strategy in AD**.**

JNK INHIBITOR	EXPERIMENTAL MODEL	FINDINGS	REFERENCES
DHA	Tg2576 mice on DHA regimen	Decreased PI3K activity- Increase of caspase-cleaved actin.	Calon et al. 2004
	Tg2576 mice on DHA regimen	Decrease of Aβ levels	Lim et al. 2005
	Human neural cells culture	Reduce Aβ peptides production	Lukiw et al. 2005
	Primary cell culture	Supress Aβ-induced IRS-1 inactivation and pTau pathology	Ma et al. 2009
	C57BL/6J mice on DHA regimen	Decrease of both γ and β-secretase activity	Grimm et al. 2011
	CHME3 cell culture	Increase Aβ phagocytosis and decrease TNF-α levels	Hjorth et al. 2013
	5xFAD mice	Reduction Aβ levels, senile plaque and pro-inflammatory markers. Memory improvement	Casali et al. 2015
	Primary microglia and astrocyte cells culture	Reduction of LPS-induced pro-inflammatory cytokines release	Casali et al. 2015
	Human PBMC cell culture	Enhance Aβ phagocytosis	Fiala et al. 2015
	APP/PS1 mice on DHA regimen	Reduced Aβ pathology and cognitive improvement	Teng et al. 2015
	Tg2576 mice on DHA regimen	Changes in brain lipid content in cortex and hippocampus	Bascoul-Colombo et al. 2016
EPA	SH-SY5Y cell culture	Increase of cell survival	Kou et al. 2008
	CHME3 cell culture	Increase Aβ phagocytosis and decrease TNF-α levels	Hjorth et al. 2013
	Human PBMCs	Enhance Aβ phagocytosis by PBMCs	Fiala et al. 2015

CHME3: Human microglial cells line; IRS-1: Insulin receptor substrate-1; LPS: Lipopolysaccharide; PBMC: Peripheral blood mononuclear cells; PI3K: phophoinositol 3 kinase

As explained above, management of chronic neuroinflammation is essential in the development and progression of AD. PUFAs are involved in inflammatory resolution by inducing Aβ phagocytosis, inhibiting pro-inflammatory molecules, and increasing neuronal survival as well as memory improvement (Fraga et al., 2017). In this way, DHA supplementation in APP/PS1 mice reduced Aβ aggregation (Teng et al., 2015). Moreover, DHA treatment decreased TNF-α levels, while EPA increased the production of brain-derived neurotrophic factor (BDNF) in CHME3 microglial cells and both reduce the expression of pro-inflammatory markers, such as CD40 (Hjorth et al., 2013). The administration of ω-3 PUFAs prevented IRS-1 inactivation and pTau pathology by inhibition of the JNK signaling *in vitro* (neuronal cultures from embryonic Sprague Dawley rats), *in vivo* models (3xTransgenic AD mice) and post-mortem human AD samples (Ma et al. 2009).

Regarding clinical trials, the evidence observed *in vitro* and *in vivo* AD experimental models becomes controversial. EPA and DHA supplementation for six months did not reduce cognitive impairment, but seems that longer period of time and the supplementation in early stage of dementia are able to improve AD pathology (Burckhardt et al., 2016; de Souza Fernandes et al., 2015). Despite the absence of clear evidence, the effect of ω-3 PUFAs supplementations should be further explored in both *in vivo* and *in vitro* studies.

4.2. Specialized pro-resolving lipid mediators

ω-3 PUFAs serve as precursors of others molecules involved in resolution of inflammation, namely specialized pro-resolving lipid mediators (SPMs) which have also beneficial effects on inflammatory-related diseases, such as obesity, MetS or neurodegenerative disorders. It is noteworthy to mention that unlike their precursors, these SPMs exert potent actions at pico to nanomolar range, suggesting a potential powerful therapeutic approach to improve obesity and metabolic-related diseases thanks to its anti-inflammatory effect. These novel bioactive lipid mediators are classified reliant on their precursor (**Figure 7**).

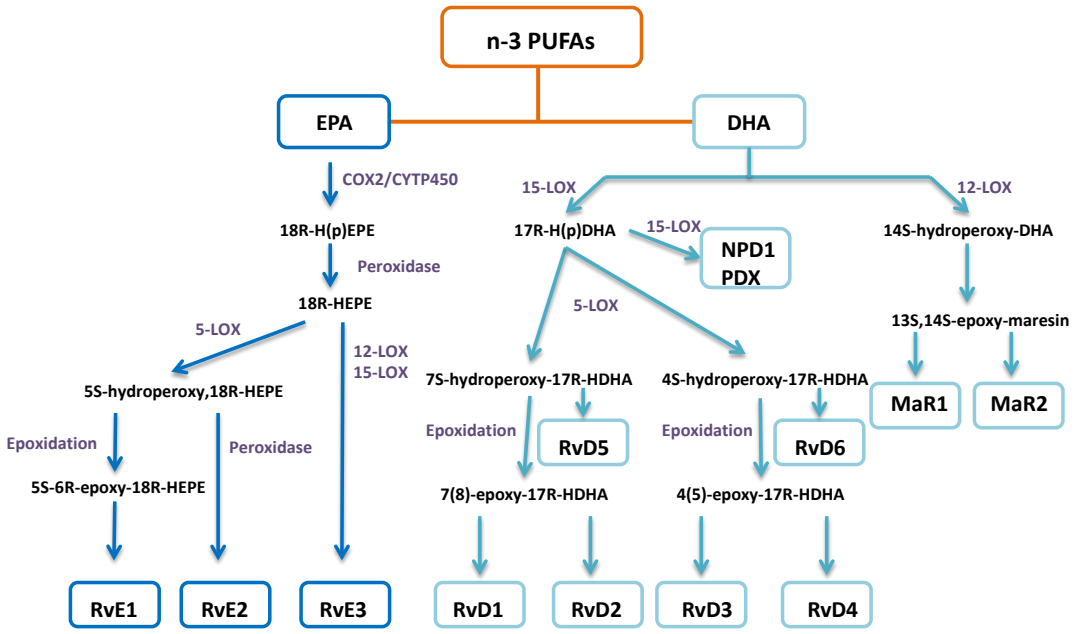

Figure 7. Human specialized pro-resolving lipid mediators (SPMs) biosynthetic routes. SPMs derived from EPA and DHA are classified in 4 families depending on the biosynthetic routes: EPA derived resolvin (RvE), DHA derived resolving (RvD), protectin/neuroprotection (PDX/NPD) and maresins (MaR). The human enzymes involved in the production of SPMs include 15-LOX, 12-LOX, 5-LOX and COX2. COX: cyclooxiygenase; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; LOX: lipoxygensase; MaR: maresins; NPD: neuroprotectins; PDX: protectins; RvE: EPA derived resolvin; RvD: DHA derived resolving. Modified from Martinez-Fernandez et al. (2015).

EPA-derived SPMs are known as E-series Resolvins (RvE1-3) and DHA-derived lipid mediators are named as D-series Resolvins (RvD1-6), Protectins (NPD1 and PDX), and Maresins (MaR1-2) (Serhan, 2014). These SMPs derived from ω-3 PUFAs by the action of different enzymes, including the three major oxygenase enzymes in humans (5-, 12- and 15-LOX) and also through epoxidation and peroxidation reactions. Additionally, the cyclooxygenase-2 (COX2), a key enzyme in the biosynthesis of prostaglandins, and the cytochrome P450 monooxygenases also play a role in some SPMs synthesis (Serhan, 2007).

Interestingly, the ω-6 PUFA arachidonic acid, which is known to have pro-inflammatory actions, also serves as a substrate of SPMs. The arachidonic acid-derived SPMs are called lipoxins. These lipoxins promote resolution and exert similar properties as ω-3 PUFAs derivates, instead of having pro-inflammatory actions as their precursors (Borgeson et al., 2015; Chiang & Serhan, 2017; Serhan, 2017).

Historically, the resolution of inflammation was thought to be a passive process, while is now known to involve several active molecules such as SPMs, which play a major role facilitating inflamed tissues to recover homeostasis (Serhan et al, 2008). Thus, SPMs activate tissue protection and healing through resolving physiological inflammatory responses and acting as more potent anti-inflammatory agents than their precursors.

Indeed, it has been described that SPMs also present a wide distribution in human SNC, including brain and CSF, among others such as spleen or adipose tissue (Serhan, 2017), where they exert anti-inflammatory effects.

SPMs are investigated in AD on the basis of anti-inflammatory effects in neurons, inhibition of apoptosis signalling and induction of Aβ phagocytosis. In this way, RvD1 and RvE1 pretreatment reduced IL-6, IL-1β and TNF-α levels in murine microglial cells BV2 (Rey et al., 2016). In addition, RvD1 showed neuroprotective effects inhibiting Aβ-induced apoptosis (Mizwicki et al., 2013; Zhu et al., 2016). Finally, NPD1 inhibits APP-amyloidogenic processing (Zhao et al., 2011). Therefore, evidences reveal SPMs as a promising therapeutic approach in AD (**Table 6**).

Table 6. Use of different specialized pro-resolving lipid mediators (SPMs) as a possible therapeutic strategy in AD**.**

JNK INHIBITOR	EXPERIMENTAL MODEL	FINDINGS	REFERENCES
RvD1	Peripheral human macrophages	Enhancement Aβ phagocytosis and inhibition of apoptosis	Mizwicki et al. 2013
	CHME3 and SH-SY5Y cell culture	Contribution to neuronal maintenance	Zhu et al. 2016
	Murine microglial cells BV2	Reduce LPS-induced pro-inflammatory markers release	Rey et al. 2016
NPD1	Human neural cells culture	Exertion of neuroprotective and anti-apoptotic effects	Lukiw et al. 2005
	Human neuronal glial culture	Down-regulated expression of pro-inflammatory markers and inhibition of amyloidogenic processing of APP	Zhao et al. 2011
MaR1	Human macrophages culture	Reduction of Zym-induced JNK, c-Jun, p38 and NF-κB phosphorylation	Li et al. 2013a
	CHME3 and SH-SY5Y cell culture	Decrease pro-inflammatory markers and apoptosis signals. Increase of Aβ phagocytosis	Zhu et al. 2016

CHME3: Human microglial cells line; LPS: Lipopolysaccharide; SPMs: Specialized pro-resolving lipid mediators; Zym: zymosan.

4.2.1. Maresin 1 in Alzheimer's disease

The "**Ma**crophage mediator in **res**olving **in**flammation" (Maresins) are a family of lipid mediators biosynthesized from DHA via 12-LOX through macrophages action. Maresin 1 (7R,14S-dihydroxy-docosa-4Z,8E,10E,12Z,16Z,19Z-hexaenoic acid or MaR1), was the first member of this family identified. The biosynthesis of MaR1 takes place during inflammation resolution. In fact, one of the intermediate in MaR1 biosynthesis, 14S-hydroperoxydocosa-4Z,7Z,10Z,12E,16Z,19Z-hexaenoic acid (14-HpDHA) derived from endogenous DHA has been reported to be accumulated in the exudates of mouse peritonitis during resolution. Then, 14-HpDHA is converted to 13S,14S-epoxy-maresin through epoxidation (Dalli et al. 2016). Finally, an enzymatic hydrolysis through hydrolases converts 13S,14S-epoxy-maresin into MaR1. Maresins have been predominantly detected as products synthetized by monocytes/macrophages upon acute inflammatory response. MaR1 has been suggested to exert strong anti-inflammatory effects and potent pro-resolutive actions (Serhan, 2009). Additionally, it has been related to have pro-healing properties at similar extent to other members of the ω-3 PUFAs-derived SPMs (Serhan et al., 2012; Tang et al., 2013).

Deficiencies in SPMs levels, particularly MaR1, in AD brain turns into a decrease in the resolution of inflammation and deprivation of protective and restorative signals in neurons, being able to contribute to AD pathogenesis. Decreased levels of MaR1 have been observed in hippocampus and entorhinal cortex of AD cases (Zhu et al., 2016). Importantly, as described above, MaR1 effects have been described related to AD pathology (**Table 6**). MaR1 reduced neuronal apoptosis and improved cell survival in SH-SY5Y neuroblastoma cells. Moreover, Aβ–induced microglial activation was downregulated by MaR1, because pro-inflammatory markers decline, such as CD40 or CD11. Additionally, MaR1 induced Aβ phagocytosis (Wang et al., 2015; Zhu et al., 2016).

The mechanism of action of MaR1 has not still been established, but its similar structure to PD1 and RvD1 suggests that MaR1 might bind to peroxisome proliferator associated receptor (PPAR-γ). PPAR-γ is a receptor for several different PUFAs and SPMs, including DHA or RvD1, which was shown to be involved in neuroprotection. On the other hand, supporting this idea, a study on human macrophages described that MaR1 and RvD1 decreased in zym-induced phosphorylation of JNK and p38, an effect

mediated by NF-κB inhibition (Li et al. 2013a). Keeping in mind that Aβ increases the phosphorylation of JNK and p38 in CHME3 microglial cells and taking into account that ω-3 PUFAs are considered natural inhibitors of JNK, it is suggested that MaR1 could be a potential compound for AD treatment (**Table 6**).

HYPOTHESIS AND AIMS

1. JUSTIFICATION FOR THE STUDY

AD is the main cause of dementia and there is currently no treatment. The different clinical trials performed so far have failed and none of them have been able to reverse or halt dementia in patients. For this reason, it is essential to propose new strategies or therapeutic approaches for the disease (Scheltens et al., 2016).

In last years, different studies have demonstrated the involvement of MAPK pathways in the mechanisms of neuronal death and neuroinflammation, characteristics of neurodegenerative diseases, such as AD (Ferrer et al., 2001; Lin et al., 2013). In this context, chronic neuroinflammation induces neuronal damage through the activation of different signaling pathways. Among them, JNK activation could play important role in neurodegenerative diseases. Changes in the brain of AD patients, such as rupture of the BBB or Aβ aggregation, have been reported to induce JNK activation (Zhang & Jiang, 2015).

Activation of JNK could lead to maturation of NFTs, formation of senile plaques and mitochondrial stress. In addition pJNK produces neuroinflammation and activation of caspases that induce apoptosis. Therefore, inhibition of JNK is postulated as a promising therapeutic strategy that should be investigated in AD.

2. HYPOTHESIS

Based on all the observations previously mentioned, the hypothesis of the present study is that an over-activation of JNK may be contributing to the neuropathology in AD, and that JNK inhibition could constitute a new therapeutic target in AD.

3. AIMS

The main aim of the present study is to validate JNK as a biological target for the diagnosis and treatment of AD, as well as to determine the ability of ω-3 PUFAs (DHA, EPA and MaR1) to reverse the functional alterations derived from JNK activation in animal models of AD.

To achieve the main objective, the proposed specific aims are:

1. To study the implication of JNK in the pathological processes of AD.

   1.1. Analysis of pJNK levels in mouse models of AD.

   Levels of activated JNK (pJNK) will be measured in a familiar type AD mouse model (Tg2576) and a sporadic type AD mouse model (SAMP8).

   1.2. Measurement of pJNK in AD and other dementias.

   Levels of pJNK will be measured in post-mortem human samples of AD. The selectivity of this activation will be assessed by studying pJNK levels in other dementias (Lewy body dementia, frontotemporal dementia and vascular dementia).

   1.3. Characterization of pJNK as a potential new AD biomarker.

   To achieve this aim, levels of pJNK will be analyzed in cerebrospinal fluid (CSF) of control, mild cognitive impairment (MCI), progressive mild cognitive impairment (PMCI) and AD.

2. To investigate the relationship between pJNK and Aβ in AD.

   2.1. Measurement of Aβ levels and its relation to pJNK in AD murine models

   To this end the same mouse models as in objective 1.1. will be used.

   2.2. Assessment of Aβ levels in AD and other dementias.

   To perform this aim Aβ levels and its relation to pJNK will be analyzed in the same postmortem brain tissue described in aim 1.2.

   2.3. Study of the co-localization of Aβ and pJNK.

   To this end immunohistochemistry analysis will be performed in murine models of AD and postmortem brain tissue.

2.4. Investigate the causal relationship between Aβ increase and pJNK activation.

To achieve this aim $A\beta_{42}$ will be ICV injected to wild type mice in order to study the subsequently JNK activation.

3. Validate JNK inhibition as a treatment for AD.

3.1. Three JNK inhibitors of ω-3 PUFAs group (DHA, EPA and MaR1) will be studied in order to assess in their ability to reverse the cognitive impairment showed by the mouse model of sporadic AD (SAMP8).

3.2. To demonstrate the plausible mechanism underlying the beneficial effects of ω-3 PUFAs on cognition. To achieve this aim, Tau hyperphosphorylation and endoplasmic reticulum (ER) stress markers will be analyzed.

EXPERIMENTAL DESIGN AND METHODS

1. HUMAN SAMPLES

Brain tissues were obtained from the Brains for Dementia Research Initiative Network (BDR). At death, informed consent had been obtained from the patients' next-of-kin before collection of brains. To exclude the effect of ageing, controls were split in mature and old subgroups according to their mean age (mature 65.10 ± 3.86; old 77.14 ± 2.77). All cases were selected based on clinic-pathological consensus diagnoses. AD cases were clinically diagnosed on the basis of meeting the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria for a diagnosis of probable or definite AD, LBD according to international consensus criteria and FTD according to Movement Disorders Society criteria. VaD was defined by the presence of multiple or cystic infarcts. Neuropathological assessment was performed according to standardized neuropathological scoring/grading systems, including neurofibrillary Braak staging (threads and tangles), CERAD scores (neuritic plaques), Newcastle/McKeith Criteria for Lewy body disease (Lewy bodies and neurites), National Institute on Aging—Alzheimer's Association guidelines and phases of Aβ deposition (as described in Howlett et al., 2014). Frontal (Brodmann Area, BA10) cortex were dissected free of meninges. All tissue used had a brain pH>6.1, condition used as an indication of tissue quality in post-mortem research (Lewis, 2002). The demographic characteristics are described in detail in **Table 7.**

Table 7. Demographic features of patients.

DIAGNOSIS	SAMPLE SIZE (n)	GENDER (MALE/FEMALE)	AGE AT DEATH	PMD (h)
Controls				
Mature	10	5/5	65.10 ± 3.86	34.10 ± 4.91
Old	16	6/10	77.14 ± 2.77	49.42 ± 6.82
AD	16	5/11	81.20 ± 1.90	90.90 ± 6.21
FTD	11	6/5	61.91 ± 2.48	31.00 ± 4.38
LBD	8	4/4	74.50 ± 2.73	28.12 ± 4.27
VaD	8	4/4	72.12 ± 3.77	29.12 ± 7.53

AD: Alzheimer's disease; FTD: frontotemporal dementia; LBD: Lewy body dementia; PMD: post-mortem delay (hours); VaD: vascular dementia.

Immunohistochemical studies were performed in brain slices from AD patients at Brack V-VI stage obtained from Banco de Tejidos Neurológicos de Navarra (Spain).

Cerebrospinal fluid (CSF) samples were collected from 74 patients included in a study conducted by the Karolinska University Hospital (Sweden). As shown in **Table 8**, 22 patients with subjective cognitive impairment (SCI) were grouped as controls because they didn't present complaints on objective cognitive tasks, 24 patients as mild cognitive impairment (MCI), 9 progressive MCI evolving to AD (PMCI), and 19 as AD. The different dementia states were diagnosed according to DSM-IV and NINCDS-ADRDA criteria as previously described (Gil-Bea et al., 2010a). CSF samples were obtained by lumbar puncture from L3/L4 o L4/L5 interspaces in the mornings. After disposal of the first milliliter, the following 10 mL were collected in polypropylene tubes. No sample containing more than 500 erythrocytes/µL of CSF was used. Samples were gently mixed to avoid gradient effects and centrifuged at 2000 x g at 4ºC for 10 min to eliminate cells an insoluble material. Supernatants were aliquoted, immediately frozen and stored at -80ºC.

Table 8. Clinical and demographic characterization of cerebrospinal fluid (CSF) donors.

CHARACTERISTICS	SCI	MCI	PMCI	AD
Sample size (n)	22	24	9	19
Gender (male/female)	9/13	16/8	3/6	6/13
Age (years)	57.14 ± 6.7	61.52 ± 8.5	63.31 ± 2.2	69.23 ± 7.4
$A\beta_{42}$ levels (ng/L)	852.4 ± 31.6	676.4 ± 49.1 **	424.0 ± 36.3 **,^^	477.3 ± 30.8**,^^
Tau total (ng/L)	275.5 ± 23.9	409.5 ± 120.5	466.5 ± 74.9	733.0 ± 100.5**,^
p-Tau_{181} (ng/L)	51.6 ± 4.0	56.2 ± 8.6	71.4 ± 15.4	98.7 ± 11.6**,^^

Numeric values are presented as the number of patients or mean ± SEM. The current data of different protein measurements in CSF were determined in the same samples reported by our group in previous studies (Gil-Bea et al., 2010a).*p<0.05 vs. SCI; **p<0.01 vs. SCI; ^p<0.05 vs. MCI; ^^p<0.01 vs. MCI.
$A\beta_{42}$: β-amyloid; AD: Alzheimer's disease; MCI: stable mild-cognitive impairment. PMCI: mild-cognitive impairment with AD progression; SCI: controls, subjective cognitive impairment.

2. ANIMALS

Animals were housed in a temperature (21 ± 1ºC) and humidity (55 ± 1%) controlled room on a 12h light/dark cycle. Experimental procedures were conducted in accordance with the European and Spanish regulations (2003/65/EC; 1201/2005) for the care and use of laboratory animals and approved by the Ethical Committee of University of Navarra (068-11).

2.1. Tg2576 mice

In the present study, 9- and 16-month-old Tg2576 AD transgenic mice with the Swedish mutation and wild-type (WT) mice with the same background (C57BL/6J) were used. The sample size was 34 mice distributed in four different groups: 9-months-old WT mice (n=10), 9-months-old Tg2576 mice (n=9), 16-months-old WT mice (n=7) and 16-months-old Tg2576 mice (n=8). This AD mouse model over-expresses the human APP, thus Aβ accumulation is exponentially associated to ageing. This mouse model has a normal development, but age-associated cognitive deficits are found. Studies have reported an impaired spatial learning and working memory by 9 months of age (Hsiao et al., 1996). This fact coincides with Aβ deposition and formation of senile plaques in time (Foley et al., 2015). However, NFTs and neuronal loss is absent in this model (Irizarry et al., 1997). Gliosis is another hallmark of this murine model: microglial density is increased in the hippocampus and frontal and occipital cortex from 10 months old (**Figure 8**; Frautschy et al., 1998).

2.2. SAMP8 mice

As sporadic AD murine model, the SAMP8 mouse model developed by professor Takeda from the department of pathology of Kyoto's University has been chosen. The Jackson Laboratory (Bar Harbor, Maine, USA) donated several pairs of AKR/J mice to professor Takeda, some of which showed features of an early senescence after sister-brother mating continued: loss of activity, alopecia and lack of glossiness, skin coarseness, periophthalmic lesions, increased lordokyphosis of the spine and a shortened life span, despite the relatively low incidence of thymic lymphoma. These litters were selected as progenitors of the "senescence-prone" (P series) and the rest of the litters were selected as "senescence-resistant" (R series) progenitors. Eleven senescence-prone subtypes of mice and seven senescence-resistant strains were obtained from the different P and R series, respectively. Life spam from the SAMP ranges from 10 to 17 months and is markedly shorter tan SAMR, which ranges from 19 months to 21 months. Due to its phenotypical characteristics, SAMP8 mice have

received attention in dementia research. Indeed, this mouse model shows most of the characteristics present in AD patients such as age-related learning and memory deficits, oxidative stress, neuroinflammation, as well as the main AD hallmarks which are Tau hyperphosphorylation and Aβ accumulation (Flood & Morley, 1998; Takeda et al., 1981).

The time line of the appearance of the phenotypical characteristics in the SAMP8 mice is the following: oxidative stress appears at 2 month of age; hippocampal cognitive deficit, as well as, Tau phosphorylation start at 6 months of age; increased soluble Aβ , as well as, gliosis at 8 months of age and, finally, Aβ peptide accumulation begins after 12 months of age (Pallas et al., 2008; **Figure 8**). Due to this phenotype, the model is not only suitable for studying age-related learning and memory deficits, but may also be useful for studying Aβ-mediated effects in cognitive decline. Therefore, as these latter effects are of great relevance to AD, in the last years SAMP8 mice have been widely used as an AD mouse model.

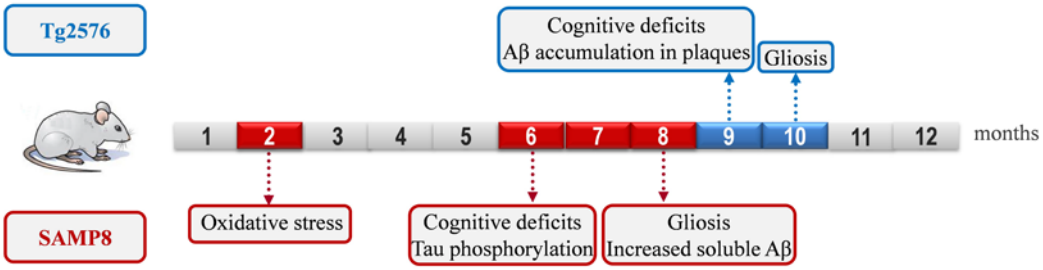

Figure 8. Phenotypical characteristics developed by Tg2576 and SAMP8 mouse models. Schematic representation of the time line of the appearance of the phenotypical characteristics in the two AD murine models: Tg2576 and SAMP8.

The characterization of pJNK and Aβ levels in the sporadic AD mouse model were carried out using a total of 66 mice which were divided in 6 subgroups: SAMR1 6-months-old (n=12), SAMP8 6-months-old (n=10), SAMR1 10-months-old (n=17) and SAMP8 10-months-old (n=8). Number of animals was dependent on availability.

In order to study the ability of ω-3 PUFAs (DHA, EPA and MaR1) to reverse the functional alterations derived from JNK activation, 9-months-old SAMP8 and SAMR1 mice were chosen, divided in different groups depending on the treatment: SAMR1 Saline (n=10), SAMP8 Saline (n=8), SAMP8 MaR1 (n=8), SAMP8 DHA (n=8) and SAMP8 EPA (n=8).

Mice were orally treated with 200 mg/kg of DHA, 200 mg/kg of EPA and 25 μg/kg of MaR1 for 20 days. The drugs were purchased from Cayman Chemical (Michigan, USA). Behavioral test carried out with these animals are described in section 4.

3. Aβ INTRACEREBROVENTRICULAR INJECTION IN WT MICE

ICV injection of Aβ was performed in 14 WT (C57BL/6N) mice of 9-months-old divided in two groups (Sham and Aβ injected) in order to study the causal relationship between pJNK and amyloidogenesis.

$A\beta_{42}$ peptide was purchased by Bachem laboratories (Bubendorf, Switzerland). For assuring monomerization, 1 mg of $A\beta_{42}$ protein was dissolved to 1 mM in hexafluoroisopropanol (HFIP) and aliquoted into microcentrifuge tubes, then the HFIP was evaporated, and the peptides were stored at −20°C until use. For oligomeric assembly, concentrated peptides were suspended in DMSO and then diluted to 100 μM in PBS and incubated at 4°C for 24 h. ICV injection of $A\beta_{42}$ (300 pmol in 3 μL sterile dH_2O containing 10% DMSO) was stereotaxically performed in both lateral ventricles of 9 month-old-male WT mice (anterior–posterior, +0.3 mm; lateral, 1.0 mm; horizontal, 3.0 mm from the bregma). Sham animals received equivalent amounts of sterile phosphate buffer saline. Mice were sacrificed 7 days after the injection.

4. BEHAVIORAL TEST

Behavioral experiments were conducted between 09:00 h and 13:00 h. Observers were blind to the treatment. All behavioral tests were carried out in the same cohort of animals (**Figure 9**).

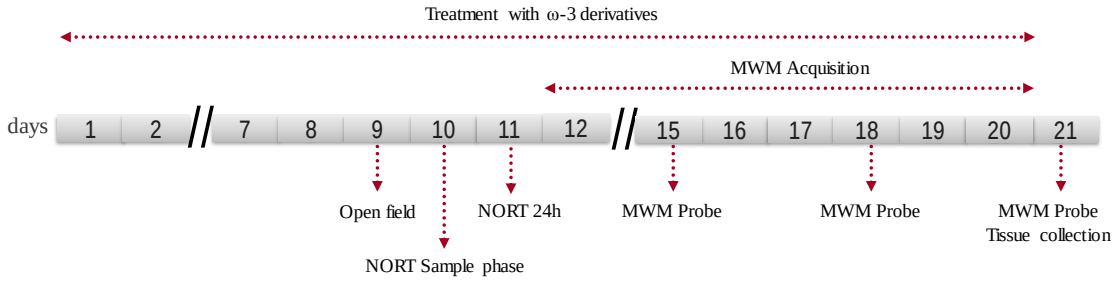

Figure 9. Schematic representation of the experimental design. MWM, Morris water maze; NORT, novel object recognition test.

The behavioral tests were conducted before daily oral administration of different treatments.

4.1. Open field

Locomotor activity was measured for 30 min in an open field (35 × 35 cm, 45 cm height) made of black wood, using a video-tracking system (Ethovision 11.5, Noldus Information Technology B.V., The Netherlands), in a softly illuminated room. Total path velocity (cm/s) was analyzed.

4.2. Morris water maze (MWM)

The MWM, a hippocampus-dependent learning task, was used to test spatial memory and to evaluate the working and reference memory functions.

The water maze is a circular pool (diameter of 145 cm) filled with water (21–22°C) and virtually divided into four equal quadrants identified as northeast, northwest, southeast, and southwest.

To test learning capacity, hidden-platform training was conducted with the platform placed in the northeast quadrant 1 cm below the water surface over 9 consecutive days (4 trials/day). Several large visual cues were placed in the room to guide the mice to the hidden platform. Each trial was finished when the mouse reached the platform (escape latency) or after 60 s, whichever came first. Mice failing to reach the platform were guided onto it. After each trial mice remained on the

platform for 15 s. To test memory, probe trials were performed at the 4th, 7th and last day of the test (10th day). In the probe trials the platform was removed from the pool and mice were allowed to swim for 60 s. The percentage of time spent in the target quadrant was recorded. All trials were monitored by a video camera set above the center of the pool and connected to a video tracking system (Ethovision 11.5; Noldus Information Technology B.V, Wageningen, Netherlands).

4.3. Novel object recognition test (NORT)

The open field consisted of a square divided into four sections (35 cm × 35 cm × 45 cm each) with black walls. On the previous day to the experiment, animals were familiarized with the square for 30 min. The test consists of 2 trials of 5 min: sample phase and 24 h trial. During the first trial, two identical objects were placed inside the cubicle, and the mice were allowed to explore. The second task took place 24 h later in which one object was replaced by another and the exploration time was recorded for 5 min. Results were expressed as percentage of time spent exploring the new object with respect to the total exploration time (discrimination index). It is important to highlight that the exploration was considered complete when the nose of the mouse was oriented within 2 cm of the object. This behavioral test was carried out using video-tracking system (Ethovision 11.5; Noldus Information Technology B.V, Wageningen, Netherlands).

5. BIOCHEMICAL MEASUREMENTS

5.1. Tissue collection

Mice were sacrificed by decapitation between 09:00–12:00 h. Brains were removed and dissected on ice to obtain the hippocampus and frontal cortex and stored at −80°C. For immunohistochemistry assays, left hemispheres from 5 mice per

group were fixed by immersion in 4% paraformaldehyde in 0.1 M PBS (pH=7.4) for 24 h followed by 20% sucrose solution. Brains were cut into series of 40 µm slides.

5.2. Western blotting

Total tissue homogenates were obtained by homogenizing the cortex or hippocampus in ice-cold RIPA buffer (50 mM Tris-HCl pH=7.4, 0.25% DOC, 1% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 µg/ml leupeptin, 1 µg/ml aprotinin, 1 mM Na_3VO_4, 1 mM NaF), centrifuged at 14000 × *g* 4°C for 20 min, and the supernatant was aliquoted and frozen at −80°C.

BA10 from patients or frontal cortex of Tg2576 mice homogenates were separated on 7.5% polyacrylamide gels. The primary antibodies used are described in **Table 9**. Secondary antibodies conjugated to IRDye 800CW or IRDye 680CW (LI-COR Biosciences, Lincoln, NE) were diluted to 1:5000 in TBS with 5% BSA. Bands were visualized using Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). β-actin (mouse monoclonal, 1:10000, Sigma-Aldrich) was used as an internal control.

Table 9: List of primary antibodies used for Western Blot studies.

PROTEIN	PRIMARY ANTIBODY DILUTION	MOLECULAR WEIGHT	COMPANY
pJNK (Thr183/Tyr185)	1:1000	46, 54kDa	Cell Signaling Technology, MA, USA
JNK	1:1000	46, 54kDa	Cell Signaling Technology, MA, USA
p-c-Jun (Ser73)	1:500	48kDa	Cell Signaling Technology, MA, USA
pTau AT8 (Ser202/Tyr205)	1:1000	79kDa	Thermo Fisher Scientific, MA, USA
Tau	1:1000	45 to 60 kDa	Sigma-Aldrich, St. Louis, MO, USA
pIRE1 (Ser724)	1:1000	110kDa	Abcam, Cambridge, MA, USA

Hippocampus of SAMP8 and SAMR1 mice homogenates (30 µg of protein) were separated by electrophoresis on polyacrylamide gels (7.5%). Membranes were probed overnight at 4 °C with the corresponding primary antibodies (**Table 9**). Secondary antibodies conjugated to IRDye 800CW or IRDye 680CW (LI-COR Biosciences, Lincoln, NE) were diluted to 1:5000 in TBS with 5% BSA. Bands were visualized using Odyssey

Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). β-actin (mouse monoclonal, 1:10000, Sigma-Aldrich) was used as an internal control.

For the visualization of ICV injected Aβ oligomers, increasing concentrations (1, 2.5, 5, 8 and 10 μM) of previously aggregated $A\beta_{42}$ solution was subjected to SDS-PAGE electrophoresis in non-termal denatured conditions (samples were not boiled before loading). The separated proteins were transferred to nitrocellulose membranes for determining the presence of different Aβ aggregates with 6E10 as primary antibody (Abcam, Cambridge, MA, USA). Blots were incubated and revealed under the conditions previously described.

5.3. Measurement of Aβ levels

In human samples and SAMP8 mice samples, $A\beta_{42}$ levels were determined using a commercially available high-sensitive ELISA kit (Wako Pure Chemical Industries, Tokyo, Japan) following manufacturer instructions.

In Tg2576 mice samples, human $A\beta_{42}$ levels were measured using a commercially available ultra-sensitive ELISA kit (Thermo Fisher Scientific Waltham, MA, USA) following manufacturer instructions.

5.4. Measurement of pJNK levels in cerebrospinal fluid (CSF)

pJNK levels were determined in CSF of 74 patients using a commercially available JNK (pT183/Y185 + Total) ELISA kit (Abcam, Cambridge, UK) with several modifications implemented by our group in order to reach the detection limits.

In order to obtain detectable pJNK levels, CSF samples were concentrated 3 times by centrifugal vacuum. Savant Speed Vac is a concentrator equipment that removes solvents from the samples leading to higher sample concentration. For our experiment, 60 μL of CSF samples were concentrated during 55 min at 43^{o}C avoiding protein denaturation due to high temperatures. Concentrated CSF samples were subjected to the ELISA kit above mentioned, following manufacturer instructions.

5.5. Immunofluorescence staining

For immunofluorescence, free-floating brain sections were washed (3 × 10 min) with PBS 0.1 M (pH=7.4) and incubated in blocking solution (PBS containing 0.3% Triton X-100, 0.1% BSA, and 2% normal donkey serum) for 2 h at room temperature. For 6E10 immunostaining, sections were incubated in 70% formic acid for 7 min to expose the epitope. Primary and secondary antibodies were diluted in the blocking solution. Sections were incubated with the primary antibody overnight at 4°C, washed with PBS and incubated with the secondary antibody for 2 h at room temperature, protected from light. The primary antibodies used were anti-pJNK (rabbit monoclonal, 1:250, Cell Signaling Technology, Beverly, MA) and 6E10 (mouse monoclonal, 1:250, Covance, Princeton, NJ). Secondary antibodies used were Alexa Fluor 488 Donkey anti-rabbit and Alexa Fluor 546 Donkey anti-mouse (1:200, Invitrogen-Molecular Probes, Eugene, OR).

In order to develop the human brain sections immunofluorescence, our group set up the paraffin embedded brain sections staining. Slides were dewaxed, washing with xilol, decreasing concentrations of ethanol (100%, 90%, 70%) and water during 5 min every time. In order to block the endogenous peroxidase activity, sections were washed with hydrogen peroxide 3% during 5 min at 37ºC and, then, immersed in dH2O (2 x 5 min) and 0,1M PBS pH=7,4 (2 x 5min).

With the purpose of antigen retrieval, slides were treated with 0,01 M citrate buffer pH=6, microwaved 2 min and, then, washed with dH_2O (2 x 5 min) and 0,1 M PBS pH=7.4 (2 x 5 min). After, they were incubated with blocking solution (PBS containing 0.3% Triton X-100 0.1% BSA and 2% normal donkey serum) for 1 h at room temperature. Primary and secondary antibodies were diluted in the blocking solution. Sections were incubated with the primary antibody overnight at 4ºC, washed with PBS and incubated with the secondary antibody for 2 h at room temperature, protected from light. Finally, black sudan treatment was applied on brain sections and they were washed with 70% ethanol 1 min and dH_2O (3 x 5 min).

The primary antibodies employed were anti-pJNK (rabbit monoclonal, 1:250, Cell Signaling Technology, Beverly, MA) and 6E10 (mouse monoclonal, 1:250, Covance, Princeton, NJ). Secondary antibodies used were Alexa Fluor 488 Donkey anti-rabbit

and Alexa Fluor 546 Donkey anti-mouse (1:250, Invitrogen-Molecular Probes, Eugene, OR).

To ensure comparable immunostaining, sections were processed together under identical conditions. Fluorescence signals were detected with confocal microscope LSM 510 Meta (Carl Zeiss, Oberkochen, Germany).

6. STATISTICAL ANALYSIS

Data were analyzed by SPSS for Windows 15.0 and normality was checked by Shapiro–Wilk's test ($p<0.05$).

In the acquisition phase of the Morris water maze, over-all treatment effects were examined by two-way repeated measures ANOVA (treatment x trial). Differences between trials within groups were analyzed using a factorial ANOVA with replicates. Data in the retention phase were analyzed with one-way ANOVA.

Neurochemical data was analyzed by Student´s t-test, one- or two-way ANOVA.

Correlation between variables was investigated by Pearson's or Spearman´s correlation coefficients, depending upon the normality of variables.

RESULTS

1. SPECIFIC INCREASE OF pJNK IN AD

As already described in introduction, JNK is involved in gene expression, inflammation, cell proliferation and apoptosis; therefore it is suggested that JNK activation plays a key role in neurodegenerative disorders, especially AD. The present study aimed to evaluate the expression of activated JNK (pJNK) in AD experimental models and human samples, in order to determine its possible implication in AD pathology.

1.1. Increased pJNK levels in AD experimental mouse models

As depicted in **Figure 10A**, significant increased levels of pJNK were found in 9-month-old and 16-month-old Tg2576 mice frontal cortex compared to WT animals (two-way ANOVA, main effect of genotype, $F_{(1,16)}$ =5.467; $p<0.05$). Moreover, pJNK levels significantly increase in 16-month-old group compared with 9-month-old mice (two-way ANOVA, main effect of age, $F_{(1,16)}$=84.48; $p<0.001$).

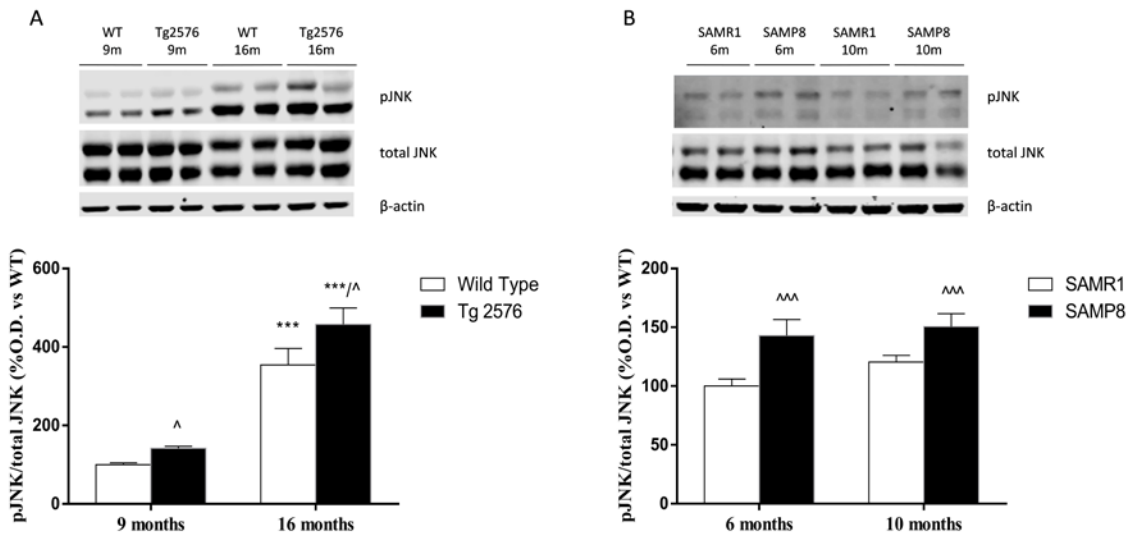

Figure 10. JNK immunoblotting in 9-month-old and 16-month-old wild-type (WT) and Tg2576 mice frontal cortex (panel A) and in 6-month-old and 10-month-old SAMP8 mice frontal cortex (panel B). In each panel, a representative picture of western blot is shown. Results are expressed as % optical density (O.D.) of correspondent controls and normalized to total levels of JNK. ^main effect of genotype; * main effect of age, two-way ANOVA.

In the other mouse model analyzed, a significant increase in pJNK levels in SAMP8 mice in all ages was observed (two-way ANOVA, main effect of genotype, $F_{(1,28)}$= 14.08; p<0.001; **Figure 10B**).

Therefore, pJNK is increased in both AD experimental mouse models used.

1.2. Specific increases in pJNK levels in AD human samples

In order to confirm that the increase in JNK activation observed in both AD mouse models is also observed in AD patients, pJNK levels were measured in frontal cortex (BA10) of post-mortem samples.

The demographic characteristics of brain samples used were detailed in **Table 7**. There were significant differences between age at death among the different pathological conditions (one-way ANOVA; $F_{(3,42)}$=11.065, p<0.001), being AD cases older than the rest of the groups (p<0.01). In addition, it is to note that in controls, pJNK expression correlated with age (Pearson's, r=0.435; p<0.05, n=26). In order to match for age in all pathological conditions, two subset of controls were used, one being considered mature controls (age at death=65.1±3.86, n=10), being these the samples used as controls for the FTD, VaD and LBD samples, and the other group was named old controls (age at death=77.14±2.77, n=16) and was used as control for AD samples. There were no statistical differences regarding sex or post-mortem delay between groups.

Significant increases in pJNK levels were seen (Student t test, p<0.05) in the frontal cortex of patients with AD compared with controls (**Figure 11A**). Total levels of JNK did not differ between controls and AD group. pJNK levels in AD did not differ by sex (male/female, Student t test, p>0.05).

Based in the literature, increases in pJNK levels could be considered a common denominator for all types of dementia. However, our results do not support this possible hypothesis because pJNK levels were similar (one-way ANOVA; $F_{(3,31)}$= 1.210; p>0.05) in all dementia groups (LBD, FTD or VaD) compared to control samples (**Figure 11B**). Total levels of JNK did not differ from controls in any of the pathological groups.

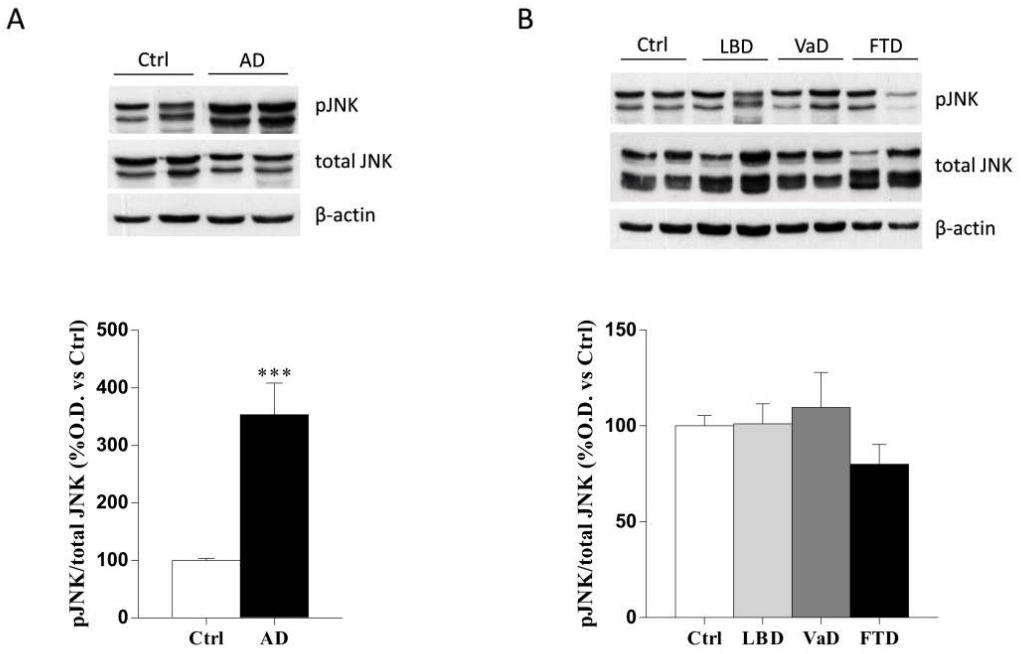

Figure 11. JNK immunoblotting in control and Alzheimer´s disease (AD) frontal cortex (BA10) (panel A) and in Vascular dementia (VaD), Lewy body dementia (LBD) and Frontotemporal dementia (FTD) (panel B). In each panel, a representative picture of western blot is shown. Results are expressed as % optical density (O.D.) of controls and normalized to total levels of JNK. ***p<0.001, Student´s t-test.

For this reason, it could be suggested that the increase of pJNK levels in frontal cortex is specific of AD and no other dementias.

1.3. pJNK as a potential AD biomarker

pJNK was measured in CSF samples. It is noteworthy that we needed to develop technique implementations to achieve this aim, explained in detail in methods.

As described in **Figure 12**, our results revealed increased CSF pJNK levels in AD compared with the control (SCI) and MCI groups (One-way ANOVA, $F_{(3,70)}$=4.484, p<0.01; Tukey test p<0.05).

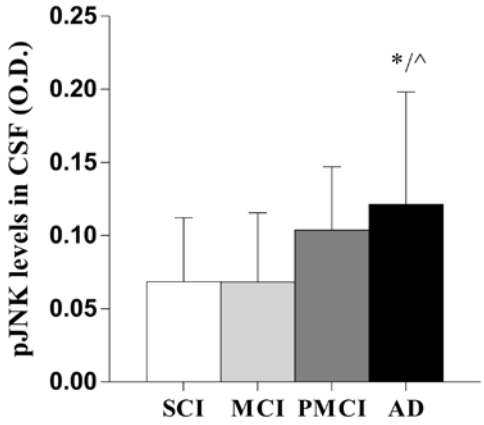

Figure 12. CSF levels of pJNK in patients diagnosed with clinical and prodromal stages of AD. SCI: Controls, subjective cognitive impairment; MCI: Stable mild- cognitive impairment; PMCI: Mild-cognitive impairment with AD progression; AD: Alzheimer's disease. Data are expressed as total levels of pJNK as optical density (O.D.). *p<0.05 vs. SCI, ^p<0.05 vs. MCI; one-way ANOVA; Tukey p<0.05.

2. RELATIONSHIP BETWEEN pJNK AND Aβ LEVELS IN AD

2.1. Aβ levels in mice models of AD

As it has been described before, both AD mouse models (Tg2576 and SAMP8) assessed in the present work show most of the characteristics present in AD patients, such as Aβ peptide accumulation, one of the main AD hallmarks. Aβ peptide originates from the proteolysis of APP and it has the ability to accumulate in Tg2576 model while it remains soluble in SAMP8 mice without aggregation.

As depicted in **Figure 13A**, significant increases in $A\beta_{42}$ levels were observed in 9-month-old and 16-month-old Tg2576 mice frontal cortex compared to wild-type animals (two-way ANOVA, main effect of genotype, $F_{(1,22)}$ =181.2; p<0.001). Moreover, it was observed that this increase follows an age fashion as $A\beta_{42}$ levels are significantly higher in 16-month-old mice with respect to 9-month-old mice (two-way ANOVA, main effect of age, $F_{(1,22)}$ =12.04; p<0.01). Aβ levels in 9-months-old WT were 7.18 ± 1.13 pg/mL.

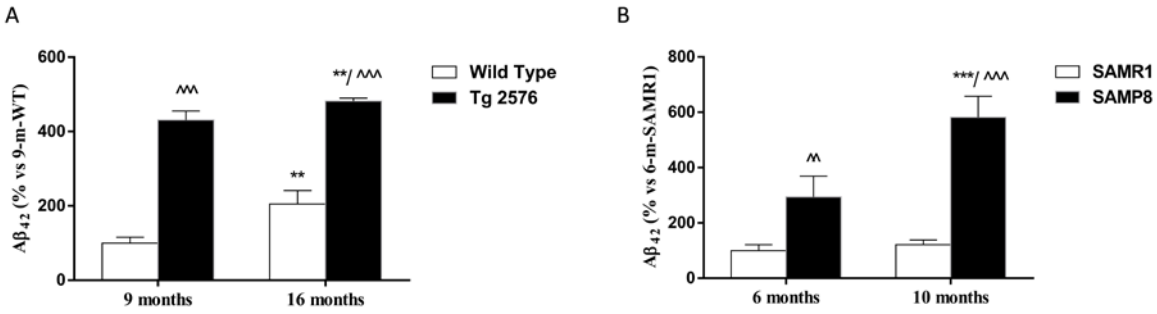

Figure 13. Aβ levels in mouse models of AD. In panel A, $A\beta_{42}$ levels in 9-month-old and 16-month-old wild-type (WT) and Tg2576 mice frontal cortex. ^main effect of genotype; *main effect of age; two-way ANOVA. In panel B, $A\beta_{42}$ levels in 6-month-old and 10-month-old SAMR1 and SAMP8 mice frontal cortex. ^vs. 6-month-old SAMR1; *vs. 6-month-old SAMP8; two-way ANOVA; Tukey $p<0.05$. Levels of $A\beta_{42}$ are expressed as % versus its corresponding control.

Interestingly, regarding $A\beta_{42}$ levels in sporadic mouse model of AD, significant interaction between age and strain was observed (two-way ANOVA, $F_{(1,27)} = 11.41$; $p<0.01$; **Figure 13B**). Further analysis showed increased levels of Aβ peptides in 10-month-old SAMP8 mice compared to the rest of the groups (Tukey, $p<0.001$) and 6-month-old SAMP8 mice in comparison with 6-month-old SAMR1 mice (Tukey, $p<0.01$). Aβ levels in 6-month-old SAMR1 were 6.40 ± 2.02 pg/mL.

Therefore, the increase observed in pJNK and Aβ levels present a very similar pattern in both AD mouse models, reinforcing the possible relationship between pJNK and Aβ in AD pathology.

2.2. Aβ levels in human samples of AD and other dementias

$A\beta_{42}$ levels were significantly increased in AD cases compared to controls (Student t-test $p<0.05$; **Figure 14A**) and not in other dementias (one-way ANOVA; $F_{(3,36)}=1.210$, $p>0.05$; **Figure 14B**).

There was a significant correlation between enhanced pJNK expression in BA10 and $A\beta_{42}$ levels (Spearman´s rho = 0.733, $p<0.05$, n=16) in AD. Interestingly, no correlation was found between pJNK expression in BA10 and $A\beta_{42}$ levels in any other

type of dementia (Spearman´s rho = 0.143, p>0.05, Spearman´s rho = -0.143, p>0.05 and Spearman´s rho = 0.405, p>0.05 for LBD, FTD and VaD, respectively).

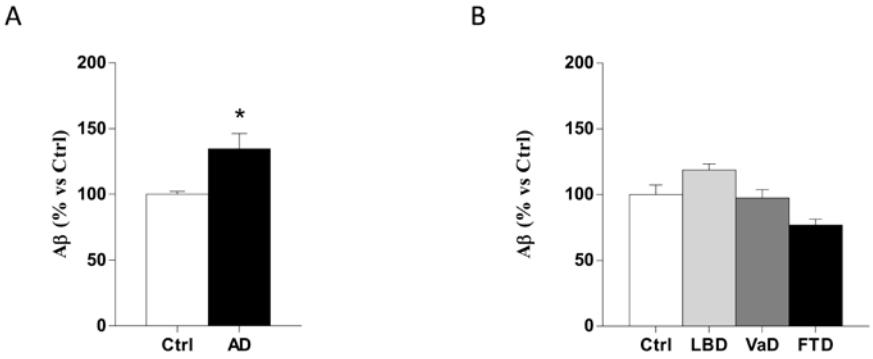

Figure 14. Aβ levels in Alzheimer´s disease (AD) cases (panel A) and in Vascular dementia (VaD), Lewy body dementia (LBD) and Frontotemporal dementia (FTD) (panel B). Levels of $A\beta_{42}$ are expressed as % versus its corresponding controls (Ctrl). *p<0.05, Student´s t-test.

These observations allow us to propose the positive correlation among pJNK levels and Aβ as a characteristic exclusive of the pathology of AD, underlining the similarity between the experimental mouse models and the pathogenesis in patients.

2.3. pJNK and Aβ co-localize in AD human samples and in Tg2576 mouse model

In order to deepen in the understanding of the positive correlation between pJNK and Aβ levels, co-localization studies of both compounds in frontal cortex from patients and AD mouse model brains were performed. Co-localization studies were not performed in SAMP8 mouse model because murine Aβ does not accumulate in plaques and are not detectable in immunohistochemical analysis.

Our inmunohistochemical results revealed that pJNK in Tg2576 mice brains was detected around the amyloid plaque. In matched aged control mice, pJNK immunolabelling was not seen (**Figure 15)**.

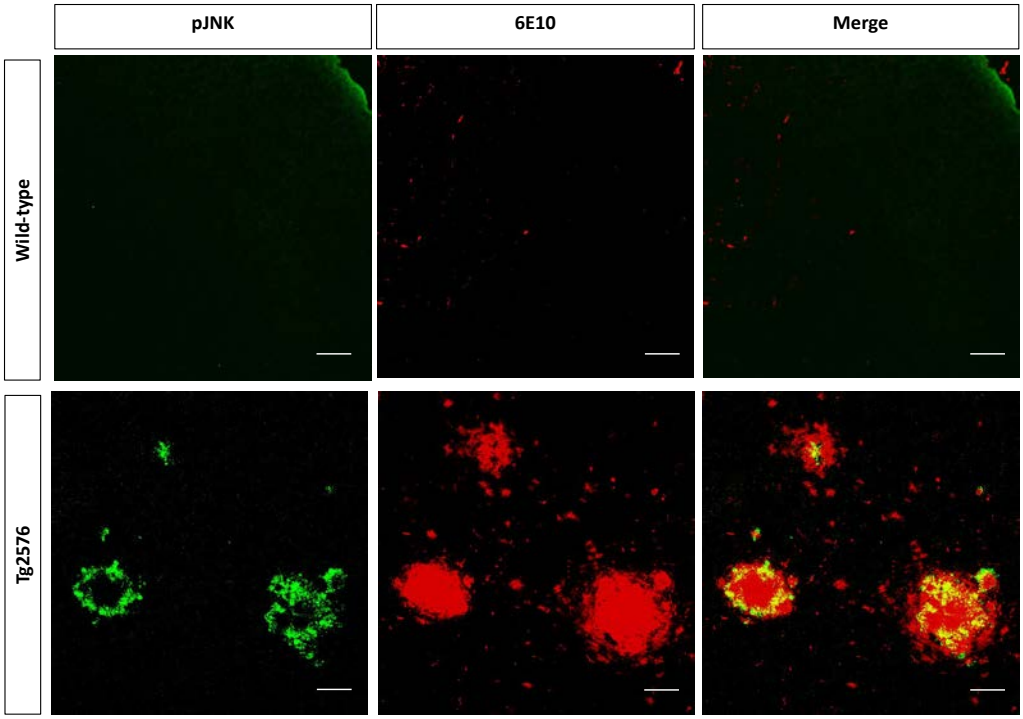

Figure 15. pJNK and 6E10 (β-amyloid marker) immunostaining in frontal cortex of Tg2576. Scale bar 50 µm.

The same pattern was observed in human samples, as AD patients presented pJNK and senile plaques co-localization (**Figure 16)**, reinforcing the idea of a strong association between activated JNK and Aβ in AD.

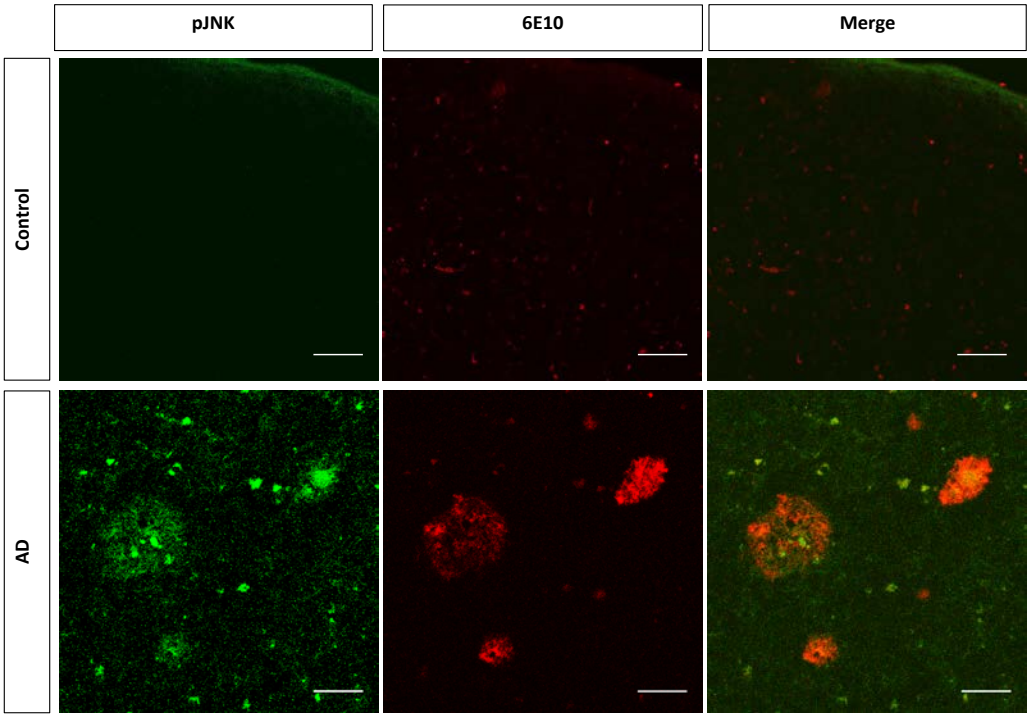

Figure 16. pJNK and 6E10 (β-amyloid marker) immunostaining in BA10 of Alzheimer's Disease (AD) cases. Scale bar 50 µm.

2.4. $A\beta_{42}$ intracerebroventricular administration increases pJNK levels in wild type mice frontal cortex

The next question was to assess whether an increase of Aβ could lead to pJNK elevation.

The injected $A\beta_{42}$ was previously aggregated to obtain the highly toxic oligomer species. To study the oligomeric species formed in the incubation conditions used, western blot was performed and several bands corresponding to the different aggregation forms could be observed (**Figure 17**).

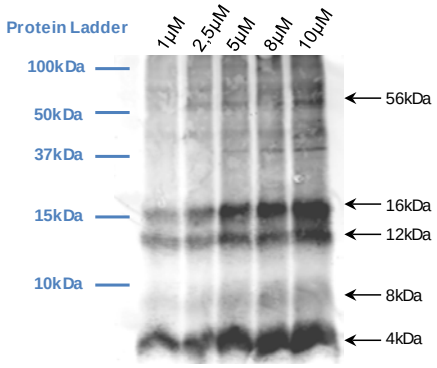

Figure 17. $A\beta_{42}$ aggregation and toxic oligomer formation. Aβ immunoblotting (6E10) of different Aβ concentration evaluated.

In order to achieve this aim, western blot was performed using the 6E10 antibody. Increasing concentrations of protein (1 µM, 2.5 µM, 5 µM, 8 µM and 10 µM) were tested in order to be able to discriminate all the oligomeric species present in the sample (**Figure 17**).

The bands under 10 kDa could correspond to the monomeric (4 kDa) and dimeric (8 kDa) forms of $A\beta_{42}$. Bands under and above 15 kDa correlate in size with $A\beta_{42}$ in its trimeric (12 kDa) and tetrameric (16 kDa) forms. The band above 50 kDA is closed to the expected 56 kDa band of the dodecameric Aβ form (Aβ*56), currently considered as one of the most toxic oligomers of Aβ.

As depicted in **Figure 18**, pJNK levels increased in frontal cortex of WT mice after ICV administration of $A\beta_{42}$ (Student t test $p<0.05$). Consistent with JNK activation *in vivo* in both AD and animal model brains, ICV injection of Aβ to WT mice activates JNK, suggesting a causal relationship between both.

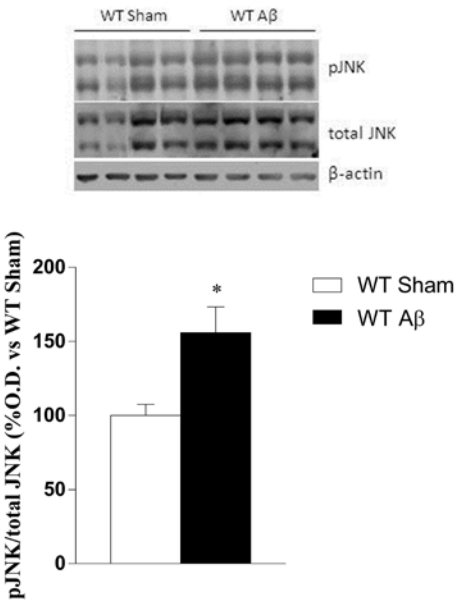

Figure 18. JNK immunoblotting in wild-type (WT) mice frontal cortex after ICV administration of $A\beta_{42}$. Results are expressed as % optical density (O.D.) of sham and normalized total levels of JNK. *p<0.05, Student´s t-test.

3. JNK INHIBITION RESTORES COGNITIVE IMPAIRMENT IN AN AD MOUSE MODEL

After proving the strong association between activated JNK and Aβ levels in AD, the last aim of the present work was to study the consequences of JNK inhibition in AD. The ω-3 PUFAs are natural JNK inhibitors with reported benefits in several diseases. Thus, two ω-3 PUFAs (DHA and EPA) and one specialized pro-resolving lipid mediator (MaR1) were selected to assess the effects of JNK inhibition in the sporadic AD mouse model, SAMP8.

3.1. Effects of ω-3 polyunsaturated fatty acids in cognition

In order to study the potential beneficial effects of ω-3 PUFAs on the cognitive deficits observed in the SAMP8 model, 9 months old SAMP8 and SAMR1 mice were treated for 20 days with DHA, EPA or MaR1.

Locomotor activity was measured as velocity in open field. No differences between groups were found (one-way ANOVA; $F_{(4,41)}$=1.963; p=0.122), indicating that purported behavioral performance differences between SAMP8 and SAMR1 are not due to locomotor activity alterations (**Figure 19**).

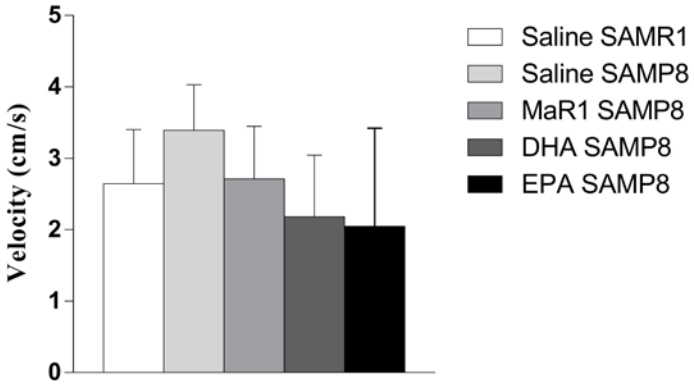

Figure 19. Effect of MaR1, DHA and EPA treatment in locomotor activity. Average velocity in the open field. Data are presented as mean ± SEM. DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; MaR1: maresin 1.

There was no difference between groups in the discrimination index in the sample trial in NORT ($F_{(4,41)}$=2.006; p=0.116) indicating no preference for any of the objects (**Figure 20**).

Recognition memory was significantly impaired at 24 h interval in SAMP8 mice, as shown by a significantly decreased discrimination index (one-way ANOVA; $F_{(4,41)}$=5.472, p<0.01; Tukey's p< 0.05 vs. SAMR1 Saline; **Figure 20**), that was reversed by MaR1 and DHA. Interestingly, cognitive improvement was not observed in SAMP8 treated with EPA (Tukey's ^p<0.05 vs. SAMP8 Saline), establishing marked differences between the ω-3 PUFAs tested.

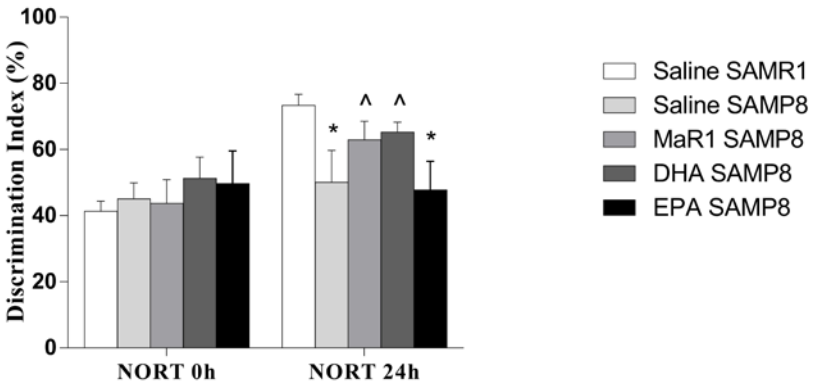

Figure 20. Effect of MaR1, DHA and EPA treatment in cognition on NORT. Sample phase and 24 h interval of NORT. Data are presented as discrimination index (percentage of time exploring the new object/total exploration time). *vs. SAMR1 Saline, ^vs. SAMP8 Saline; Tukey's test, $p<0.05$; one-way ANOVA. NORT: Novel object recognition test; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; MaR1: maresin 1.

In the MWM task, no significant differences among groups were found during the days of visible-platform training, indicating that all groups are able to perform the test correctly (data not shown).

In the acquisition phase of the test, significant difference between strains was found (repeated measurements ANOVA; $F_{(4,37)}=22.20$; $p<0.001$) where SAMP8 mice showed higher scape latency compared to SAMR1 mice, indicating a cognitive impairment characteristic of this mouse model (**Figure 21A**). Interestingly, when SAMP8 mice were treated with DHA or MaR1, lower scape latency was observed, indicating a cognitive performance improvement. However, this improvement was not enough to reach SAMR1 scape latency (Dunnet $p<0.05$, vs. SAMR1 Saline group; **Figure 21A**).

In the retention phase, no significant differences were found among groups in the first probe trial (day 3 of the MWM task) (one-way ANOVA; $F_{(4,41)}=0.219$; $p=0.926$). Nevertheless, on the second probe trial (Day 7), SAMP8 saline group showed a statistically significant decrease compared with SAMR1 saline group in the time swam in the quadrant were the platform used to be located, indicative of a memory deficit of this AD mouse model. Interestingly, DHA was the only treatment able to reverse the observed cognitive impairment (one-way ANOVA, $F_{(4,41)}=5.150$, $p<0.01$). The same results were observed in the last probe trial (Day 10), i.e. the memory deficit observed in SAMP8 mice was completely reversed by DHA (one-way ANOVA, $F_{(4,41)}=6.146$,

p<0.001). These results indicates that DHA administered for 20 days is able to restore the cognitive function of SAMP8 mice in the MWM test (Tukey's p<0.05 in all cases; **Figure 21B**).

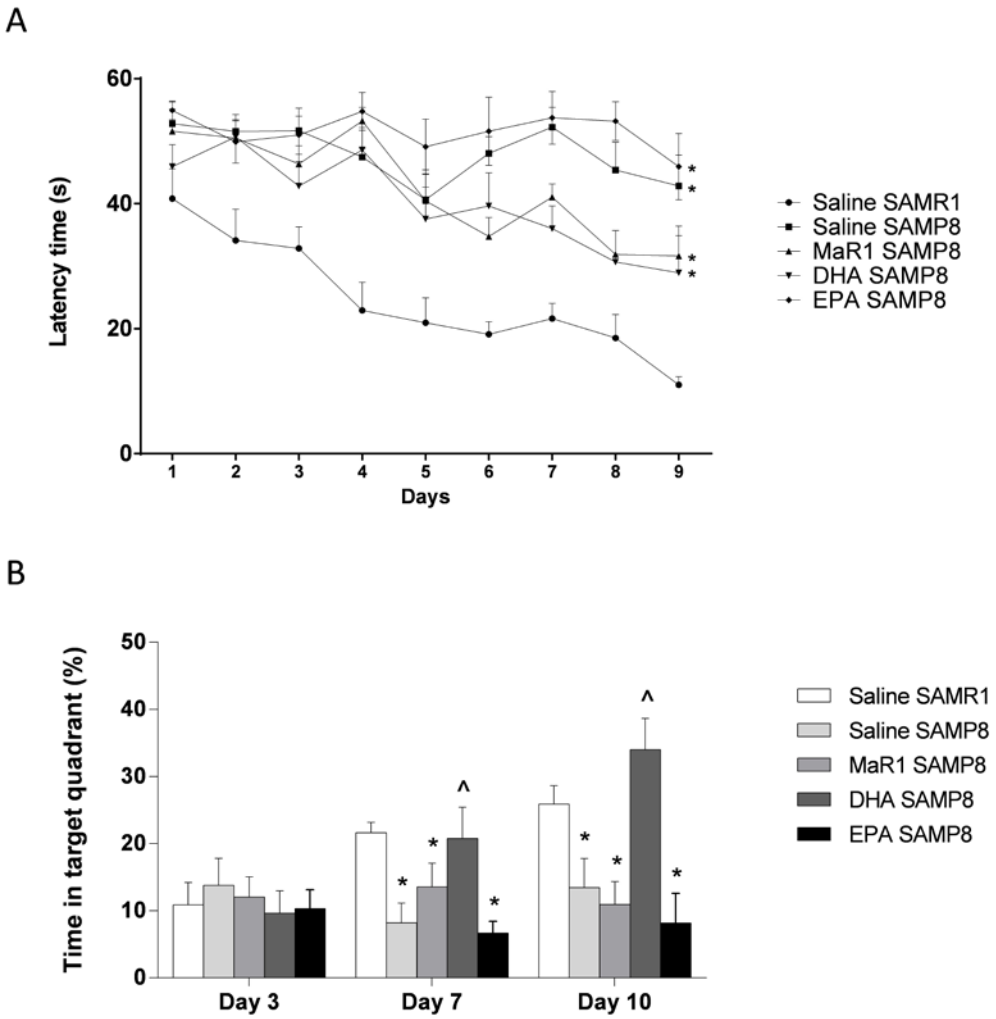

Figure 21. Effect of MaR1, DHA and EPA treatment in cognition. In panel A and B, cognitive performance assessed by MWM acquisition phase and retention phase, respectively. Data are presented as mean ± SEM. In panel A, *vs. SAMR1 Saline; repeated measure two-way ANOVA; Dunnet test p<0.05. In panel B, *vs. SAMR1 Saline, ^vs. SAMP8 Saline; Tukey's test, p<0.05; one-way ANOVA. MWM: Morris water maze; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; MaR1: maresin 1.

3.2. Involvement of pJNK on cognitive improvement

Recently, ω-3 PUFAs have been suggested as JNK inhibitors. Therefore, we next aimed to evaluate the effect of MaR1, DHA and EPA on pJNK levels in SAMP8 mice hippocampus, the main brain region involved in learning and memory.

In order to confirm this data and study a possible pJNK inhibition induced by PUFAs, activated and total JNK levels were measured by immunoblotting analysis. According with previous data, pJNK levels were significantly increased in SAMP8 mice compared to SAMR1 mice. Only DHA treatment was able to significantly reverse pJNK levels (one-way ANOVA, $F_{(4,41)}$=5.501, p<0.01; Tukey's p<0.05; **Figure 22**).

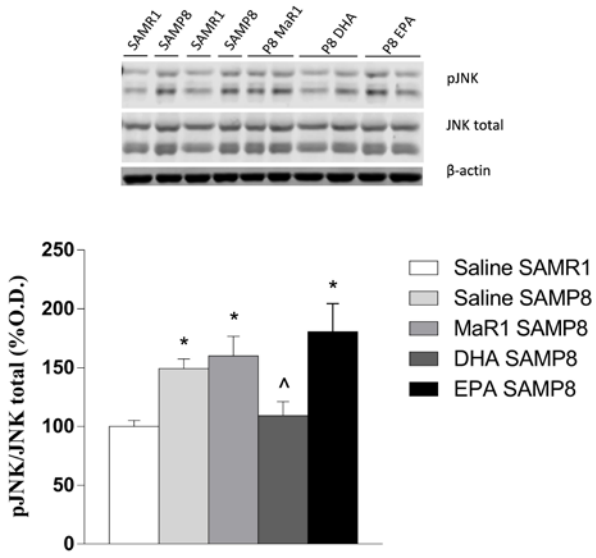

Figure 22. JNK immunoblotting in MaR1- DHA- and EPA-treated SAMP8 and SAMR1 mice hippocampus. A representative picture of western blot is shown. Results are expressed as % optical density (O.D.) of correspondent controls (SAMR1 mice) and normalized to total levels of JNK. *vs. SAMR1 Saline, ^vs. SAMP8 Saline; one-way ANOVA, Tukey p<0.05.

This result confirms the possible effects of DHA as JNK inhibitor, but also indicates that this is not a general characteristic of ω-3 PUFAs, since EPA and MaR1 do not achieve the same effect.

Interestingly, there was a significant and negative correlation between pJNK levels and cognitive decline, i.e. the reduction of pJNK levels in SAMP8 hippocampus and improvement in cognitive function in 24 h interval NORT (Spearman´s rho = -0.601, p<0.01, n=40) and last day of retention probe in MWM (Day 10) (Spearman´s rho = -0.560, p<0.01, n=40).

3.3. Mechanisms and consequences of JNK inhibition by ω-3 derivatives

The mechanism by which ω-3 derivatives (DHA) could inhibit JNK activation would involve ERS, direct inhibition of the enzyme or any other step (see **Figure 2**). The involvement of ERS can be checked by measuring the pIRE1α, a marker of ERS. Significant increase of pIRE1α was shown in SAMP8 mice compared to SAMR1 group. However, no effect of treatment was observed in any case (one-way ANOVA, $F_{(4,41)}$=5.917, p<0.001; Tukey's p<0.05; **Figure 23**). These results point to a direct inhibition of JNK activation by ω-3 derivatives.

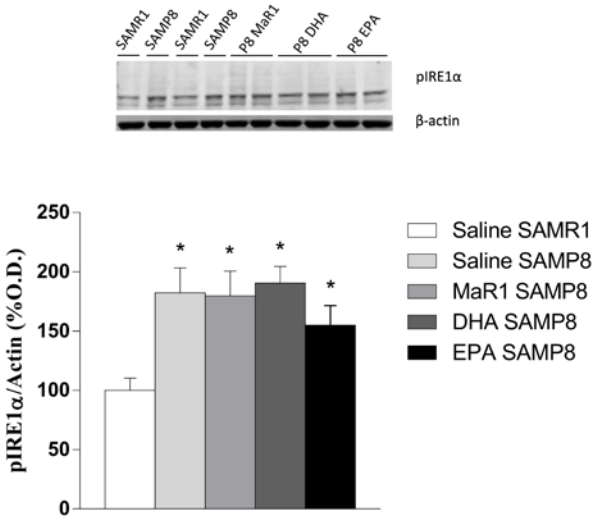

Figure 23. Activated IRE1α immunoblotting in MaR1- DHA- and EPA-treated SAMP8 and SAMR1 mice hippocampus. A representative picture of western blot is shown. Results are expressed as % optical density (O.D.) of correspondent controls (SAMR1 mice) and normalized to total levels of JNK. *vs. SAMR1 Saline; one-way ANOVA, Tukey p<0.05.

In order to assess the biochemical outcomes after JNK inhibition by DHA, activated c-Jun (p-c-Jun) was measured. c-Jun is a nuclear transcriptional factor and one of the main JNK substrates. DHA treatment was able to reverse the increase of p-c-Jun levels observed in SAMP8 strain in comparison with SAMR1 group (one-way ANOVA, $F_{(4,41)}$=2.639, p<0.05; Tukey's p<0.05; **Figure 24**).

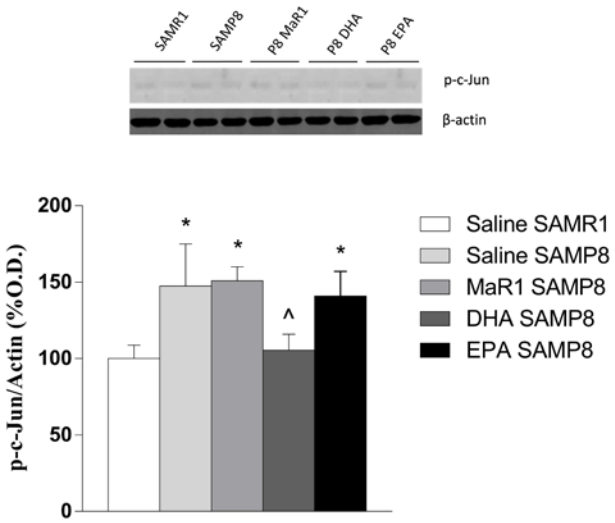

Figure 24. Activated c-Jun immunoblotting in MaR1- DHA- and EPA-treated SAMP8 and SAMR1 mice hippocampus. A representative picture of western blot is shown. Results are expressed as % optical density (O.D.) of correspondent controls (SAMR1 mice) and normalized to total levels of β-actin. *vs. SAMR1 Saline, ^vs. SAMP8 Saline; one-way ANOVA, Tukey p<0.05.

As it is described in the introduction, activated JNK induces Tau hyperphosphorylation, contributing to decline in AD pathology. Regarding our results, a significant increase in AT8 was observed in SAMP8 animals when compared to control SAMR1 strain. However, unlike to our expectations, AT8 phosphorylation was not reversed after DHA treatment (one-way ANOVA, $F_{(4,41)}$=3.131, p<0.05; Tukey's p<0.05; **Figure 25**).

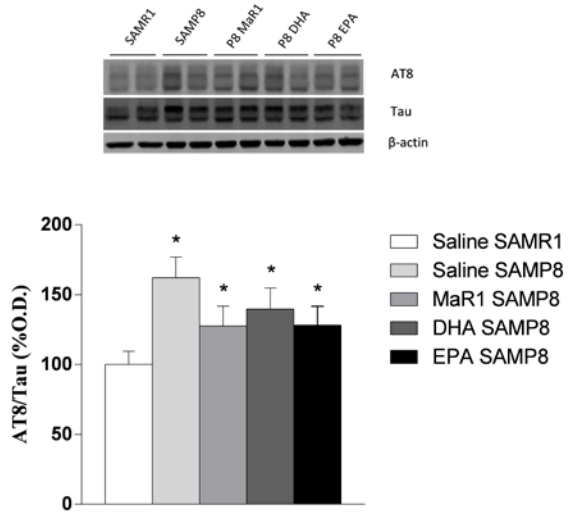

Figure 25. Phosphorylated Tau (AT8) immunoblotting in MaR1- DHA- and EPA-treated SAMP8 and SAMR1 mice hippocampus. A representative picture of western blot is shown. Results are expressed as % optical density (O.D.) of correspondent controls (SAMR1 mice) and normalized to total levels of Tau. *vs. SAMR1 Saline; one-way ANOVA, Tukey p<0.05.

DISCUSSION

Many studies have pointed out the emerging role of JNK in the development of neurodegenerative processes due to its implication in stress-triggered response (Cui et al., 2007; Pearson et al., 2006), neuronal apoptosis (Antoniou et al., 2011), caspase activation (Nishina et al., 2004; Pearson et al., 2006), mitochondrial oxidative burst, gene modulation (Cui et al., 2007) or its involvement in maturing process of NFTs (Kolarova et al., 2012; Mondragon-Rodriguez et al., 2008; Reynolds et al., 2000; Stoothoff & Johnson, 2005). Moreover, many different molecules and biological mediators associated to markers of neurodegeneration have proved to directly activate the JNK-c-Jun cascade such as cytokines, reactive oxygen intermediates or Aβ peptide (Marques et al., 2003; Okazawa & Estus, 2002; Sahara et al., 2008; Tamagno et al., 2003). Therefore, JNK has been proposed a promising target in the field of neurodegenerative disorders (Yarza et al., 2016).

Increases in Aβ levels remain a clear pathological mark, albeit unspecific, in the pathological development of AD, which has been clearly related to neuronal stress and subsequent pathological perpetuator (Bloom, 2014). *In vitro* discoveries revealed that pJNK increases after treatment with Aβ in primary cortical and hippocampal cultures from C57BL/6 mice, in primary cortical cell cultures from Wistar rat and in SH-SY5Y neuroblastoma cells (Morishima et al., 2001; Suwanna et al., 2014; Xu et al., 2015). In AD experimental models, research using a mouse model of AD that incorporates the Swedish APP mutation and a mutant presenilin-1 (PS1) –Tg2576- has demonstrated that JNK activation is associated with increased levels of senile plaques (Savage et al., 2002). Regarding SAMP8 mouse model, it is widely characterized that this mouse model shows most of the characteristics present in AD patients such as elevated Aβ peptide (Orejana et al., 2015) and interestingly, this Aβ over-production might be associated with enhanced pJNK levels (Orejana et al., 2013; Tajes et al., 2010). According with these data in the present study it has been demonstrated both Aβ and pJNK increases in the two AD mouse models used: the familiar AD model Tg2576 and the sporadic AD model SAMP8. Based in the above mentioned literature and according to our results, it is tempting to speculate that Aβ accumulation could be the cause of elevated pJNK levels observed in those mice, therefore, the next step was to investigate this question.

There is extensive evidence that Aβ induces the activation of JNK in familiar AD mouse models (Guglielmotto et al., 2011; Hwang et al., 2004; Savage et al., 2002). Moreover, JNK3 activation is involved in a positive feedback loop of $A\beta_{42}$ production (Yoon et al., 2012). In accordance with these data, it has been described that ICV $A\beta_{42}$ injection induces astroglial and microglial activation, and as consequence, neuroinflammation and neurocognitive impairment (Frozza et al., 2013). In this line, our study reported a significant increase of pJNK levels after ICV $A\beta_{42}$ injection in healthy mice, according with the published literature (Bicca et al., 2015; Frozza et al., 2013) and suggesting that pJNK activation is the consequence rather than the cause of Aβ accumulation. However, some other studies reported the decrease in $A\beta_{42}$ brain levels after JNK inhibition, which might suggest that the activation of JNK triggers the Aβ cascade leading to neuronal death (Ebenezer et al., 2010; Mazzitelli et al., 2011). Moreover, it has been described that $A\beta_{42}$ is able to increase BACE-1 expression through JNK activation, which leads to establish a vicious cycle between JNK and Aβ (Guglielmotto et al., 2011). Although the mutual feedback between JNK and $A\beta_{42}$ in AD has been extensively probed, it remains unclear which of either JNK or Aβ appears first in the disease onset. The results observed in CSF samples may shed some light to this dichotomy.

Thank to basic research studies, AD is progressively more characterized. However, there are still few clinical trials sponsored by pharmaceuticals industry, which highlights the need to develop clinical tests to diagnose the disease before the manifestation of the symptoms and thus improve the life quality of the patients. Since the alteration of $A\beta_{42}$ and Tau levels in CSF of AD patients were discovered, there is a clear need to develop new biomarkers that are minimally invasive and that reflect other pathological mechanisms of the disease such as neurodegeneration, neuroinflammation or synaptic dysfunction (Mattsson et al., 2015). To date, many CSF biomarkers have been described in AD: VLP-1, GAP-43, SANP-25, IL-3, IL-6, IL-1β or YKL-40 (Alcolea et al., 2015; Blennow et al., 2010; Craig-Schapiro et al., 2010; Killick et al., 2014; Lee et al., 2008; Tang et al., 2008; Zetterberg, 2015). As a matter of fact, several biomarkers have been measured in our CSF samples, as shown in **Table 8**. When determining the levels of pJNK in CSF in patients diagnosed with AD or

prodromal stages of AD, it has been presently demonstrated a significant increase of pJNK levels in AD group compared with control and MCI groups. Interestingly, PMCI levels are higher, although not significantly, than both MCI and control groups, pointing to JNK as a plausible prodromal biomarker of AD. This result suggests the need of increasing the number of participants in this group, as it is presently rather small.

In contrast to our findings, a previous work reported undetectable CSF levels of pJNK in AD patients (Gourmaud et al., 2015). Indeed, in the present work, the manufacturer's protocol needed to be adapted to reach detectable JNK concentration. To this end, CSF samples were concentrated in a step prior to the performance of the enzyme-immunoassay, being this concentration of the samples a crucial step in the technique development. This previous step can be useful for any other CSF measurement as it allows preserving the chemical and physical characteristics of the samples. It should be noted that the sensitivity and selectivity is an essential requirement in the procedures carried out. As future perspectives, it might be possible to propose the validation of this modified method, consolidating pJNK measurement in CSF as AD biomarker.

One of the aims of the present work was to study if the activation of JNK is a central feature not only in AD but also other types of dementias, i.e. VaD, LBD and FTD. Confirming previously published works, we found increased expression of pJNK in human post-mortem brain samples from AD patients and a positive correlation with Aβ levels (Killick et al., 2014; Zhu et al., 2001). Interestingly this increase of pJNK appeared to be specific of AD, as no alteration in this kinase was observed in the other dementias. This reinforces the idea of a direct link between pJNK and Aβ, i.e. all our experiments suggest that increased Aβ levels in AD could lead to an increased activation of JNK, which in turn could result in neuroinflammation and neurodegeneration.

The discovery of a lack of involvement of pJNK in VaD is interesting and novel. There are still several open questions regarding the pathophysiology of VaD and the possible role of Aβ in the pathophysiology of the disease. Indeed, whether expression of Aβ and the co-existence of underlying AD-related pathology play a crucial role in the

pathogenesis of VaD remains still controversial. Several reports suggest that there are no significant differences in serum and CSF levels of $A\beta_{42}$ when comparing VaD patients with the corresponding controls (Jia et al., 2005; Paraskevas et al., 2009; Uslu et al., 2012). In fact, cortical microinfarcts that are frequently found in AD, with particular predilection for the occipital lobe, show amyloid burden whereas VaD-associated subcortical infarcts do not express amyloid (Okamoto et al., 2009). Consistent with these observations, in the present work it was not found increases in amyloid expression beyond the limits observed in normal elderly brains. However, it has been proposed that, in late stages, VaD patients may develop AD pathology that could reflect an overlap between histopathological features of both VaD and AD (Lewis et al. 2006).

It is widely accepted that the dementia spectrum varies from pure forms of AD and LBD to intermediate forms of both, sharing either Lewy body pathology or AD-related pathology. Although in our hands we did not found Aβ elevation in LBD samples, it has been described that between 50 and 90% of the LBD cases show in some extent increased Aβ production without other AD pathological markers such as NFTs and other AD-related histopathological changes (Halliday et al., 2011; Kovari et al., 2009). On the other hand, the results obtained in the present work showing the lack of activation of JNK in samples from LBD are in agreement with a previous study (Ferrer et al., 2001). In fact, some authors have suggested the possible role of α-synuclein aggregates in preventing JNK activation and, therefore, inhibiting JNK-mediated signaling (Hashimoto et al. 2002). Interestingly, another study found activated JNK within LBD samples when they also show AD-related pathology (i.e. increases in Aβ and Tau levels), showing that pJNK is exclusively present in areas affected by AD-related pathology and no neurons affected by Lewy bodies without AD-markers showed pJNK staining (Ferrer et al., 2001). These results highlight a presumable specificity of pJNK towards AD and concomitant AD-related pathology in mixed type dementias.

Regarding FTD, different classifications have been proposed according to clinical and histopathological features expressed. Three different subtypes are distinguished according to the predominant inclusions found in the pathological examination

(Koedam et al., 2013). The Tau positive variant shows Tau pathology similar to AD cases whereas the other subtypes (TDP-43 and DPR subtypes) do not. As a matter of fact, Tau subtype shows increased production and expression of amyloid leading to formation of Aβ although plaques are absent (Vitali et al., 2004). No literature published so far supports an underlying production of Aβ in the other FTD subtypes, which is congruent with the results of our study, where no increases in amyloid production were found. In fact, imaging studies using 18F-Florbetapir (an amyloid labeling radiotracer) showed a lower expression of cortical Aβ-labeling in FTD compared to controls (Kobylecki et al., 2015). In this way, the presence of Aβ within the Tau positive variant in FTD could be the representation of a mixed variant of FTD that includes AD-related pathology. Opposite to our data, some studies have shown positive staining for pJNK in the Tau positive variant of FTD (Atzori et al., 2001; Ferrer et al., 2001; Lagalwar et al., 2007) and in the TDP-43 variant (Parker et al., 2012). It is important to note that TDP-43 inclusions are not specifically expressed in FTD variants, as production of this misfolded-protein subtype has also been described in other dementia sub-entities such as AD (Arai et al., 2009; Jung et al., 2014) and LBD (Higashi et al., 2007). In fact, some reports describe an expression-frequency of TDP-43 inclusions that varies from 20 to 57% in AD cases (Amador-Ortiz et al., 2007; Josephs et al., 2014). Moreover, in regard to TDP-43 inclusions, most frequently they are present mainly in both temporal and limbic structures (Hu et al., 2008), whereas aggregates are rarely seen within frontal cortex (Josephs et al., 2014). Therefore, the lack of activation of JNK found in this study could be related to the region studied, as our study was conducted in the frontal cortex (BA10).

Co-localization studies were performed to further support the relationship between Aβ and pJNK. Literature published so far (Mehan et al., 2011; Yarza et al., 2016) highlights the hypothesis that increased expression of pJNK could underlie the AD pathology, as it has been demonstrated the co-localization of pJNK with the main AD histopathological markers, i.e., senile plaques and NFTs (Killick et al., 2014; Zhu et al., 2001) and even with TDP-43 inclusions, which, as mentioned before, seem to be present in a significant percentage of AD cases (Amador-Ortiz et al., 2007; Josephs et al., 2014).

Supporting the tight and specific relation between pJNK and Aβ in AD, this work showed the co-localization between pJNK with the senile plaque in the BA10 region of AD patients. In addition, immunohistochemistry in the frontal cortex of Tg2576 mice, an amyloidogenic transgenic model, reproduced the co- localization of pJNK and Aβ with a pattern in which pJNK appears to be located around the senile plaque. These data supports the idea of a tight link between Aβ peptide accumulation and the neuroinflammation present in AD, in accordance with previous studies where it has been also reported the co-localization of pJNK with the amyloid deposits (Savage et al., 2002). The pattern showed by pJNK staining is another key point, as the co-localization of activated JNK not only appears inside the senile plaques in Tg2576 mice (Savage et al., 2002), but also co-localizes with dystrophic neurites coinciding with the damage of neuritic processes and neuronal death (Yoon et al., 2012). This suggests a possible role of pJNK in the inflammation surrounding the plaque and the cell death that occurs within that area. Moreover, the similar staining pattern observed in our hands both in animals and human brain samples indicates the similarity and the validity of Tg2576 as AD murine model.

Although, SAMP8 mouse model does not show Aβ accumulation in form of senile plaques, the current project demonstrated the enhanced pJNK expression in SAMP8 strain that mirrors the elevation in Aβ levels. The disadvantages of this model rely on the fact that the produced Aβ peptides does not accumulate in form of senile plaques (and therefore it is not possible to perform plaques immunohistochemistry) and that they only develop certain features of the disease. Indeed, in the SAMP8 mouse it is still unknown the final cause by which progressive cognitive decline and neurodegenerative changes are developed. In spite of this, the two selected models showed increased pJNK levels indicating that are adequate tools to try to understand the implication of this kinase in AD onset and development.

After our characterization about the specific increase of pJNK levels in AD and its association with Aβ deposition, it was next proposed to study JNK inhibition as therapeutic strategy for the treatment of AD. As described in the introduction of this work, the ω-3 fatty acids (EPA and DHA) are considered natural JNK inhibitors and they have demonstrated beneficial effects in experimental models of neurodegenerative

diseases. However, most clinical trials did not meet the expectations and did not reproduce the good results observed in preclinical stages (Eckert et al. 2013). The quality of evidence from clinical trials and the duration of supplementation with ω-3 PUFAs has been questioned in relation to the cognitive function of patients included in those studies (Burckhardt et al., 2016). In addition, it has been shown that ω-3 PUFAs are capable of being metabolized in vivo to a novel series of bioactive lipid mediators known as resolvins (Rv), protectins (PDX) and maresines (MaR) (Serhan, 2014). These lipid mediators not only have potent anti-inflammatory effects, but also actively participate in the resolution of tissue inflammation, and may contribute to the beneficial actions of ω-3 PUFAs in AD. Recent studies have revealed that the administration of some of these SPMs as RvD1 and NPD1 are found to be beneficial in counteracting the AD pathology (Rey et al., 2016; Zhao et al., 2011; Zhu et al., 2016). However, the metabolic actions of MaR1 and its possible therapeutic actions in neurodegeneration have been poorly studied. For these reason, it was decided to assess the effects of EPA and DHA together with MaR1, a novel DHA metabolite which has not been previously tested for the treatment of AD, in SAMP8 mice.

It has been already described that EPA pre-treatment induces neuroprotective actions against irradiation and LPS-induced dysfunction in rat hippocampus through JNK and caspases activation reduction and a subsequent apoptosis inhibition (Lonergan et al., 2002; Lonergan et al., 2004). In the same way, pre-treatment with EPA exhibited neuroprotective effects by inhibiting the mitochondrial apoptotic pathway in PC12 cells. Moreover, EPA-enriched diet during 3 months restored cognitive function and reduced anxiety in SAMP8 mice, evaluated in MWM and open field test, respectively (Wu et al., 2014).

In contrast to previous publications (Zhang & Jiang, 2015), in our hands, EPA did not show beneficial effects on SAMP8 memory deficits. Supporting our data, recent studies discovered that EPA decreases cell viability in a dose-dependent way inducing apoptosis in HepG2 cells, a human hepatoma cell line. The study showed that EPA-induced ROS production which caused cytoplasmic calcium concentration elevation and JNK activation leading to mitochondrial-dysfunction triggered by Cyt C release and caspase activation (Zhang & Jiang, 2015). Notably, this hypothesis could explain the

different effects of EPA and DHA over the risk to suffer AD observed in a clinical randomized trial, where it has been shown that only DHA and not EPA is able to prevent AD development in elderly population (Morris et al., 2003).

On the other hand, DHA promotes cell survival through the induction of anti-apoptotic and neuroprotective gene expression. Certain evidence has already related ω-3 fatty acids with Aβ, i.e. DHA limits the production of APP avoiding the production and accumulation of Aβ (Cole et al. 2009). Moreover, several studies reported the beneficial impact of DHA diet on synaptic plasticity by increase of pre and postsynaptic markers in Tg2576 mice leading to memory improvement in MWM (Calon et al., 2004; Lim et al., 2005). Aged 3x-Tg-AD mice that accumulate human Aβ showed improved cognition and less dysfunction of entorhinal cortex neurons after daily DHA feeding for 8-10 months (Arsenault et al., 2011). Finally, SAMP8 mice fed with ω-3 FA-enriched fish oil during 2 months demonstrated memory improvement in a passive avoidance test and increased survival in SAMP8 strain which presented shortened life-span. Specifically, these beneficial effects were attributed to DHA and not to other dietary fatty acids (EPA, DPA or ALA) since it was the only fatty acid that appeared increased in the brain lipid composition of these animals (Petursdottir et al., 2008; Ueda et al., 2011). Indeed, age-dependant decrease of DHA in SAMP8 hippocampus was detected in comparison with SAMR1 which is in agreement with several clinical trials that reported a positive association between decreases in ω-3 fatty acid brain content and cognitive decline (Petursdottir et al. 2007; Cole et al. 2009).

Albeit MaR1 is not proved in AD mice model or patients, its anti-inflammatory effects are widely accepted and it has been proposed as a potential therapeutic tool in neurodegenerative diseases due to its higher potency compared with its upstream precursor DHA. MaR1 improved cell survival in SH-SY5Y culture and reduced Aβ levels, which is a clear AD hallmark (Wang et al. 2015; Zhu et al. 2016).

As expected, in our hands, SAMP8 mice showed cognitive deficit in the NORT and MWM test, and only treatment with DHA was able to counteract these deficits. Surprisingly, MaR1 was ineffective. Maybe a higher dose and/or longer treatment would be essential to detect a positive effect of MaR1 (mice were treated only for 20 days). These results were quite surprising because being MaR1 the active metabolite

of DHA, a stronger effect that the one observed after DHA treatment was expected. However, MaR1 could be acting at other levels such as decreasing neutrophil infiltration, increasing macrophage phagocytosis, inhibiting NF-κB as well as limiting pro-inflammatory cytokines production among others (Martínez-Fernández et al., 2017).

Therefore, our data demonstrates that DHA improves cognitive function in SAMP8 mice, remaining controversial the effect of MaR1 on memory. Based on these evidences, the next step was to try to understand the mechanisms underlying the cognitive improvement observed in DHA-treated SAMP8 mice.

As ω-3 fatty acids have been proposed as natural JNK inhibitors, the first aim in the mechanism seeking was to explore the involvement of JNK in these actions. Regarding our results, SAMP8 mice showed significant increase of pJNK levels (Orejana et al., 2013). This increase was reversed only after DHA administration. Interestingly, higher pJNK levels correlate negatively with mice cognitive status, while a decrease in pJNK expression after DHA treatment is associated with cognitive improvement. On the contrary, pJNK levels were not reversed after EPA and MaR1 treatment and their cognitive impairment persisted.

It has been postulated that JNK can be activated intracellularly by ERS; however, the exact mechanism is still unknown. ERS has been implicated in abnormal protein processing and neuronal death in cognitive dysfunction. The UPR response starts, when three ERS sensors located on the ER membrane are phosphorylated: IRE1α, PERK and ATF6. It has been suggested that misfolded proteins accumulation such Aβ initiates IRE1α activation inducing UPR which is the adaptive response to ERS. The effect of IRE1 pathways results in JNK activation through IRE1-TNF receptor associated factor 2 (TRAF2)-ASK signaling or due to the calcium dysregulation in response to ERS modulation (Shah et al., 2017). Notably, it has been proposed that DHA protected SH-SY5Y cell from thapsigargin induced ERS that resulted in decrease of mitochondrial membrane potential representing an early hallmark of apoptosis (Eckert et al., 2011).

Activated IRE1α measurement aimed to assess the ERS implication in JNK activation in our AD experimental model and its plausible inhibition after ω-3 fatty acids treatment. According to a recent study, SAMP8 strain presents elevation in ERS

markers in comparison with SAMR1 mice, assessed as higher IRE1α phosphorylation (Zhang et al., 2017). However, in the present study, DHA treatment did not reverse the increase in pIRE1α levels, suggesting that DHA is a direct JNK inhibitor and it is not able to act in an upstream level in the JNK pathway (**Figure 26**). According with these results, Torres et al. did not find significant changes in pIRE1α levels in SH-SY5Y cells after DHA derivate metabolite treatment (Torres et al., 2015).

Upon its activation, JNK may act at the cytosolic level, since it is its subcellular location, but it may also perform a translocation to the nucleus in order to carry out its kinase activity in this cell compartment (Zeke et al., 2016). c-Jun has been described as the first and main substrate of JNK (Dérijard et al., 1994). c-Jun is a transcription factor that is phosphorylated by JNK activity. Previous studies have shown that cell stress produces an increase in p-c-Jun and that JNK inhibitors succeed in decreasing c-Jun phosphorylation induced by cellular stress (Barros-Miñones et al., 2013; Reddy et al., 2013). JNK also acts on other transcription factors such as ATF2, FOXO3, FOXO4 or Stat3, among others (Zeke et al., 2016). In this way, JNK can regulate the transcription of many target genes involved in differentiation, growth or apoptosis, altering the balance between survival and neuronal death. Indeed, c-Jun activation promotes neuronal apoptosis and DHA was able to reverse the increased p-c-Jun levels in SAMP8 mice suggesting the mechanism of action through which DHA can exert its beneficial effects on cognition.

At cytosolic level, it has been observed that pJNK leads to hyperphosphorylation of Tau and results in neurofibrillary tangles maturation, a characteristic event in AD. It has been proposed that the onset of amyloidosis induces Tau hyperphosphorylation, thereby suggesting that pathological changes consequent to amyloid deposition such as neuroinflammation or oxidative stress may be potential events involved in abnormal Tau processing, where JNK activation might play a key role (Zhang et al. 2016; Ma et al. 2009).

Tau can be phosphorylated at multiple sites by several kinases, but in particular, JNK is known to phosphorylate Tau at Ser202/Thr205 and Ser422 and these two sites are strictly associated with AD pathogenesis. Phosphorylation at Ser202/Thr205 residues of Tau, recognized by AT8 antibody, displays at early stage of the disease,

while phosphorylation at Ser422 is related with tangle formation at the end-stage of neurodegeneration (Ploia et al. 2011; Ma et al. 2009). Some authors also related Ser396/Ser404 Tau phosphorylation, recognized by PHF-1 antibody, with JNK activation in AD or other tautopathies (Bellucci et al., 2007; Tatebayashi et al., 2006).

Regarding our AD mouse model, it is widely accepted that the brains of the SAMP8 strains presents hyperphosphorylation of Tau protein (Morley et al., 2012). Specifically, these animals are characterized by an increase in Tau phosphorylation at Ser202/Thr205 (AT8 epitope). In this line it has been described a reduction of pJNK-induced Tau phosphorylation (AT8) by D-JNKi-1 treatment, a well-standardized JNK inhibitor (Orejana et al., 2013). Following this evidences, in the present study, AT8 antibody was chosen for the assessment of Tau aberrant phosphorylation, as it has been demonstrated to be the most appropriated antibody for the measurement of p-JNK-induced Tau phosphorylation (Ploia et al., 2011). Unexpectedly, although our data reveals that SAMP8 mice presented increased levels of AT8 in concordance with previous studies, DHA induced JNK inhibition did not restore this effect.

It could be proposed that other Tau phosphorylation (Ser422 or PHF-1) could be restored after DHA induced JNK inhibition in SAMP8 mice. Indeed, Ploia et al. did not observe alteration of Ser422 Tau phosphorylation in TgCRND8 after D-JNKi-1 administration, but Ser422 Tau phosphorylation was reduced by the same treatment in AD human fibroblasts (Ploia et al., 2011). In addition, the JNK inhibitor SP600125 significantly inhibits Tau phosphorylation at PHF-1 site in SH-SY5Y cells (Tatebayashi et al., 2006). Interestingly, it has been described that after pretreatment of hippocampal cultured neurons with DHA during 48h, the Aβ-induced Ser422 Tau phosphorylation was suppressed trough JNK inhibition. DHA showed a trend for suppression of Ser422 Tau phosphorylation and Ser616 IRS-1 phosphorylation in JNK-dependent pathway when it is oral administrated during 4 months to HFD-fed 3xTg-AD mice (Ma et al. 2009). Therefore, further experiments are required to study the implication of JNK inhibition in Tau hyperphosphorylation in AD.

Concluding remarks

In summary (**Figure 26**), based on our results, a clear relationship between Aβ and pJNK is hereby reported highlighting the direct role of JNK within AD pathogenesis. Even though the etiology of AD remains elusive, the nosogenic basis of AD seems to be related to neuron apoptosis and loss of synaptic terminals that results in neuroinflammation and neurodegeneration (Yarza et al., 2016).

The JNK cascade could be understood as an axis in the molecular development of AD and other neurodegenerative pathologies. Its implication at different stages of the disease makes clear its importance within neuronal dysregulation, metabolic disruption as well as in development of its neuropathological hallmarks.

It has been suggested that elevated levels of oxidative stress can activate different apoptotic pathways mediated by JNK activation (Marques et al., 2003; Sahara et al., 2008). Our study showed a significant increase in levels of pJNK and Aβ specific for AD, which is not observed in all other dementias, with a positive correlation between these levels. In addition, an age- and genotype-dependent increase in pJNK and Aβ levels is observed in AD transgenic mouse models. Moreover, the positive strong correlation between both pathological processes is supported by the co-localization of pJNK and Aβ in both human and mice brains. Finally, the increase of activated JNK in WT mice after ICV Aβ administration further supports the close relation between them. Considering all the above mentioned results, the increase of pJNK levels could be a direct consequence of Aβ increase in AD. Moreover, the specificity of increasing levels of pJNK in AD allows us to propose it as a possible CSF biomarker for the detection of the disease.

Several studies and clinical investigations have suggested beneficial effects of ω-3 PUFAs on neurodegenerative diseases, such as AD. The beneficial effects of DHA have been demonstrated in AD experimental models, but the therapeutic effect in clinical trials is controversial (Casali et al., 2015; Hjorth et al., 2013; Teng et al., 2015). In the present work, it has been proposed that the therapeutic actions of DHA could be related to JNK inhibition. As described in **Figure 26**, thanks to lipid nature of DHA, it is able to cross BBB and to disseminate into the neuron. At cytosolic level, DHA exerts its

action directly inhibiting JNK phosphorylation and, as consequence, decreasing p-c-Jun levels which promotes survival in neurons.

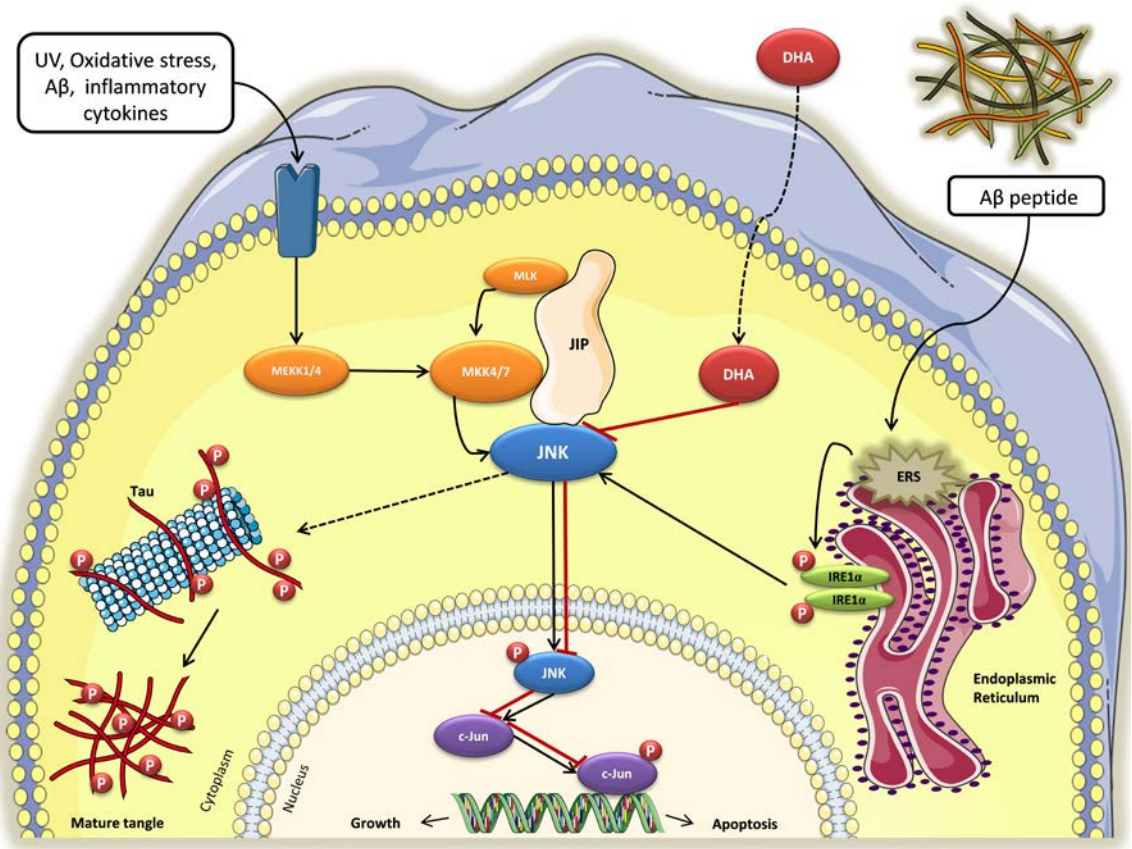

Figure 26. Schematic proposed DHA mechanism of action. DHA acts inhibiting JNK phosphorylation and, in consequence, decreasing c-Jun activation at nuclear level.

Altogether, the present results remark the specific implication of JNK in AD-related pathology and, thus, reinforce the idea that JNK could represent a promising therapeutic target in AD. Moreover, DHA administration showed cognitive improvement in AD mice model by JNK inhibition, pointing DHA as a novel therapeutic approach. Nevertheless, further investigation is needed in order to elucidate the mechanism of action of DHA and to determine the real extension of the rationale behind proposing JNK-related drugs as specific treatments of AD.

Nowadays, different pharmacological agents are available for experimental and preclinical use assessing the possible role of JNK as a plausible therapeutic target in AD. Significant progress in the design of selective JNK inhibitors versus other kinases

has been achieved within the past years. However, directed inhibition of JNK isoforms in specific tissues is still an open task.

The fact that JNK3 is specifically expressed in the CNS and its activation by stress-stimuli renders is an attractive and potential target for treating AD. It is possible to speculate that JNK3 specific inhibition will reduce the possible side-effects of a systemic JNK inhibition. Although there is no consensus in the literature if there is a need for whether isoform selectivity is needed for the treatment of AD, the answer to this question can only be obtained when such compounds are available.

CONCLUSIONS

From the results obtained in the present Doctoral Thesis it can be concluded that:

1. Age- and genotype-increased activated JNK (pJNK) levels were observed in experimental mouse models of AD: Tg2576, a transgenic amyloidogenic model and SAMP8, a sporadic AD based on accelerated senescence. This increase parallels the enhanced Aβ levels.
2. Specific increase of pJNK levels in AD postmortem and not in other dementias was found.
3. Significantly increased pJNK levels were observed in CSF of AD patients compared with subject cognitive impairment (controls) and stable mild cognitive impairment groups.
4. Aβ levels were increased in human frontal cortex samples of AD patients, and these Aβ levels positively correlated to pJNK levels.
5. Aβ and pJNK co-localize in frontal cortex of Alzheimer's disease brains. Aβ and pJNK also co-localize in frontal cortex of Tg2576 mice. In both cases the same immunolabelling pattern was observed which shown activated JNK around the senile plaque.
6. Intracerebroventricular administration of β-amyloid increased JNK expression in wild-type mice.
7. SAMP8 mice showed cognitive deficits in NORT and MWM test that were only reversed by docosahexaenoic acid (DHA), and not other ω-3 polyunsaturated fatty acids, treatment.
8. Increased pJNK levels in SAMP8 mice were only reversed by DHA treatment.

As general conclusion, pJNK expression is selectively increased in AD and not in other dementias, probably as a consequence of increased levels of Aβ in this type of dementia. JNK could be considered as a new AD biomarker and pharmacological inhibition of JNK with DHA, an ω-3 fatty acid derivative, could be a new therapeutic approach for the treatment of AD.

REFERENCES

Abete, I., Goyenechea, E., Zulet, M. A., & Martínez, J. A. (2011). Obesity and metabolic syndrome: Potential benefit from specific nutritional components. *Nutrition, Metabolism and Cardiovascular Diseases*, *21*, B1–B15. https://doi.org/10.1016/j.numecd.2011.05.001

Akhter, R., Sanphui, P., Das, H., Saha, P., & Biswas, S. C. (2015). The regulation of p53 up-regulated modulator of apoptosis by JNK/c-Jun pathway in β-amyloid-induced neuron death. *Journal of Neurochemistry*, *134*(6), 1091–103. https://doi.org/10.1111/jnc.13128

Alcolea, D., Martínez-Lage, P., Sánchez-Juan, P., Olazarán, J., Antúnez, C., Izagirre, A., et al. (2015). Amyloid precursor protein metabolism and inflammation markers in preclinical Alzheimer disease. *Neurology*, *85*(7), 626–633. https://doi.org/10.1212/WNL.0000000000001859

Amador-Ortiz, C., Lin, W.-L., Ahmed, Z., Personett, D., Davies, P., Duara, R., et al. (2007). TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Annals of Neurology*, *61*(5), 435–45. https://doi.org/10.1002/ana.21154

Antoniou, X., Falconi, M., Di Marino, D., & Borsello, T. (2011). JNK3 as a therapeutic target for neurodegenerative diseases. *Journal of Alzheimer's Disease : JAD*, *24*(4), 633–42. https://doi.org/10.3233/JAD-2011-091567

Arai, T., Mackenzie, I. R. A., Hasegawa, M., Nonoka, T., Niizato, K., Tsuchiya, K., et al. (2009). Phosphorylated TDP-43 in Alzheimer's disease and dementia with Lewy bodies. *Acta Neuropathologica*, *117*(2), 125–36. https://doi.org/10.1007/s00401-008-0480-1

Arsenault, D., Julien, C., Tremblay, C., & Calon, F. (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

Arthur, P. G., Matich, G. P., Pang, W. W., Yu, D.-Y., & Bogoyevitch, M. A. (2007). Necrotic death of neurons following an excitotoxic insult is prevented by a peptide inhibitor of c-jun N-terminal kinase. *Journal of Neurochemistry*, *102*(1), 65–76. https://doi.org/10.1111/j.1471-4159.2007.04618.x

Atzori, C., Ghetti, B., Piva, R., Srinivasan, A. N., Zolo, P., Delisle, M. B., et al. (2001). Activation of the JNK/p38 pathway occurs in diseases characterized by tau protein pathology and is related to tau phosphorylation but not to apoptosis. *Journal of*

Neuropathology and Experimental Neurology, *60*(12), 1190–7.

Bagyinszky, E., Giau, V. Van, Shim, K., Suk, K., An, S. S. A., & Kim, S. (2017). Role of inflammatory molecules in the Alzheimer's disease progression and diagnosis. *Journal of the Neurological Sciences*, *376*, 242–254. https://doi.org/10.1016/j.jns.2017.03.031

Bain, J., McLauchlan, H., Elliott, M., & Cohen, P. (2003). The specificities of protein kinase inhibitors: an update. *The Biochemical Journal*, *371*(Pt 1), 199–204. https://doi.org/10.1042/BJ20021535

Bamji-Mirza, M., Callaghan, D., Najem, D., Shen, S., Hasim, M. S., Yang, Z., et al. (2014). Stimulation of insulin signaling and inhibition of JNK-AP1 activation protect cells from amyloid-β-induced signaling dysregulation and inflammatory response. *Journal of Alzheimer's Disease : JAD*, *40*(1), 105–22. https://doi.org/10.3233/JAD-131949

Barr, R. K., Kendrick, T. S., & Bogoyevitch, M. A. (2002). Identification of the critical features of a small peptide inhibitor of JNK activity. *The Journal of Biological Chemistry*, *277*(13), 10987–97. https://doi.org/10.1074/jbc.M107565200

Barros-Miñones, L., Orejana, L., Goñi-Allo, B., Suquía, V., Hervías, I., Aguirre, N., et al. (2013). Modulation of the ASK1-MKK3/6-p38/MAPK signalling pathway mediates sildenafil protection against chemical hypoxia caused by malonate. *British Journal of Pharmacology*, *168*(8), 1820–34. https://doi.org/10.1111/bph.12071

Bascoul-Colombo, C., Guschina, I. A., Maskrey, B. H., Good, M., O'Donnell, V. B., & Harwood, J. L. (2016). Dietary DHA supplementation causes selective changes in phospholipids from different brain regions in both wild type mice and the Tg2576 mouse model of Alzheimer's disease. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1861*(6), 524–537. https://doi.org/10.1016/j.bbalip.2016.03.005

Bellucci, A., Rosi, M. C., Grossi, C., Fiorentini, A., Luccarini, I., & Casamenti, F. (2007). Abnormal processing of tau in the brain of aged TgCRND8 mice. *Neurobiology of Disease*, *27*(3), 328–338. https://doi.org/10.1016/j.nbd.2007.06.008

Bevilaqua, L. R. M., Kerr, D. S., Medina, J. H., Izquierdo, I., & Cammarota, M. (2003). Inhibition of hippocampal Jun N-terminal kinase enhances short-term memory but blocks long-term memory formation and retrieval of an inhibitory avoidance

task. *The European Journal of Neuroscience*, *17*(4), 897–902.

Bicca, M. A., Costa, R., Loch-Neckel, G., Figueiredo, C. P., Medeiros, R., & Calixto, J. B. (2015). B2 receptor blockage prevents Aβ-induced cognitive impairment by neuroinflammation inhibition. *Behavioural Brain Research*, *278*, 482–491. https://doi.org/10.1016/j.bbr.2014.10.040

Blennow, H. K., Zetterberg, Blennow, K., Blennow, K., Hampel, H., Weiner, M., et al. (2010). Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nature Publishing Group*, *6*(16104), 131–144. https://doi.org/10.1038/nrneurol.2010.4

Bloom, G. S. (2014). Amyloid-β and tau: the trigger and bullet in Alzheimer disease pathogenesis. *JAMA Neurology*, *71*(4), 505–8. https://doi.org/10.1001/jamaneurol.2013.5847

Bodles, A. M., & Barger, S. W. (2005). Secreted beta-amyloid precursor protein activates microglia via JNK and p38-MAPK. *Neurobiology of Aging*, *26*(1), 9–16. https://doi.org/10.1016/j.neurobiolaging.2004.02.022

Bogoyevitch, M. A., & Arthur, P. G. (2008). Inhibitors of c-Jun N-terminal kinases: JuNK no more? *Biochimica et Biophysica Acta*, *1784*(1), 76–93. https://doi.org/10.1016/j.bbapap.2007.09.013

Bogoyevitch, M. A., Boehm, I., Oakley, A., Ketterman, A. J., & Barr, R. K. (2004). Targeting the JNK MAPK cascade for inhibition: basic science and therapeutic potential. *Biochimica et Biophysica Acta*, *1697*(1–2), 89–101. https://doi.org/10.1016/j.bbapap.2003.11.016

Bogoyevitch, M. A., Ngoei, K. R. W., Zhao, T. T., Yeap, Y. Y. C., & Ng, D. C. H. (2010). c-Jun N-terminal kinase (JNK) signaling: Recent advances and challenges. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, *1804*(3), 463–475. https://doi.org/10.1016/j.bbapap.2009.11.002

Borgeson, E., Johnson, A. M. F., Lee, Y. S., Till, A., Syed, G. H., Ali-Shah, S. T., et al. (2015). Lipoxin A4 Attenuates Obesity-Induced Adipose Inflammation and Associated Liver and Kidney Disease. *Cell Metabolism*, *22*(1), 125–137. https://doi.org/10.1016/j.cmet.2015.05.003

Borsello, T., & Bonny, C. (2004). Use of cell-permeable peptides to prevent neuronal degeneration. *Trends in Molecular Medicine*, *10*(5), 239–244. https://doi.org/10.1016/j.molmed.2004.03.008

Borsello, T., Clarke, P. G. H., Hirt, L., Vercelli, A., Repici, M., Schorderet, D. F., et al. (2003). A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia. *Nature Medicine*, *9*(9), 1180–1186. https://doi.org/10.1038/nm911

Bozyczko-Coyne, D., O'Kane, T. M., Wu, Z. L., Dobrzanski, P., Murthy, S., Vaught, J. L., et al. (2001). CEP-1347/KT-7515, an inhibitor of SAPK/JNK pathway activation, promotes survival and blocks multiple events associated with Abeta-induced cortical neuron apoptosis. *Journal of Neurochemistry*, *77*(3), 849–63.

Brecht, S., Kirchhof, R., Chromik, A., Willesen, M., Nicolaus, T., Raivich, G., et al. (2005). Specific pathophysiological functions of JNK isoforms in the brain. *European Journal of Neuroscience*, *21*(2), 363–377. https://doi.org/10.1111/j.1460-9568.2005.03857.x

Burckhardt, M., Herke, M., Wustmann, T., Watzke, S., Langer, G., & Fink, A. (2016). Omega-3 fatty acids for the treatment of dementia. In M. Burckhardt (Ed.), *Cochrane Database of Systematic Reviews* (Vol. 4, p. CD009002). Chichester, UK: John Wiley & Sons, Ltd. https://doi.org/10.1002/14651858.CD009002.pub3

Burdge, G. C., Jones, A. E., & Wootton, S. A. (2002). Eicosapentaenoic and docosapentaenoic acids are the principal products of α-linolenic acid metabolism in young men. *British Journal of Nutrition*, *88*(4), 355. https://doi.org/10.1079/BJN2002662

Burdge, G. C., & Wootton, S. A. (2002). Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *British Journal of Nutrition*, *88*(4), 411. https://doi.org/10.1079/BJN2002689

Calder, P. C. (2015). Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1851*(4), 469–484. https://doi.org/10.1016/j.bbalip.2014.08.010

Calon, F., Lim, G. P., Yang, F., Morihara, T., Teter, B., Ubeda, O., et al. (2004). Docosahexaenoic Acid Protects from Dendritic Pathology in an Alzheimer's Disease Mouse Model. *Neuron*, *43*(5), 633–645. https://doi.org/10.1016/j.neuron.2004.08.013

Cao, M., Liu, F., Ji, F., Liang, J., Liu, L., Wu, Q., et al. (2013). Effect of c-Jun N-terminal kinase (JNK)/p38 mitogen-activated protein kinase (p38 MAPK) in morphine-induced tau protein hyperphosphorylation. *Behavioural Brain Research*, *237*, 249–255. https://doi.org/10.1016/j.bbr.2012.09.040

Carter, C. L., Resnick, E. M., Mallampalli, M., & Kalbarczyk, A. (2012). Sex and Gender Differences in Alzheimer's Disease: Recommendations for Future Research. *Journal of Women's Health*, *21*(10), 1018–1023. https://doi.org/10.1089/jwh.2012.3789

Casali, B. T., Corona, A. W., Mariani, M. M., Karlo, J. C., Ghosal, K., & Landreth, G. E. (2015). Omega-3 Fatty Acids Augment the Actions of Nuclear Receptor Agonists in a Mouse Model of Alzheimer's Disease. *Journal of Neuroscience*, *35*(24), 9173–9181. https://doi.org/10.1523/JNEUROSCI.1000-15.2015

Cederholm, T., Salem, N., & Palmblad, J. (2013). Omega-3 Fatty Acids in the Prevention of Cognitive Decline in Humans. *Advances in Nutrition: An International Review Journal*, *4*(6), 672–676. https://doi.org/10.3945/an.113.004556

Chae, S. S., Yoo, C. B., Jo, C., Yun, S.-M., Jo, S. A., & Koh, Y. H. (2010). Caspases-2 and -8 are involved in the presenilin1/γ-secretase-dependent cleavage of amyloid precursor protein after the induction of apoptosis. *Journal of Neuroscience Research*, *88*(9), NA-NA. https://doi.org/10.1002/jnr.22356

Chen, J.-T., Lu, D.-H., Chia, C.-P., Ruan, D.-Y., Sabapathy, K., & Xiao, Z.-C. (2005). Impaired long-term potentiation in c-Jun N-terminal kinase 2-deficient mice. *Journal of Neurochemistry*, *93*(2), 463–73. https://doi.org/10.1111/j.1471-4159.2005.03037.x

Chen, Y.-R., & Tan, T.-H. (1998). Inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway by curcumin. *Oncogene*, *17*(2), 173–178. https://doi.org/10.1038/sj.onc.1201941

Chiang, N., & Serhan, C. N. (2017). Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. *Molecular Aspects of Medicine*. https://doi.org/10.1016/j.mam.2017.03.005

Cho, E. S., Jang, Y. J., Kang, N. J., Hwang, M. K., Kim, Y. T., Lee, K. W., et al. (2009). Cocoa procyanidins attenuate 4-hydroxynonenal-induced apoptosis of PC12 cells by directly inhibiting mitogen-activated protein kinase kinase 4 activity. *Free*

Radical Biology & Medicine, *46*(10), 1319–27. https://doi.org/10.1016/j.freeradbiomed.2009.02.010

Chuang, J. Y., Huang, Y. L., Yen, W. L., Chiang, I. P., Tsai, M. H., & Tang, C. H. (2014). Syk/JNK/AP-1 Signaling Pathway Mediates Interleukin-6-Promoted Cell Migration in Oral Squamous Cell Carcinoma. *International Journal of Molecular Sciences*, *15*(1), 545–559. https://doi.org/10.3390/ijms15010545

Coffey, E. T. (2014). Nuclear and cytosolic JNK signalling in neurons. *Nature Reviews. Neuroscience*, *15*(5), 285–99. https://doi.org/10.1038/nrn3729

Cole, G. M., Ma, Q.-L., & Frautschy, S. A. (2009). Omega-3 fatty acids and dementia. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *81*(2–3), 213–221. https://doi.org/10.1016/j.plefa.2009.05.015

Colombo, A., Bastone, A., Ploia, C., Sclip, A., Salmona, M., Forloni, G., et al. (2009). JNK regulates APP cleavage and degradation in a model of Alzheimer's disease. *Neurobiology of Disease*, *33*(3), 518–525. https://doi.org/10.1016/j.nbd.2008.12.014

Corsinovi, L., Biasi, F., Poli, G., Leonarduzzi, G., & Isaia, G. (2011). Dietary lipids and their oxidized products in Alzheimer's disease. *Molecular Nutrition & Food Research*, *55 Suppl 2*(S2), S161-72. https://doi.org/10.1002/mnfr.201100208

Costello, D. A., & Herron, C. E. (2004). The role of c-Jun N-terminal kinase in the Aβ-mediated impairment of LTP and regulation of synaptic transmission in the hippocampus. *Neuropharmacology*, *46*(5), 655–662. https://doi.org/10.1016/j.neuropharm.2003.11.016

Craig-Schapiro, R., Perrin, R. J., Roe, C. M., Xiong, C., Carter, D., Cairns, N. J., et al. (2010). YKL-40: A novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biological Psychiatry*, *68*(10), 903–912. https://doi.org/10.1016/j.biopsych.2010.08.025

Cui, J., Zhang, M., Zhang, Y.-Q., & Xu, Z.-H. (2007). JNK pathway: diseases and therapeutic potential. *Acta Pharmacologica Sinica*, *28*(5), 601–8. https://doi.org/10.1111/j.1745-7254.2007.00579.x

Das, H. K., Tchedre, K., & Mueller, B. (2012). Repression of transcription of presenilin-1 inhibits γ-secretase independent ER Ca2+ leak that is impaired by FAD mutations. *Journal of Neurochemistry*, *122*(3), 487–500. https://doi.org/10.1111/j.1471-

4159.2012.07794.x

Davis, R. J. (2000). Signal transduction by the JNK group of MAP kinases. *Cell*, *103*(2), 239–52. https://doi.org/doi: 10.1016/S0092-8674(00)00116-1

De Girolamo, L. A., & Billett, E. E. (2006). Role of extracellular-regulated kinase and c-jun NH2-terminal kinase in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurofilament phosphorylation. *Journal of Neuroscience Research*, *83*(4), 680–693. https://doi.org/10.1002/jnr.20765

de Souza, R. J., Mente, A., Maroleanu, A., Cozma, A. I., Ha, V., Kishibe, T., et al. (2015). Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ (Clinical Research Ed.)*, *351*, h3978.

de Souza Fernandes, D. P., Canaan Rezende, F. A., Pereira Rocha, G., De Santis Filgueiras, M., Silva Moreira, P. R., & Gonçalves Alfenas, R. de C. (2015). Effect of eicosapentaenoic acd and docosahexaenoic acid supplementatations to control cognitive decline in dementia and Alzheimer's disease: a systematic review. *Nutricion Hospitalaria*, *32*(2), 528–33. https://doi.org/10.3305/nh.2015.32.2.9111

Dérijard, B., Hibi, M., Wu, I. H., Barrett, T., Su, B., Deng, T., et al. (1994). JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell*, *76*(6), 1025–37.

Dhanasekaran, D. N., & Reddy, E. P. (2008). JNK signaling in apoptosis. *Oncogene*, *27*(48), 6245–51. https://doi.org/10.1038/onc.2008.301

Dhikav, V., & Anand, K. S. (2007). Glucocorticoids may initiate Alzheimer's disease: A Potential therapeutic role for mifepristone (RU-486). *Medical Hypotheses*, *68*(5), 1088–1092. https://doi.org/10.1016/j.mehy.2006.09.038

Dickens, M., Rogers, J. S., Cavanagh, J., Raitano, A., Xia, Z., Halpern, J. R., et al. (1997). A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science*, *277*(5326), 693–6.

Ebenezer, P. J., Weidner, A. M., LeVine, H., Markesbery, W. R., Murphy, M. P., Zhang, L., et al. (2010). Neuron specific toxicity of oligomeric amyloid-β: role for JUN-kinase and oxidative stress. *Journal of Alzheimer's Disease : JAD*, *22*(3), 839–48. https://doi.org/10.3233/JAD-2010-101161

Eckert, G. P., Chang, S., Eckmann, J., Copanaki, E., Hagl, S., Hener, U., et al. (2011).

Liposome-incorporated DHA increases neuronal survival by enhancing non-amyloidogenic APP processing. *Biochimica et Biophysica Acta*, *1808*(1), 236–43. https://doi.org/10.1016/j.bbamem.2010.10.014

Eckert, G. P., Lipka, U., & Muller, W. E. (2013). Omega-3 fatty acids in neurodegenerative diseases: Focus on mitochondria. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, *88*(1), 105–114. https://doi.org/10.1016/j.plefa.2012.05.006

Eminel, S., Roemer, L., Waetzig, V., & Herdegen, T. (2008). c-Jun N-terminal kinases trigger both degeneration and neurite outgrowth in primary hippocampal and cortical neurons. *Journal of Neurochemistry*, *104*(4), 957–69. https://doi.org/10.1111/j.1471-4159.2007.05101.x

Engstrom, W., Ward, A., & Moorwood, K. (2010). The role of scaffold proteins in JNK signalling. *Cell Proliferation*, *43*(1), 56–66. https://doi.org/10.1111/j.1365-2184.2009.00654.x

Feld, M., Krawczyk, M. C., Sol Fustiñana, M., Blake, M. G., Baratti, C. M., Romano, A., et al. (2014). Decrease of ERK/MAPK overactivation in prefrontal cortex reverses early memory deficit in a mouse model of Alzheimer's disease. *Journal of Alzheimer's Disease : JAD*, *40*(1), 69–82. https://doi.org/10.3233/JAD-131076

Feng, H.-L., Fan, H., Dang, H.-Z., Chen, X.-P., Ren, Y., Yang, J.-D., et al. (2014). Neuroprotective effect of curcumin to Aβ of double transgenic mice with Alzheimer's disease. *China Journal of Chinese Materia Medica*, *39*(19), 3846–9.

Ferrer, I., Blanco, R., Carmona, M., & Puig, B. (2001). Phosphorylated mitogen-activated protein kinase (MAPK/ERK-P), protein kinase of 38kDa (p38-P), stress-activated protein kinase (SAPK/JNK-P), and calcium/calmodulin-dependent kinase II (CaM kinase II) are differentially expressed in tau deposits in neurons. *Journal of Neural Transmission*, *108*(12), 1397–1415. https://doi.org/10.1007/s007020100016

Fiala, M., Terrando, N., & Dalli, J. (2015). Specialized Pro-Resolving Mediators from Omega-3 Fatty Acids Improve Amyloid-β Phagocytosis and Regulate Inflammation in Patients with Minor Cognitive Impairment. *Journal of Alzheimer's Disease*, *48*(2), 293–301. https://doi.org/10.3233/JAD-150367

Flood, J. F., & Morley, J. E. (1998). Learning and memory in the SAMP8 mouse.

Neuroscience and Biobehavioral Reviews, *22*(1), 1–20.

Foley, A. M., Ammar, Z. M., Lee, R. H., & Mitchell, C. S. (2015). Systematic review of the relationship between amyloid-β levels and measures of transgenic mouse cognitive deficit in Alzheimer's disease. *Journal of Alzheimer's Disease : JAD*, *44*(3), 787–95. https://doi.org/10.3233/JAD-142208

Fraga, V. G., Carvalho, M. das G., Caramelli, P., de Sousa, L. P., & Gomes, K. B. (2017). Resolution of inflammation, n-3 fatty acid supplementation and Alzheimer disease: A narrative review. *Journal of Neuroimmunology*, *310*, 111–119. https://doi.org/10.1016/j.jneuroim.2017.07.005

Frautschy, S. A., Horn, D. L., Sigel, J. J., Harris-White, M. E., Mendoza, J. J., Yang, F., et al. (1998). Protease inhibitor coinfusion with amyloid beta-protein results in enhanced deposition and toxicity in rat brain. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *18*(20), 8311–21.

Frozza, R. L., Bernardi, A., Hoppe, J. B., Meneghetti, A. B., Matté, A., Battastini, A. M. O., et al. (2013). Neuroprotective Effects of Resveratrol Against Aβ Administration in Rats are Improved by Lipid-Core Nanocapsules. *Molecular Neurobiology*, *47*(3), 1066–1080. https://doi.org/10.1007/s12035-013-8401-2

Funakoshi-Tago, M., Nagata, T., Tago, K., Tsukada, M., Tanaka, K., Nakamura, S., et al. (2012). Fullerene derivative prevents cellular transformation induced by JAK2 V617F mutant through inhibiting c-Jun N-terminal kinase pathway. *Cellular Signalling*, *24*(11), 2024–2034. https://doi.org/10.1016/j.cellsig.2012.06.014

Ganguly, A., Oo, T. F., Rzhetskaya, M., Pratt, R., Yarygina, O., Momoi, T., et al. (2004). CEP11004, a novel inhibitor of the mixed lineage kinases, suppresses apoptotic death in dopamine neurons of the substantia nigra induced by 6-hydroxydopamine. *Journal of Neurochemistry*, *88*(2), 469–80.

Gehringer, M., Muth, F., Koch, P., & Laufer, S. A. (2015). c-Jun N-terminal kinase inhibitors: a patent review (2010 – 2014). *Expert Opinion on Therapeutic Patents*, *25*(8), 849–872. https://doi.org/10.1517/13543776.2015.1039984

Gil-Bea, F., Aisa, B., Solomon, A., Solas, M., Mugueta, C., Winblad, B., et al. (2010a). HPA Axis Dysregulation Associated to Apolipoprotein E4 Genotype in Alzheimer's Disease. *Journal of Alzheimer's Disease*, *22*(3), 829–838. https://doi.org/10.3233/JAD-2010-100663

Gil-Bea, F. J., Solas, M., Solomon, A., Mugueta, C., Winblad, B., Kivipelto, M., et al. (2010b). Insulin levels are decreased in the cerebrospinal fluid of women with prodomal Alzheimer's disease. *Journal of Alzheimer's Disease : JAD*, *22*(2), 405–13. https://doi.org/10.3233/JAD-2010-100795

Giuliani, D., Ottani, A., Zaffe, D., Galantucci, M., Strinati, F., Lodi, R., et al. (2013). Hydrogen sulfide slows down progression of experimental Alzheimer's disease by targeting multiple pathophysiological mechanisms. *Neurobiology of Learning and Memory*, *104*, 82–91. https://doi.org/10.1016/j.nlm.2013.05.006

Goodfellow, V. S., Loweth, C. J., Ravula, S. B., Wiemann, T., Nguyen, T., Xu, Y., et al. (2013). Discovery, Synthesis, and Characterization of an Orally Bioavailable, Brain Penetrant Inhibitor of Mixed Lineage Kinase 3. *Journal of Medicinal Chemistry*, *56*(20), 8032–8048. https://doi.org/10.1021/jm401094t

Goodman, Y., & Mattson, M. P. (1994). Staurosporine and K-252 compounds protect hippocampal neurons against amyloid beta-peptide toxicity and oxidative injury. *Brain Research*, *650*(1), 170–4.

Gourmaud, S., Paquet, C., Dumurgier, J., Pace, C., Bouras, C., Gray, F., et al. (2015). Increased levels of cerebrospinal fluid JNK3 associated with amyloid pathology: Links to cognitive decline. *Journal of Psychiatry and Neuroscience*, *40*(3), 151–161. https://doi.org/10.1503/jpn.140062

Gregor, M. F., & Hotamisligil, G. S. (2011). Inflammatory mechanisms in obesity. *Annual Review of Immunology*, *29*(1), 415–45. https://doi.org/10.1146/annurev-immunol-031210-101322

Grimm, M. O. W., Kuchenbecker, J., Grösgen, S., Burg, V. K., Hundsdörfer, B., Rothhaar, T. L., et al. (2011). Docosahexaenoic acid reduces amyloid beta production via multiple pleiotropic mechanisms. *The Journal of Biological Chemistry*, *286*(16), 14028–39. https://doi.org/10.1074/jbc.M110.182329

Guglielmotto, M., Monteleone, D., Giliberto, L., Fornaro, M., Borghi, R., Tamagno, E., et al. (2011). Amyloid-β_{42} activates the expression of BACE1 through the JNK pathway. *Journal of Alzheimer's Disease : JAD*, *27*(4), 871–83. https://doi.org/10.3233/JAD-2011-110884

Guillozet-Bongaarts, A. L., Cahill, M. E., Cryns, V. L., Reynolds, M. R., Berry, R. W., & Binder, L. I. (2006). Pseudophosphorylation of tau at serine 422 inhibits caspase

cleavage: in vitro evidence and implications for tangle formation in vivo. *Journal of Neurochemistry*, *97*(4), 1005–14. https://doi.org/10.1111/j.1471-4159.2006.03784.x

Guillozet-Bongaarts, A. L., Garcia-Sierra, F., Reynolds, M. R., Horowitz, P. M., Fu, Y., Wang, T., et al. (2005). Tau truncation during neurofibrillary tangle evolution in Alzheimer's disease. *Neurobiology of Aging*, *26*(7), 1015–1022. https://doi.org/10.1016/j.neurobiolaging.2004.09.019

Haas, C. (2012). Strategies, development, and pitfalls of therapeutic options for Alzheimer's disease. *Journal of Alzheimer's Disease*, *28*(2), 241–281. https://doi.org/10.3233/JAD-2011-110986

Halliday, G. M., Holton, J. L., Revesz, T., & Dickson, D. W. (2011). Neuropathology underlying clinical variability in patients with synucleinopathies. *Acta Neuropathologica*, *122*(2), 187–204. https://doi.org/10.1007/s00401-011-0852-9

Hashimoto, M., Hsu, L. J., Rockenstein, E., Takenouchi, T., Mallory, M., & Masliah, E. (2002). α-synuclein protects against oxidative stress via inactivation of the c-Jun N-terminal kinase stress-signaling pathway in neuronal cells. *Journal of Biological Chemistry*, *277*(13), 11465–11472. https://doi.org/10.1074/jbc.M111428200

Hashimoto, Y., Niikura, T., Chiba, T., Tsukamoto, E., Kadowaki, H., Nishitoh, H., et al. (2003). The Cytoplasmic Domain of Alzheimer's Amyloid- Protein Precursor Causes Sustained Apoptosis Signal-Regulating Kinase 1/c-Jun NH2-Terminal Kinase-Mediated Neurotoxic Signal via Dimerization. *Journal of Pharmacology and Experimental Therapeutics*, *306*(3), 889–902. https://doi.org/10.1124/jpet.103.051383

Higashi, S., Iseki, E., Yamamoto, R., Minegishi, M., Hino, H., Fujisawa, K., et al. (2007). Concurrence of TDP-43, tau and alpha-synuclein pathology in brains of Alzheimer's disease and dementia with Lewy bodies. *Brain Research*, *1184*(1), 284–294. https://doi.org/10.1016/j.brainres.2007.09.048

Hjorth, E., Zhu, M., Toro, V. C., Vedin, I., Palmblad, J., Cederholm, T., et al. (2013). Omega-3 fatty acids enhance phagocytosis of Alzheimer's disease-related amyloid-β42 by human microglia and decrease inflammatory markers. *Journal of Alzheimer's Disease : JAD*, *35*(4), 697–713. https://doi.org/10.3233/JAD-130131

Holzberg, D., Knight, C. G., Dittrich-Breiholz, O., Schneider, H., Dörrie, A., Hoffmann, E.,

et al. (2003). Disruption of the c-JUN-JNK Complex by a Cell-permeable Peptide Containing the c-JUN δ Domain Induces Apoptosis and Affects a Distinct Set of Interleukin-1-induced Inflammatory Genes. *Journal of Biological Chemistry*, *278*(41), 40213–40223. https://doi.org/10.1074/jbc.M304058200

Hong, Y. K., Lee, S., Park, S. H., Lee, J. H., Han, S. Y., Kim, S. T., et al. (2012). Inhibition of JNK/dFOXO pathway and caspases rescues neurological impairments in Drosophila Alzheimer's disease model. *Biochemical and Biophysical Research Communications*, *419*(1), 49–53. https://doi.org/10.1016/j.bbrc.2012.01.122

Howlett, D. R., Whitfield, D., Johnson, M., Attems, J., O'Brien, J. T., Aarsland, D., et al. (2014). Regional Multiple Pathology Scores Are Associated with Cognitive Decline in Lewy Body Dementias. *Brain Pathology*, *25*, 401–408. https://doi.org/10.1111/bpa.12182

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., et al. (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science*, *274*(5284), 99–102.

Hu, W. T., Josephs, K. A., Knopman, D. S., Boeve, B. F., Dickson, D. W., Petersen, R. C., et al. (2008). Temporal lobar predominance of TDP-43 neuronal cytoplasmic inclusions in Alzheimer disease. *Acta Neuropathologica*, *116*(2), 215–220. https://doi.org/10.1007/s00401-008-0400-4

Hwang, D. Y., Cho, J. S., Lee, S. H., Chae, K. R., Lim, H. J., Min, S. H., et al. (2004). Aberrant expressions of pathogenic phenotype in Alzheimer's diseased transgenic mice carrying NSE-controlled APPsw. *Experimental Neurology*, *186*(1), 20–32. https://doi.org/10.1016/j.expneurol.2003.09.021

Irizarry, M. C., McNamara, M., Fedorchak, K., Hsiao, K., & Hyman, B. T. (1997). APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *Journal of Neuropathology and Experimental Neurology*, *56*(9), 965–73.

Jia, J. P., Meng, R., Sun, Y. X., Sun, W. J., Ji, X. M., & Jia, L. F. (2005). Cerebrospinal fluid tau, Aβ1-42 and inflammatory cytokines in patients with Alzheimer's disease and vascular dementia. *Neuroscience Letters*, *383*(1–2), 12–16. https://doi.org/10.1016/j.neulet.2005.03.051

Josephs, K. A., Murray, M. E., Whitwell, J. L., Parisi, J. E., Petrucelli, L., Jack, C. R., et al.

(2014). Staging TDP-43 pathology in Alzheimer's disease. *Acta Neuropathologica*, *127*(3), 441–450. https://doi.org/10.1007/s00401-013-1211-9

Jump, D. B., Depner, C. M., & Tripathy, S. (2012). Omega-3 fatty acid supplementation and cardiovascular disease. *Journal of Lipid Research*, *53*(12), 2525–45. https://doi.org/10.1194/jlr.R027904

Jung, Y., Dickson, D. W., Murray, M. E., Whitwell, J. L., Knopman, D. S., Boeve, B. F., et al. (2014). TDP-43 in Alzheimer's disease is not associated with clinical FTLD or Parkinsonism. *Journal of Neurology*, *261*(7), 1344–1348. https://doi.org/10.1007/s00415-014-7352-5

Kempuraj, D., Thangavel, R., Natteru, P. A., Selvakumar, G. P., Saeed, D., Zahoor, H., et al. (2016). Neuroinflammation Induces Neurodegeneration. *Journal of Neurology, Neurosurgery and Spine*, *1*(1).

Killick, R., Ribe, E. M., Al-Shawi, R., Malik, B., Hooper, C., Fernandes, C., et al. (2014). Clusterin regulates β-amyloid toxicity via Dickkopf-1-driven induction of the wnt-PCP-JNK pathway. *Molecular Psychiatry*, *19*(1), 88–98. https://doi.org/10.1038/mp.2012.163

Kim, J.-H., Chae, M., Choi, A.-R., Sik Kim, H., & Yoon, S. (2014). SP600125 overcomes antimitotic drug-resistance in cancer cells by increasing apoptosis with independence of P-gp inhibition. *European Journal of Pharmacology*, *723*, 141–147. https://doi.org/10.1016/j.ejphar.2013.11.026

Kobylecki, C., Langheinrich, T., Hinz, R., Vardy, E. R. L. C., Brown, G., Martino, M.-E., et al. (2015). 18F-Florbetapir PET in Patients with Frontotemporal Dementia and Alzheimer Disease. *Journal of Nuclear Medicine*, *56*(3), 386–391. https://doi.org/10.2967/jnumed.114.147454

Koch, P., Gehringer, M., & Laufer, S. A. (2015). Inhibitors of c-Jun N-Terminal Kinases: An Update. *Journal of Medicinal Chemistry*, *58*(1), 72–95. https://doi.org/10.1021/jm501212r

Koedam, E. L. G. E., van der Vlies, A. E., van der Flier, W. M., Verwey, N. A., Koene, T., Scheltens, P., et al. (2013). Cognitive correlates of cerebrospinal fluid biomarkers in frontotemporal dementia. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association*, *9*(3), 269–75. https://doi.org/10.1016/j.jalz.2011.12.007

Kolarova, M., García-Sierra, F., Bartos, A., Ricny, J., & Ripova, D. (2012). Structure and

pathology of tau protein in Alzheimer disease. *International Journal of Alzheimer's Disease*, *2012*. https://doi.org/10.1155/2012/731526

Kou, W., Luchtman, D., & Song, C. (2008). Eicosapentaenoic acid (EPA) increases cell viability and expression of neurotrophin receptors in retinoic acid and brain-derived neurotrophic factor differentiated SH-SY5Y cells. *European Journal of Nutrition*, *47*(2), 104–13. https://doi.org/10.1007/s00394-008-0703-1

Kovari, E., Horvath, J., & Bouras, C. (2009). Neuropathology of Lewy body disorders. *Brain Research Bulletin*, *80*(4–5), 203–210. https://doi.org/10.1016/j.brainresbull.2009.06.018

Kuan, C. Y., Yang, D. D., Samanta Roy, D. R., Davis, R. J., Rakic, P., & Flavell, R. A. (1999). The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron*, *22*(4), 667–76.

Lagalwar, S., Berry, R. W., & Binder, L. I. (2007). Relation of hippocampal phospho-SAPK/JNK granules in Alzheimer's disease and tauopathies to granulovacuolar degeneration bodies. *Acta Neuropathologica*, *113*(1), 63–73. https://doi.org/10.1007/s00401-006-0159-4

Lagalwar, S., Guillozet-Bongaarts, A. L., Berry, R. W., & Binder, L. I. (2006). Formation of Phospho-SAPK/JNK Granules in the Hippocampus Is an Early Event in Alzheimer Disease. *Journal of Neuropathology & Experimental Neurology*, *65*(5), 455–464. https://doi.org/10.1097/01.jnen.0000229236.98124.d8

Lee, H. mi, Kim, K.-S., & Kim, J. (2014). A comparative study of the effects of inhibitory cytokines on human natural killer cells and the mechanistic features of transforming growth factor-beta. *Cellular Immunology*, *290*(1), 52–61. https://doi.org/10.1016/j.cellimm.2014.05.001

Lee, J. M., Blennow, K., Andreasen, N., Laterza, O., Modur, V., Olander, J., et al. (2008). The brain injury biomarker VLP-1 is increased in the cerebrospinal fluid of Alzheimer disease patients. *Clinical Chemistry*, *54*(10), 1617–1623. https://doi.org/10.1373/clinchem.2008.104497

Lewis, D. A. (2002). The human brain revisited: Opportunities and challenges in postmortem studies of psychiatric disorders. *Neuropsychopharmacology*, *26*(2), 143–154. https://doi.org/10.1016/S0893-133X(01)00393-1

Lewis, H., Beher, D., Cookson, N., Oakley, A., Piggott, M., Morris, C. M., et al. (2006).

Quantification of Alzheimer pathology in ageing and dementia: Age-related accumulation of beta-amyloid(42) peptide in vascular dementia. *Neuropathology and Applied Neurobiology*, *32*(2), 103–118. https://doi.org/10.1111/j.1365-2990.2006.00696.x

Li, Y., Dalli, J., Chiang, N., Baron, R. M., Quintana, C., & Serhan, C. N. (2013a). Plasticity of leukocytic exudates in resolving acute inflammation is regulated by MicroRNA and proresolving mediators. *Immunity*, *39*(5), 885–98. https://doi.org/10.1016/j.immuni.2013.10.011

Li, Y., Wang, F., Liu, C., Zeng, M., Han, X., Luo, T., et al. (2013b). JNK pathway may be involved in isoflurane-induced apoptosis in the hippocampi of neonatal rats. *Neuroscience Letters*, *545*, 17–22. https://doi.org/10.1016/j.neulet.2013.04.008

Lim, G. P., Calon, F., Morihara, T., Yang, F., Teter, B., Ubeda, O., et al. (2005). A Diet Enriched with the Omega-3 Fatty Acid Docosahexaenoic Acid Reduces Amyloid Burden in an Aged Alzheimer Mouse Model. *Journal of Neuroscience*, *25*(12), 3032–3040. https://doi.org/10.1523/JNEUROSCI.4225-04.2005

Lin, W., Ding, M., Xue, J., & Leng, W. (2013). The role of TLR2/JNK/NF-κB pathway in amyloid β peptide-induced inflammatory response in mouse NG108-15 neural cells. *International Immunopharmacology*, *17*(3), 880–884. https://doi.org/10.1016/j.intimp.2013.09.016

Lonergan, P. E., Martin, D. S. D., Horrobin, D. F., & Lynch, M. A. (2002). Neuroprotective effect of eicosapentaenoic acid in hippocampus of rats exposed to gamma-irradiation. *The Journal of Biological Chemistry*, *277*(23), 20804–11. https://doi.org/10.1074/jbc.M202387200

Lonergan, P. E., Martin, D. S. D., Horrobin, D. F., & Lynch, M. A. (2004). Neuroprotective actions of eicosapentaenoic acid on lipopolysaccharide-induced dysfunction in rat hippocampus. *Journal of Neurochemistry*, *91*(1), 20–9. https://doi.org/10.1111/j.1471-4159.2004.02689.x

Lorente-Cebrián, S., Costa, A. G. V., Navas-Carretero, S., Zabala, M., Laiglesia, L. M., Martínez, J. A., et al. (2015). An update on the role of omega-3 fatty acids on inflammatory and degenerative diseases. *Journal of Physiology and Biochemistry*, *71*(2), 341–349. https://doi.org/10.1007/s13105-015-0395-y

Lorente-Cebrián, S., Costa, A. G. V., Navas-Carretero, S., Zabala, M., Martínez, J. A., &

Moreno-Aliaga, M. J. (2013). Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. *Journal of Physiology and Biochemistry*, *69*(3), 633–651. https://doi.org/10.1007/s13105-013-0265-4

Lukiw, W. J., Cui, J.-G., Marcheselli, V. L., Bodker, M., Botkjaer, A., Gotlinger, K., et al. (2005). A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. *Journal of Clinical Investigation*, *115*(10), 2774–2783. https://doi.org/10.1172/JCI25420

Ma, G., Chen, S., Wang, X., Ba, M., Yang, H., & Lu, G. (2005). Short-term interleukin-1(beta) increases the release of secreted APP(alpha) via MEK1/2-dependent and JNK-dependent alpha-secretase cleavage in neuroglioma U251 cells. *Journal of Neuroscience Research*, *80*(5), 683–92. https://doi.org/10.1002/jnr.20515

Ma, Q.-L., Yang, F., Rosario, E. R., Ubeda, O. J., Beech, W., Gant, D. J., 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. *Journal of Neuroscience*, *29*(28), 9078–9089. https://doi.org/10.1523/JNEUROSCI.1071-09.2009

Maiti, P., Manna, J., Veleri, S., & Frautschy, S. (2014). Molecular Chaperone Dysfunction in Neurodegenerative Diseases and Effects of Curcumin. *BioMed Research International*, *2014*, 1–14. https://doi.org/10.1155/2014/495091

Marker, D. F., Tremblay, M.-E., Puccini, J. M., Barbieri, J., Gantz Marker, M. A., Loweth, C. J., et al. (2013). The New Small-Molecule Mixed-Lineage Kinase 3 Inhibitor URMC-099 Is Neuroprotective and Anti-Inflammatory in Models of Human Immunodeficiency Virus-Associated Neurocognitive Disorders. *Journal of Neuroscience*, *33*(24), 9998–10010. https://doi.org/10.1523/JNEUROSCI.0598-13.2013

Marques, C. A., Keil, U., Bonert, A., Steiner, B., Haass, C., Muller, W. E., et al. (2003). Neurotoxic mechanisms caused by the alzheimer's disease-linked Swedish amyloid precursor protein. Mutation oxidative stress, caspases, and the JNK pathway. *Journal of Biological Chemistry*, *278*(30), 28294–28302. https://doi.org/10.1074/jbc.M212265200

Martínez-Fernández, L., González-Muniesa, P., Laiglesia, L. M., Sáinz, N., Prieto-

Hontoria, P. L., Escoté, X., et al. (2017). Maresin 1 improves insulin sensitivity and attenuates adipose tissue inflammation in *ob/ob* and diet-induced obese mice. *The FASEB Journal*, *31*(5), 2135–2145. https://doi.org/10.1096/fj.201600859R

Martínez-Fernández, L., Laiglesia, L. M., Huerta, A. E., Martínez, J. A., & Moreno-Aliaga, M. J. (2015). Omega-3 fatty acids and adipose tissue function in obesity and metabolic syndrome. *Prostaglandins & Other Lipid Mediators*, *121*(Pt A), 24–41. https://doi.org/10.1016/j.prostaglandins.2015.07.003

Martisova, E., Aisa, B., Guereñu, G., & Ramírez, M. J. (2013). Effects of early maternal separation on biobehavioral and neuropathological markers of Alzheimer's disease in adult male rats. *Current Alzheimer Research*, *10*(4), 420–32.

Mattsson, N., Carrillo, M. C., Dean, R. A., Devous Sr, M. D., Nikolcheva, T., Pesini, P., et al. (2015). Revolutionizing Alzheimer's disease and clinical trials through biomarkers. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*, *1*(4), 1–8. https://doi.org/10.1016/j.dadm.2015.09.001

Mazzitelli, S., Xu, P., Ferrer, I., Davis, R. J., & Tournier, C. (2011). The Loss of c-Jun N-Terminal Protein Kinase Activity Prevents the Amyloidogenic Cleavage of Amyloid Precursor Protein and the Formation of Amyloid Plaques In Vivo. *Journal of Neuroscience*, *31*(47), 16969–16976. https://doi.org/10.1523/JNEUROSCI.4491-11.2011

Meade, A. J., Meloni, B. P., Mastaglia, F. L., Watt, P. M., & Knuckey, N. W. (2010). AP-1 inhibitory peptides attenuate in vitro cortical neuronal cell death induced by kainic acid. *Brain Research*, *1360*, 8–16. https://doi.org/10.1016/j.brainres.2010.09.007

Mehan, S., Meena, H., Sharma, D., & Sankhla, R. (2011). JNK: A stress-activated protein kinase therapeutic strategies and involvement in Alzheimer's and various neurodegenerative abnormalities. *Journal of Molecular Neuroscience*, *43*(3), 376–390. https://doi.org/10.1007/s12031-010-9454-6

Meloni, B. P., Craig, A. J., Milech, N., Hopkins, R. M., Watt, P. M., & Knuckey, N. W. (2014). The Neuroprotective Efficacy of Cell-Penetrating Peptides TAT, Penetratin, Arg-9, and Pep-1 in Glutamic Acid, Kainic Acid, and In Vitro Ischemia Injury Models Using Primary Cortical Neuronal Cultures. *Cellular and Molecular Neurobiology*, *34*(2), 173–181. https://doi.org/10.1007/s10571-013-9999-3

Miller, A. A., & Spencer, S. J. (2014). Obesity and neuroinflammation: A pathway to cognitive impairment. *Brain, Behavior, and Immunity*, *42*, 10–21. https://doi.org/10.1016/j.bbi.2014.04.001

Mizwicki, M. T., Liu, G., Fiala, M., Magpantay, L., Sayre, J., Siani, A., et al. (2013). 1α,25-dihydroxyvitamin D3 and resolvin D1 retune the balance between amyloid-β phagocytosis and inflammation in Alzheimer's disease patients. *Journal of Alzheimer's Disease : JAD*, *34*(1), 155–70. https://doi.org/10.3233/JAD-121735

Mondragon-Rodriguez, S., Basurto-Islas, G., Santa-Maria, I., Mena, R., Binder, L. I., Avila, J., et al. (2008). Cleavage and conformational changes of tau protein follow phosphorylation during Alzheimer's disease. *International Journal of Experimental Pathology*, *89*(2), 81–90. https://doi.org/10.1111/j.1365-2613.2007.00568.x

Morishima, Y., Gotoh, Y., Zieg, J., Barrett, T., Takano, H., Flavell, R., et al. (2001). Beta-amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand. *The Journal of Neuroscience*, *21*(19), 7551–7560. https://doi.org/21/19/7551

Morley, J. E., Farr, S. A., Kumar, V. B., & Armbrecht, H. J. (2012). The SAMP8 mouse: a model to develop therapeutic interventions for Alzheimer's disease. *Current Pharmaceutical Design*, *18*(8), 1123–30.

Morris, M. C., Evans, D. A., Bienias, J. L., Tangney, C. C., Bennett, D. A., Wilson, R. S., et al. (2003). Consumption of Fish and n-3 Fatty Acids and Risk of Incident Alzheimer Disease. *Archives of Neurology*, *60*(7), 940. https://doi.org/10.1001/archneur.60.7.940

Mudher, A., Chapman, S., Richardson, J., Asuni, A., Gibb, G., Pollard, C., et al. (2001). Dishevelled regulates the metabolism of amyloid precursor protein via protein kinase C/mitogen-activated protein kinase and c-Jun terminal kinase. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *21*(14), 4987–95.

Newman, A. B., Fitzpatrick, A. L., Lopez, O., Jackson, S., Lyketsos, C., Jagust, W., et al. (2005). Dementia and Alzheimer's Disease Incidence in Relationship to Cardiovascular Disease in the Cardiovascular Health Study Cohort. *Journal of the American Geriatrics Society*, *53*(7), 1101–1107. https://doi.org/10.1111/j.1532-5415.2005.53360.x

Nishina, H., Wada, T., & Katada, T. (2004). Physiological roles of SAPK/JNK signaling pathway. *Journal of Biochemistry*, *136*(2), 123–126. https://doi.org/10.1093/jb/mvh117

Okada, M., Sato, A., Shibuya, K., Watanabe, E., Seino, S., Suzuki, S., et al. (2014). JNK contributes to temozolomide resistance of stem-like glioblastoma cells via regulation of MGMT expression. *International Journal of Oncology*, *44*(2), 591–599. https://doi.org/10.3892/ijo.2013.2209

Okamoto, Y., Ihara, M., Fujita, Y., Ito, H., Takahashi, R., & Tomimoto, H. (2009). Cortical microinfarcts in Alzheimer's disease and subcortical vascular dementia. *Neuroreport*, *20*(11), 990–6. https://doi.org/10.1097/WNR.0b013e32832d2e6a

Okazawa, H., & Estus, S. (2002). The JNK / c-Jun cascade and Alzheimer ' s disease. *American Journal of Alzheimer's Disease and Other Dementias*, *17*(2).

Orejana, L., Barros-Miñones, L., Aguirre, N., & Puerta, E. (2013). Implication of JNK pathway on tau pathology and cognitive decline in a senescence-accelerated mouse model. *Experimental Gerontology*, *48*(6), 565–571. https://doi.org/10.1016/j.exger.2013.03.001

Orejana, L., Barros-Miñones, L., Jordan, J., Cedazo-Minguez, A., Tordera, R. M., Aguirre, N., et al. (2015). Sildenafil Decreases BACE1 and Cathepsin B Levels and Reduces APP Amyloidogenic Processing in the SAMP8 Mouse. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *70*(6), 675–85. https://doi.org/10.1093/gerona/glu106

Otth, C., Mendoza-Naranjo, A., Mujica, L., Zambrano, A., Concha, I. I., & Maccioni, R. B. (2003). Modulation of the JNK and p38 pathways by cdk5 protein kinase in a transgenic mouse model of Alzheimer's disease. *Neuroreport*, *14*(18), 2403–9. https://doi.org/10.1097/01.wnr.0000099988.54721.3c

Pallas, M., Camins, A., Smith, M. A., Perry, G., Lee, H., & Casadesus, G. (2008). From aging to Alzheimer's disease: unveiling "the switch" with the senescence-accelerated mouse model (SAMP8). *Journal of Alzheimer's Disease : JAD*, *15*(4), 615–24.

Paraskevas, G. P., Kapaki, E., Papageorgiou, S. G., Kalfakis, N., Andreadou, E., Zalonis, I., et al. (2009). CSF biomarker profile and diagnostic value in vascular dementia. *European Journal of Neurology*, *16*(2), 205–211. https://doi.org/10.1111/j.1468-

1331.2008.02387.x

Parker, S. J., Meyerowitz, J., James, J. L., Liddell, J. R., Crouch, P. J., Kanninen, K. M., et al. (2012). Endogenous TDP-43 localized to stress granules can subsequently form protein aggregates. *Neurochemistry International*, *60*(4), 415–424. https://doi.org/10.1016/j.neuint.2012.01.019

Parkinson Study Group. (2004). The safety and tolerability of a mixed lineage kinase inhibitor (CEP-1347) in PD. *Neurology*, *62*(2), 330–2.

Parkinson Study Group PRECEPT Investigators. (2007). Mixed lineage kinase inhibitor CEP-1347 fails to delay disability in early Parkinson disease. *Neurology*, *69*(15), 1480–1490. https://doi.org/10.1212/01.wnl.0000277648.63931.c0

Pearson, A. G., Byrne, U. T. E., MacGibbon, G. A., Faull, R. L. M., & Dragunow, M. (2006). Activated c-Jun is present in neurofibrillary tangles in Alzheimer's disease brains. *Neuroscience Letters*, *398*(3), 246–250. https://doi.org/10.1016/j.neulet.2006.01.031

Petursdottir, A. L., Farr, S. A., Morley, J. E., Banks, W. A., & Skuladottir, G. V. (2007). Lipid peroxidation in brain during aging in the senescence-accelerated mouse (SAM). *Neurobiology of Aging*, *28*(8), 1170–1178. https://doi.org/10.1016/j.neurobiolaging.2006.05.033

Petursdottir, A. L., Farr, S. A., Morley, J. E., Banks, W. A., & Skuladottir, G. V. (2008). Effect of dietary n-3 polyunsaturated fatty acids on brain lipid fatty acid composition, learning ability, and memory of senescence-accelerated mouse. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *63*(11), 1153–60.

Ploia, C., Antoniou, X., Sclip, A., Grande, V., Cardinetti, D., Colombo, A., et al. (2011). JNK plays a key role in tau hyperphosphorylation in Alzheimer's disease models. *Journal of Alzheimer's Disease : JAD*, *26*(2), 315–29. https://doi.org/10.3233/JAD-2011-110320

Pocivavsek, A., & Rebeck, G. W. (2009). Inhibition of c-Jun N-terminal kinase increases apoE expression in vitro and in vivo. *Biochemical and Biophysical Research Communications*, *387*(3), 516–520. https://doi.org/10.1016/j.bbrc.2009.07.048

Popp, J., Meichsner, S., Kölsch, H., Lewczuk, P., Maier, W., Kornhuber, J., et al. (2013). Cerebral and extracerebral cholesterol metabolism and CSF markers of

Alzheimer's disease. *Biochemical Pharmacology*, *86*(1), 37–42. https://doi.org/10.1016/j.bcp.2012.12.007

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., & Ferri, C. P. (2013). The global prevalence of dementia: A systematic review and metaanalysis. *Alzheimer's & Dementia*, *9*(1), 63–75.e2. https://doi.org/10.1016/j.jalz.2012.11.007

Querfurth, H. W., & Laferla, F. M. (2010). Alzheimer's Disease. *The New England Journal of Medicine*, 329–344. https://doi.org/10.1016/B978-0-12-803699-0.00045-1

Rahman, M., Zhang, Z., Mody, A. A., Su, D. M., & Das, H. K. (2012). Intraperitoneal injection of JNK-specific inhibitor SP600125 inhibits the expression of presenilin-1 and Notch signaling in mouse brain without induction of apoptosis. *Brain Research*, *1448*, 117–128. https://doi.org/10.1016/j.brainres.2012.01.066

Ramin, M., Azizi, P., Motamedi, F., Haghparast, A., & Khodagholi, F. (2011). Inhibition of JNK phosphorylation reverses memory deficit induced by β-amyloid (1–42) associated with decrease of apoptotic factors. *Behavioural Brain Research*, *217*(2), 424–431. https://doi.org/10.1016/j.bbr.2010.11.017

Reddy, C. E., Albanito, L., De Marco, P., Aiello, D., Maggiolini, M., Napoli, A., et al. (2013). Multisite phosphorylation of c-Jun at threonine 91/93/95 triggers the onset of c-Jun pro-apoptotic activity in cerebellar granule neurons. *Cell Death and Disease*, *4*(10), e852. https://doi.org/10.1038/cddis.2013.381

Rey, C., Nadjar, A., Buaud, B., Vaysse, C., Aubert, A., Pallet, V., et al. (2016). Resolvin D1 and E1 promote resolution of inflammation in microglial cells in vitro. *Brain, Behavior, and Immunity*, *55*, 249–259. https://doi.org/10.1016/j.bbi.2015.12.013

Reynolds, C. H., Betts, J. C., Blackstock, W. P., Nebreda, A. R., & Anderton, B. H. (2000). Phosphorylation sites on tau identified by nanoelectrospray mass spectrometry: Differences in vitro between the mitogen-activated protein kinases ERK2, c-Jun N-terminal kinase and P38, and glycogen synthase kinase- 3β *Journal of Neurochemistry*, *74*(4), 1587–1595. https://doi.org/10.1046/j.1471-4159.2000.0741587.x

Rochfort, S. J., Towerzey, L., Carroll, A., King, G., Michael, A., Pierens, G., et al. (2005). Latifolians A and B, Novel JNK3 Kinase Inhibitors from the Papua New Guinean Plant Gnetum Latifolium. *Journal of Natural Products*, *68*(7), 1080–1082.

https://doi.org/10.1021/np049616i

Sabio, G., Das, M., Mora, A., Zhang, Z., Jun, J. Y., Ko, H. J., et al. (2008). A Stress Signaling Pathway in Adipose Tissue Regulates Hepatic Insulin Resistance. *Science*, *322*(5907), 1539–1543. https://doi.org/10.1126/science.1160794

Sahara, N., Murayama, M., Lee, B., Park, J.-M. M., Lagalwar, S., Binder, L. I., et al. (2008). Active c-jun N-terminal kinase induces caspase cleavage of tau and additional phosphorylation by GSK-3-beta is required for tau aggregation. *European Journal of Neuroscience*, *27*(11), 2897–2906. https://doi.org/10.1111/j.1460-9568.2008.06258.x

Salkovic-Petrisic, M., Knezovic, A., Hoyer, S., & Riederer, P. (2013). What have we learned from the streptozotocin-induced animal model of sporadic Alzheimer's disease, about the therapeutic strategies in Alzheimer's research. *Journal of Neural Transmission*, *120*(1), 233–252. https://doi.org/10.1007/s00702-012-0877-9

Saporito, M. S., Hudkins, R. L., & Maroney, A. C. (2002). Discovery of CEP-1347/KT-7515, an inhibitor of the JNK/SAPK pathway for the treatment of neurodegenerative diseases. *Progress in Medicinal Chemistry*, *40*, 23–62.

Savage, M. J., Lin, Y.-G., Ciallella, J. R., Flood, D. G., & Scott, R. W. (2002). Activation of c-Jun N-terminal kinase and p38 in an Alzheimer's disease model is associated with amyloid deposition. *Journal of Neuroscience*, *22*(9), 3376–3385. https://doi.org/20026352

Scheff, S. W., Price, D. A., Schmitt, F. A., DeKosky, S. T., & Mufson, E. J. (2007). Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology*, *68*(18), 1501–1508. https://doi.org/10.1212/01.wnl.0000260698.46517.8f

Scheltens, P., Blennow, K., Breteler, M. M. B., de Strooper, B., Frisoni, G. B., Salloway, S., et al. (2016). Alzheimer's disease. *The Lancet*, *388*(10043), 505–517. https://doi.org/10.1016/S0140-6736(15)01124-1

Schwid, S. R., Bausch, J., Oakes, D., Schuchter, L., Tanner, C., Forrest, M., et al. (2010). Cancer incidence in a trial of an antiapoptotic agent for Parkinson's disease. *Movement Disorders*, *25*(12), 1801–1808. https://doi.org/10.1002/mds.23006

Sclip, A., Antoniou, X., Colombo, A., Camici, G. G., Pozzi, L., Cardinetti, D., et al. (2011).

c-Jun N-terminal Kinase Regulates Soluble Aβ Oligomers and Cognitive Impairment in AD Mouse Model. *Journal of Biological Chemistry*, *286*(51), 43871–43880. https://doi.org/10.1074/jbc.M111.297515

Sclip, A., Tozzi, A., Abaza, A., Cardinetti, D., Colombo, I., Calabresi, P., et al. (2014). c-Jun N-terminal kinase has a key role in Alzheimer disease synaptic dysfunction in vivo. *Cell Death and Disease*, *5*(1), e1019. https://doi.org/10.1038/cddis.2013.559

Serhan, C. N. (2007). Resolution Phase of Inflammation: Novel Endogenous Anti-Inflammatory and Proresolving Lipid Mediators and Pathways. *Annual Review of Immunology*, *25*(1), 101–137. https://doi.org/10.1146/annurev.immunol.25.022106.141647

Serhan, C. N. (2009). Systems approach to inflammation resolution: identification of novel anti-inflammatory and pro-resolving mediators. *Journal of Thrombosis and Haemostasis*, *7*, 44–48. https://doi.org/10.1111/j.1538-7836.2009.03396.x

Serhan, C. N. (2014). Pro-resolving lipid mediators are leads for resolution physiology. *Nature*, *510*(7503), 92–101. https://doi.org/10.1038/nature13479

Serhan, C. N. (2017). Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. *Molecular Aspects of Medicine*. https://doi.org/10.1016/j.mam.2017.03.001

Serhan, C. N., Chiang, N., & Van Dyke, T. E. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nature Reviews Immunology*, *8*(5), 349–361. https://doi.org/10.1038/nri2294

Serhan, C. N., Dalli, J., Karamnov, S., Choi, A., Park, C.-K., Xu, Z.-Z., et al. (2012). Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. *The FASEB Journal*, *26*(4), 1755–1765. https://doi.org/10.1096/fj.11-201442

Shah, S. Z. A., Zhao, D., Hussain, T., & Yang, L. (2017). The Role of Unfolded Protein Response and Mitogen-Activated Protein Kinase Signaling in Neurodegenerative Diseases with Special Focus on Prion Diseases. *Frontiers in Aging Neuroscience*, *9*, 120. https://doi.org/10.3389/fnagi.2017.00120

Shoji, M., Iwakami, N., Takeuchi, S., Waragai, M., Suzuki, M., Kanazawa, I., et al. (2000). JNK activation is associated with intracellular beta-amyloid accumulation. *Brain Research. Molecular Brain Research*, *85*(1–2), 221–33.

Siddiqui, M. A., & Reddy, P. A. (2010). Small Molecule JNK (c-Jun N-Terminal Kinase) Inhibitors. *Journal of Medicinal Chemistry*, *53*(8), 3005–3012. https://doi.org/10.1021/jm9003279

Solas, M., Aisa, B., Tordera, R. M., Mugueta, C., & Ramírez, M. J. (2013a). Stress contributes to the development of central insulin resistance during aging: implications for Alzheimer's disease. *Biochimica et Biophysica Acta*, *1832*(12), 2332–9. https://doi.org/10.1016/j.bbadis.2013.09.013

Solas, M., Gerenu, G., Gil-Bea, F. J., & Ramírez, M. J. (2013b). Mineralocorticoid receptor activation induces insulin resistance through c-Jun N-terminal kinases in response to chronic corticosterone: cognitive implications. *Journal of Neuroendocrinology*, *25*(4), 350–6. https://doi.org/10.1111/jne.12006

Stoothoff, W. H., & Johnson, G. V. W. (2005). Tau phosphorylation: Physiological and pathological consequences. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1739*(2), 280–297. https://doi.org/10.1016/j.bbadis.2004.06.017

Suwanna, N., Thangnipon, W., & Soi-ampornkul, R. (2014). Neuroprotective effects of diarylpropionitrile against beta-amyloid peptide-induced neurotoxicity in rat cultured cortical neurons. *Neuroscience Letters*, *578*, 44–49. https://doi.org/10.1016/j.neulet.2014.06.029

Sze, C.-I., Su, M., Pugazhenthi, S., Jambal, P., Hsu, L.-J., Heath, J., et al. (2004). Down-regulation of WW domain-containing oxidoreductase induces Tau phosphorylation in vitro. A potential role in Alzheimer's disease. *The Journal of Biological Chemistry*, *279*(29), 30498–506. https://doi.org/10.1074/jbc.M401399200

Tajes, M., Gutierrez-Cuesta, J., Folch, J., Ortuño-Sahagun, D., Verdaguer, E., Jiménez, A., et al. (2010). Neuroprotective role of intermittent fasting in senescence-accelerated mice P8 (SAMP8). *Experimental Gerontology*, *45*(9), 702–710. https://doi.org/10.1016/j.exger.2010.04.010

Takeda, T., Hosokawa, M., Takeshita, S., Irino, M., Higuchi, K., Matsushita, T., et al. (1981). A new murine model of accelerated senescence. *Mechanisms of Ageing and Development*, *17*(2), 183–94.

Tamagno, E., Robino, G., Obbili, A., Bardini, P., Aragno, M., Parola, M., et al. (2003). H2O2 and 4-hydroxynonenal mediate beta amyloid-induced neuronal apoptosis

by activating JNKs and p38MAPK. *Experimental Neurology*, *180*(2), 144–155. https://doi.org/10.1016/S0014-4886(02)00059-6

Tang, S. C., Lathia, J. D., Selvaraj, P. K., Jo, D. G., Mughal, M. R., Cheng, A., et al. (2008). Toll-like receptor-4 mediates neuronal apoptosis induced by amyloid beta-peptide and the membrane lipid peroxidation product 4-hydroxynonenal. *Experimental Neurology*, *213*(1), 114–121. https://doi.org/10.1016/j.expneurol.2008.05.014

Tang, Y., Zhang, M. J., Hellmann, J., Kosuri, M., Bhatnagar, A., & Spite, M. (2013). Proresolution therapy for the treatment of delayed healing of diabetic wounds. *Diabetes*, *62*(2), 618–27. https://doi.org/10.2337/db12-0684

Tatebayashi, Y., Planel, E., Chui, D.-H., Sato, S., Miyasaka, T., Sahara, N., et al. (2006). c-jun N-terminal kinase hyperphosphorylates R406W tau at the PHF-1 site during mitosis. *The FASEB Journal*, *20*(6), 762–4. https://doi.org/10.1096/fj.05-4362fje

Teng, E., Taylor, K., Bilousova, T., Weiland, D., Pham, T., Zuo, X., et al. (2015). Dietary DHA supplementation in an APP/PS1 transgenic rat model of AD reduces behavioral and Aβ pathology and modulates Aβ oligomerization. *Neurobiology of Disease*, *82*, 552–560. https://doi.org/10.1016/j.nbd.2015.09.002

Terry, R. D. (2000). Cell death or synaptic loss in Alzheimer disease. *Journal of Neuropathology and Experimental Neurology*, *59*(12), 1118–9.

Thal, L. J., Kantarci, K., Reiman, E. M., Klunk, W. E., Weiner, M. W., Zetterberg, H., et al. (2006). The role of biomarkers in clinical trials for Alzheimer disease. *Alzheimer Disease and Associated Disorders*, *20*(1), 6–15. https://doi.org/10.1097/01.wad.0000191420.61260.a8

Torres, M., Marcilla-Etxenike, A., Fiol-deRoque, M. A., Escribá, P. V., & Busquets, X. (2015). The unfolded protein response in the therapeutic effect of hydroxy-DHA against Alzheimer's disease. *Apoptosis*, *20*(5), 712–724. https://doi.org/10.1007/s10495-015-1099-z

Trakas, K., Oh, P. I., Singh, S., Risebrough, N., & Shear, N. H. (2001). The health status of obese individuals in Canada. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity*, *25*(5), 662–8. https://doi.org/10.1038/sj.ijo.0801591

Tran, H. T., Sanchez, L., & Brody, D. L. (2012). Inhibition of JNK by a peptide inhibitor

reduces traumatic brain injury-induced tauopathy in transgenic mice. *Journal of Neuropathology and Experimental Neurology*, *71*(2), 116–29. https://doi.org/10.1097/NEN.0b013e3182456aed

Troy, C. M., Rabacchi, S. A., Xu, Z., Maroney, A. C., Connors, T. J., Shelanski, M. L., et al. (2001). beta-Amyloid-induced neuronal apoptosis requires c-Jun N-terminal kinase activation. *Journal of Neurochemistry*, *77*(1), 157–64.

Ueda, Y., Wang, M.-F., Irei, A. V., Sarukura, N., Sakai, T., et al. (2011). Effect of dietary lipids on longevity and memory in the SAMP8 mice. *Journal of Nutritional Science and Vitaminology*, *57*(1), 36–41. https://doi.org/10.3177/jnsv.57.36

Uslu, S., Akarkarasu, Z. E., Ozbabalik, D., Ozkan, S., Ç Olak, O., Demirkan, E. S., et al. (2012). Levels of amyloid beta-42, interleukin-6 and tumor necrosis factor-alpha in alzheimer's disease and vascular dementia. *Neurochemical Research*, *37*(7), 1554–1559. https://doi.org/10.1007/s11064-012-0750-0

Viana, R. J. S., Nunes, A. F., & Rodrigues, C. M. P. (2012). Endoplasmic reticulum enrollment in Alzheimer's disease. *Molecular Neurobiology*, *46*(2), 522–34. https://doi.org/10.1007/s12035-012-8301-x

Vitali, A., Piccini, A., Borghi, R., Fornaro, P., Siedlak, S. L., Smith, M. et al. (2004). Soluble amyloid beta-protein is increased in frontotemporal dementia with tau gene mutations. *Journal of Alzheimer's Disease : JAD*, *6*(1), 45–51.

Vogel, J., Anand, V. S., Ludwig, B., Nawoschik, S., Dunlop, J., & Braithwaite, S. P. (2009). The JNK pathway amplifies and drives subcellular changes in tau phosphorylation. *Neuropharmacology*, *57*(5–6), 539–50. https://doi.org/10.1016/j.neuropharm.2009.07.021

Volk, A., Li, J., Xin, J., You, D., Zhang, J., Liu, X., et al. (2014). Co-inhibition of NF-κB and JNK is synergistic in TNF-expressing human AML. *The Journal of Experimental Medicine*, *211*(6), 1093–1108. https://doi.org/10.1084/jem.20130990

Vukic, V., Callaghan, D., Walker, D., Lue, L.-F., Liu, Q. Y., Couraud, P.-O., et al. (2009). Expression of inflammatory genes induced by beta-amyloid peptides in human brain endothelial cells and in Alzheimer's brain is mediated by the JNK-AP1 signaling pathway. *Neurobiology of Disease*, *34*(1), 95–106. https://doi.org/10.1016/j.nbd.2008.12.007

Waetzig, V., Zhao, Y., & Herdegen, T. (2006). The bright side of JNKs—Multitalented

mediators in neuronal sprouting, brain development and nerve fiber regeneration. *Progress in Neurobiology*, *80*(2), 84–97. https://doi.org/10.1016/j.pneurobio.2006.08.002

Wang, L. H., Besirli, C. G., & Johnson, E. M. (2004). MIXED-LINEAGE KINASES : A Target for the Prevention of Neurodegeneration. *Annual Review of Pharmacology and Toxicology*, *44*(1), 451–474. https://doi.org/10.1146/annurev.pharmtox.44.101802.121840

Wang, P., Su, C., Li, R., Wang, H., Ren, Y., Sun, H., et al. (2014). Mechanisms and effects of curcumin on spatial learning and memory improvement in APPswe/PS1dE9 mice. *Journal of Neuroscience Research*, *92*(2), 218–231. https://doi.org/10.1002/jnr.23322

Wang, X., Zhu, M., Hjorth, E., Cortés-Toro, V., Eyjolfsdottir, H., Graff, C., et al. (2015). Resolution of inflammation is altered in Alzheimer's disease. *Alzheimer's & Dementia*, *11*(1), 40–50.e2. https://doi.org/10.1016/j.jalz.2013.12.024

Westerman, M. A., Cooper-Blacketer, D., Mariash, A., Kotilinek, L., Kawarabayashi, T., Younkin, L. H., et al. (2002). The relationship between Abeta and memory in the Tg2576 mouse model of Alzheimer's disease. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(5), 1858–67.

WHO. (2017). Health topic | Dementia. Retrieved September 13, 2017, from http://www.who.int/mediacentre/factsheets/fs362/en/

Williams, L. M. (2012). Hypothalamic dysfunction in obesity. *Proceedings of the Nutrition Society*, *71*(4), 521–533. https://doi.org/10.1017/S002966511200078X

Wu, F. J., Xue, Y., Liu, X. F., Xue, C. H., Wang, J. F., Du, L., et al. (2014). The protective effect of eicosapentaenoic acid-enriched phospholipids from sea cucumber Cucumaria frondosa on oxidative stress in PC12 cells and SAMP8 mice. *Neurochemistry International*, *64*(1), 9–17. https://doi.org/10.1016/j.neuint.2013.10.015

Xiong, H., Zheng, C., Wang, J., Song, J., Zhao, G., Shen, H., et al. (2013). The neuroprotection of liraglutide on Alzheimer-like learning and memory impairment by modulating the hyperphosphorylation of tau and neurofilament proteins and insulin signaling pathways in mice. *Journal of Alzheimer's Disease : JAD*, *37*(3), 623–35. https://doi.org/10.3233/JAD-130584

Xu, N., Xiao, Z., Zou, T., & Huang, Z. (2015). Induction of GADD34 Regulates the Neurotoxicity of Amyloid beta. *American Journal of Alzheimer's Disease and Other Dementias*, *30*(3), 313–319. https://doi.org/10.1177/1533317514545616 [doi]

Xu, Y., Hou, X.-Y., Liu, Y., & Zong, Y.-Y. (2009). Different protection of K252a and N-acetyl-L-cysteine against amyloid-beta peptide-induced cortical neuron apoptosis involving inhibition of MLK3-MKK7-JNK3 signal cascades. *Journal of Neuroscience Research*, *87*(4), 918–27. https://doi.org/10.1002/jnr.21909

Yang, D. D., Kuan, C.-Y., Whitmarsh, A. J., Rinócn, M., Zheng, T. S., Davis, R. J., et al. (1997). Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature*, *389*(6653), 865–870. https://doi.org/10.1038/39899

Yang, F., Lim, G. P., Begum, A. N., Ubeda, O. J., Simmons, M. R., Ambegaokar, S. S., et al. (2005). Curcumin Inhibits Formation of Amyloid β Oligomers and Fibrils, Binds Plaques, and Reduces Amyloid in Vivo. *Journal of Biological Chemistry*, *280*(7), 5892–5901. https://doi.org/10.1074/jbc.M404751200

Yao, M., Nguyen, T., & Pike, C. (2005). Beta-Amyloid-Induced Neuronal Apoptosis Involves c-Jun N-Terminal Kinase-Dependent Downregulation of Bcl-w. *Journal of Neuroscience*, *25*(5), 1149–1158. https://doi.org/10.1523/JNEUROSCI.4736-04.2005

Yarza, R., Vela, S., Solas, M., & Ramirez, M. J. (2016). c-Jun N-terminal Kinase (JNK) Signaling as a Therapeutic Target for Alzheimer's Disease. *Frontiers in Pharmacology*, *6*(January), 1–12. https://doi.org/10.3389/fphar.2015.00321

Yoon, S. O., Park, D. J., Ryu, J. C., Ozer, H. G., Tep, C., Shin, Y. J., et al. (2012). JNK3 Perpetuates Metabolic Stress Induced by Aβ Peptides. *Neuron*, *75*(5), 824–837. https://doi.org/10.1016/j.neuron.2012.06.024

Yoshida, H., Hastie, C. J., McLauchlan, H., Cohen, P., & Goedert, M. (2004). Phosphorylation of microtubule-associated protein tau by isoforms of c-Jun N-terminal kinase (JNK). *Journal of Neurochemistry*, *90*(2), 352–358. https://doi.org/10.1111/j.1471-4159.2004.02479.x

Yu, S., Zheng, W., Xin, N., Chi, Z.-H., Wang, N.-Q., Nie, Y.-X., et al. (2010). Curcumin Prevents Dopaminergic Neuronal Death Through Inhibition of the c-Jun N-Terminal Kinase Pathway. *Rejuvenation Research*, *13*(1), 55–64.

https://doi.org/10.1089/rej.2009.0908

Zeke, A., Misheva, M., Reményi, A., & Bogoyevitch, M. A. (2016). JNK Signaling: Regulation and Functions Based on Complex Protein-Protein Partnerships. *Microbiology and Molecular Biology Reviews : MMBR*, *80*(3), 793–835. https://doi.org/10.1128/MMBR.00043-14

Zetterberg, H. (2015). Cerebrospinal fluid biomarkers for Alzheimer's disease: current limitations and recent developments. *Current Opinion Pyschiatry*, *8*(5), 402–409. https://doi.org/10.3233/JAD-2009-1177

Zhang, F., & Jiang, L. (2015). Neuroinflammation in Alzheimer's disease. *Neuropsychiatric Disease and Treatment*, *11*, 243. https://doi.org/10.2147/NDT.S75546

Zhang, S.-J., Xu, T.-T., Li, L., Xu, Y.-M., Qu, Z.-L., Wang, X.-C., et al. (2017). Bushen-Yizhi formula ameliorates cognitive dysfunction through SIRT1/ER stress pathway in SAMP8 mice. *Oncotarget*, *8*(30), 49338–49350. https://doi.org/10.18632/oncotarget.17638

Zhang, X., Tang, S., Zhang, Q., Shao, W., Han, X., Wang, Y., et al. (2016). Endoplasmic reticulum stress mediates JNK-dependent IRS-1 serine phosphorylation and results in Tau hyperphosphorylation in amyloid β oligomer-treated PC12 cells and primary neurons. *Gene*, *587*(2), 183–193. https://doi.org/10.1016/j.gene.2016.05.018

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

Zhou, Q., Wang, M., Du, Y., Zhang, W., Bai, M., Zhang, Z., et al. (2015). Inhibition of c-Jun N-terminal kinase activation reverses Alzheimer disease phenotypes in APPswe/PS1dE9 mice. *Annals of Neurology*, *77*(4), 637–654. https://doi.org/10.1002/ana.24361

Zhu, M., Wang, X., Hjorth, E., Colas, R. A., Schroeder, L., Granholm, A.-C., et al. (2016). Pro-Resolving Lipid Mediators Improve Neuronal Survival and Increase Aβ42 Phagocytosis. *Molecular Neurobiology*, *53*(4), 2733–49. https://doi.org/10.1007/s12035-015-9544-0

Zhu, X., Raina, A. K., Rottkamp, C. A., Aliev, G., Perry, G., Boux, H., et al. (2001). Activation and redistribution of c-Jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimer's disease. *Journal of Neurochemistry*, *76*(2), 435–441. https://doi.org/10.1046/j.1471-4159.2001.00046.x