Cerebrospinal fluid biomarkers in relation to signature Alzheimer's disease pathology and cognitive functions

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Thesis for the degree of Philosophiae Doctor

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Tengsl lífvísa í heila- og mænuvökva við einkennandi meingerð Alzheimer-sjúkdóms og vitræna skerðingu

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A journey of a thousand miles begins with a single step.

- Lao Tzu

Ágrip

Á undanförnum árum hefur aukin áhersla verið lögð á að skilgreina Alzheimer-sjúkdóminn út frá líffræðilegum mælikvörðum, þar sem breytingar í heila hefjast einum til tveimur áratugum áður en klínísk einkenni gera vart við sig. Klassísk meingerð sjúkdómsins einkennist af amyloid skellum, taugatrefjaflækjum og sívaxandi taugafrumudauða í heilavef. Ákveðnir lífvísar gefa til kynna þessa klassísku meingerð, þ.e. mælingar á magni amyloid beta (Aβ), fosfórýleruðu tau (P-tau) og heildar-tau (T-tau) prótína í heila- og mænuvökva.

Þó svo að hæfni þessara lífvísa til greiningar sjúkdómsins sé ásættanleg, þá breytist magn þeirra lítið á síðari stigum og sýna þar af leiðandi veika fylgni við vitræna skerðingu. Það er því mikilvægt að skoða hvort aðrir lífvísar geti betur varpað ljósi á önnur líffræðileg ferli sem liggja að baki meingerðar og klínískra einkenna sjúkdómsins. Aðalmarkmið doktorsverkefnisins var að rannsaka tengsl valdra lífvísa í heila- og mænuvökva við klassíska meingerð Alzheimer-sjúkdóms og vitræna getu meðal einstaklinga á for- eða frumstigum heilabilunar.

Nýlegar rannsóknarniðurstöður gefa til kynna að truflun í starfsemi ónæmiskerfis, lípíðefnaskipta og kólvirkra taugafrumna, leiki hlutverk í meinmyndun sjúkdómsins. Í þessari rannsókn voru lífvísar mældir í heila- og mænuvökva sem taldir eru endurspegla þessi tilteknu líffræðilegu ferli. Þátttakendur voru valdir úr gagnagrunni Íslensku MCI rannsóknarinnar. Sú rannsókn samanstóð af einstaklingum sem leitað höfðu til Minnismóttöku Landspítala Háskólasjúkrahúss og skoruðu 24-30 stig á Mini-Mental State Examination (MMSE) og 4 eða færri stig á Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) prófunum í fyrstu heimsókn. Fyrir þetta verkefni voru eingöngu þeir valdir sem farið höfðu í mænuvökvastungu. Allir þátttakendur voru flokkaðir í annað tveggja einkennasniða, eftir hlutfalli T-tau og $A\beta_{42}$ magns í heila- og mænuvökva. Þátttakendur með ákveðin hlutfallsgildi (T-tau/$A\beta_{42}$ > 0,52), tilheyrðu Alzheimer-einkennasniði og voru þar með taldir bera klassíska meingerð sjúkdómsins.

Markmið **rannsóknar I** var að skoða tengsl á milli lífvísa í heila- og mænuvökva, sem gefa til kynna taugahrörnun (neurofilament light [NFL]) og virkjun ónæmisviðbragðs (chitinase-3-like protein 1 [YKL-40], S100 calcium-binding protein B [S100B], glial fibrillary acidic protein [GFAP]), við bæði

Alzheimer-einkennasnið og vitræna getu. Þó niðurstöður hafi gefið til kynna að greiningarhæfni lífvísana væri léleg þegar kom að því að greina á milli einkennasniða, þá fundust tengsl við vitræna getu. Þessi tengsl voru þó aðallega til staðar á meðal þeirra með Alzheimer-einkennasnið. Hærra NFL magn tengdist verri útkomu á yrtu atburðaminni en hærra magn af GFAP tengdist verri útkomu í mælingu á hugrænum hraða.

Markmið **rannsóknar II** var að bera kennsl á þær tegundir lípíða í heila- og mænuvökva sem best greina á milli einkennasniða ásamt því að meta samband þeirra við mælingar sem endurspegla önnur ferli (amyloid skellur, taugahrörnun, ónæmisviðbragð, yrt atburðaminni). Ómiðuð lípíðgreining í heila- og mænuvökva var framkvæmd með massagreini tengdum við vökvaskilju. Alls greindust 1008 massatoppar og af þeim voru átta valdir sem taldir voru greina best á milli einkennasniða. Af þessum átta var þó aðeins hægt að auðkenna einn topp með mikilli vissu, þ.e. þann sem samsvaraði C18 ceramide. Niðurstöður sýndu að hærra magn af C18 ceramide tengdist bæði lægra magni af $A\beta_{42}$ og hærra magni af T-tau. Að auki tengdist hærra magn af S100B hærra magni af C18 ceramide.

Markmið **rannsóknar III** var að meta tengsl á milli virkni kólvirku ensímanna acetylcholine (AChE) og butyrylcholinesterase (BuChE) og mælinga annarra ferla (amyloid skellur, taugahrörnun, ónæmisviðbragð og yrt atburðaminni). Engin tengsl fundust á milli $A\beta_{42}$ og ensímvirkni. Aftur á móti tengdist hærra magn tau prótína hærri virkni AChE, og í minna mæli, hærri virkni BuChE. Einnig fylgdi hærra magn S100B og YKL-40 hærri virkni beggja ensíma.

Á heildina litið gefa þessar niðurstöður til kynna möguleika ólíkra lífvísa í heila- og mænuvökva til þess að betrumbæta greiningu, mælingu á framvindu og meðferð Alzheimer-sjúkdóms á byrjunarstigum. C18 ceramide gæti möguleika nýst til greiningar og meðferðar, þar sem hærra magn lípíðsins tengdist bæði klassískri meingerð sjúkdómsins og virkjun á ónæmisviðbragði. Sama gildir um kólvirku ensímin, en hærri virkni þeirra fylgdi bæði aukinni taugahrörnun ásamt virkjun á ónæmisviðbragði. NFL og GFAP gætu nýst til mælingar á framvindu, en hærra magn þeirra tengdist vitrænni skerðingu meðal einstaklinga sem báru klassíska meingerð Alzheimer-sjúkdóms.

Lykilorð:

Alzheimer-sjúkdómur, heila- og mænuvökvi, lífvísar, ónæmisviðbragð, lípíð, kólvirka kerfið

Abstract

The focus has shifted in recent years from clinical towards a biological definition of Alzheimer's disease (AD), as pathophysiological changes precede the clinical symptoms by decades. In vivo brain imaging and cerebrospinal fluid (CSF) biomarkers have been at the center of this change. The core biomarkers are amyloid beta (Aβ), phosphorylated tau (P-tau) and total tau (T-tau), reflecting signature aspects of AD pathology (Aβ plaques, neurofibrillary tangles and neurodegeneration). Although the diagnostic accuracies of these markers are satisfactory, their levels reach a plateau at later stages of the disease and do not associate well with the progression of cognitive impairment. The discovery of novel biomarkers is therefore of importance for a better understanding of different biological processes driving the pathology and clinical manifestation of the disease. The overall objective of the project was to evaluate the relationships of selected CSF biomarkers with signature AD pathology and cognitive functions among individuals at the symptomatic pre- or early stages of dementia.

Recent findings suggest that dysfunction in neuroinflammation, lipid metabolism and cholinergic neurons play a critical part in the pathogenesis of AD. In this study, we measured novel biomarkers reflecting these different biological processes. Subjects were recruited from The Icelandic MCI study, which included individuals referred to the Landspitali University Hospital (LUH) Memory Clinic. The criteria for inclusion were a score between 24-30 on the Mini-Mental State Examination (MMSE) and a score of 4.0 or less on the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) at the first visit. For this project, only subjects who underwent lumbar puncture were recruited from the cohort. All subjects were categorized as having a CSF AD profile reflecting signature AD pathology or non-AD profile, based on a cut-off point (0.52) between the ratio of T-tau and $A\beta_{42}$ values in CSF.

The aim of **Paper I** was to evaluate the association of CSF biomarkers reflecting neurodegeneration (neurofilament light [NFL]) and inflammation (chitinase-3-like protein 1 [YKL-40], S100 calcium-binding protein B [S100B], glial fibrillary acidic protein [GFAP]) with CSF AD profile and cognitive decline. Our results showed that although the markers did not accurately differentiate between the two CSF profiles, they associated in different ways with certain cognitive domains. These relationships were mainly observed among subjects with a CSF AD profile, where higher levels of NFL

associated with deficits in verbal episodic memory and higher levels of GFAP with deficits in processing speed.

The aim of **Paper II** was to identify lipid species best distinguishing between CSF profiles (AD and non-AD), and to examine their relationships with measures reflecting AD-related processes (neurodegeneration, inflammation, impairment in verbal episodic memory). Untargeted CSF lipidomic analysis was performed for the detection of mass-to-charge ratio (m/z) features, by the application of ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS). Out of 1008 features detected, eight were selected as best differentiating between the two CSF profiles. One out of the eight features was assigned to a lipid species (C18 ceramide), as it was the only one to be confirmed with high confidence. Lower levels of $A\beta_{42}$ and higher levels of T-tau related to higher levels of C18 ceramide. Additionally, a positive relationship was found beween levels of inflammatory marker S100B and C18 ceramide.

The aim of **Paper III** was to assess the association between the activity of cholinesterase enzymes acetylcholine (AChE) and butyrylcholinesterase (BuChE) in CSF and measures reflecting AD-related processes (amyloidosis, neurodegeneration, inflammation, impairment in verbal episodic memory). CSF AChE and BuChE activity did not associate with amyloid status in the brain. Higher levels of T-tau and P-tau related to higher activity of AChE, and to a lesser extent, higher activity of BuChE. Higher levels of inflammatory markers S100B and YKL-40 also related to higher levels of both AChE and BuChE activity.

Overall, these results indicate the potential value of novel CSF biomarkers regarding diagnosis, progression and treatment of AD at the early stages of the disease. Higher levels of C18 ceramide related to AD pathology and inflammatory processes, indicating a potential as a therapeutic target and an additional diagnostic marker. The activity of ACh-degrading cholinergic enzymes related to inflammatory and neurodegenerative processes. Proteins NFL and GFAP, measured in CSF, are promising markers for cognitive decline and progression among individuals with signature AD pathology.

Keywords:

Alzheimer's disease, cerebrospinal fluid, biomarkers, inflammation, lipids, cholinergic system

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The principal part of the PhD project presented in this thesis was carried out at the Faculty of Medicine, University of Iceland (UI). A significant part of the project was performed over a course of seven months at the Division of Clinical Geriatrics, Center for Alzheimer Research, NVS Department, Karolinska Institutet (KI), Huddinge, Sweden.

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List of abbreviations

Acetyl-CoA	Acetyl coenzyme A
ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
ATC	Acetylthiocholine iodide
AUC	Area under curve
Aβ	Amyloid beta
BACE1	Beta-site amyloid precursor protein cleaving enzyme 1
BBB	Blood-brain barrier
BSA	Bovine serum albumin
BTC	Butyrylthiocholine iodide
BuChE	Butyrylcholinesterase
CAA	Cerebral amyloid angiopathy
CAD	Coronary artery disease
CBD	Corticobasal degeneration
Ch	Choline
ChAT	Choline acetyltransferase
CHT	Choline transporter
CJD	Creutzfeldt-Jakob disease
CNS	Central nervous system
CSF	Cerebrospinal fluid
DLB	Dementia with Lewy bodies

CV	Coefficient of variation
DSST	Digit Symbol Substitution Test
EDTA	Ethylenediaminetetraacetic acid
EEG	Electroencephalography
ELISA	Enzyme-linked immunosorbent assay
EOAD	Early-onset Alzheimer’s disease
EPP	Ethopropazine
FDA	The U.S. Food and Drug Administration
FDG-PET	Fluorodeoxyglucose - positron emission tomography
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
GFAP	Glial fibrillary acidic protein
GWAS	Genome-wide association study
IF	Intermediate filament
IQR	Interquartile range
ISF	Interstitial fluid
IQCODE	Cognitive Decline in the Elderly
KI	Karolinska Institutet
LASSO	Least absolute shrinkage and selection operator
LBD	Lewy body dementia
LOAD	Late-onset Alzheimer’s disease
LPA	Logopenic progressive aphasia
LUH	Landspitali University Hospital
M/z	Mass-to-charge ratio
mAchR	Muscarinic acetylcholine receptor
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging

mRNA	Messenger RNA
MS	Multiple sclerosis
MTA	Medial temporal lobe atrophy
MTL	Medial temporal lobe
nAchR	Nicotinic acetylcholine receptor
NBM	Nucleus basalis of Meynert
NFH	Neurofilament heavy
NFL	Neurofilament light
NFM	Neurofilament medium
NFTs	Neurofibrillary tangles
NF-κB	Nuclear factor-κB
NIA-AA	National Institute on Aging and Alzheimer's Association
NIH	National Institutes of Health
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NMDA	N-methyl D-aspartate
PCA	Posterior cortical atrophy
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PET	Positron emission tomography
PFER	Per-family error rate
PHP	Paired helical filaments
PiB	Pittsburgh compound B
PLCG2	Phospholipase C-gamma 2
PPA	Primary progressive aphasia
PP2A	Protein phosphatase 2A
PSEN	Presenilin

P-tau	Phosphorylated tau
QC	Quality control
RAGE	Receptor for advanced glycation end products
RAVLT	Rey Auditory Verbal Learning Test
ROCF	Rey-Osterrieth Complex Figure
S100B	S100 calcium-binding protein B
SCD-I	Subjective Cognitive Decline Initiative
SCI	Subjective cognitive impairment
SM	Sphingomyelin
SMase	Sphingomyelinase
TBI	Traumatic brain injury
TBS	Tris-buffered saline
TLR	Toll-like receptor
TMT	Trail Making Test
TREM2	Triggering receptor expressed on myeloid cells 2
T-tau	Total tau
UI	University of Iceland
UPLC-MS	Ultra-performance liquid chromatography-tandem mass spectrometry
VAT	Vesicular acetylcholine transporter
VD	Vascular dementia
YKL-40	Chitinase-3-like protein 1
WFSP	The World Federation of Societies of Biological Psychiatry

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List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-III):

I. Teitsdottir, U. D., Jonsdottir, M. K., Lund, S. H., Darreh-Shori, T., Snaedal, J., & Petersen, P. H. (2020). Association of glial and neuronal degeneration markers with Alzheimer's disease cerebrospinal fluid profile and cognitive functions. Alzheimer's research & therapy, 12(1), 92. doi:10.1186/s13195-020-00657-8

II. Teitsdottir, U. D., Halldorsson, S., Rolfsson, O., Lund, S. H., Jonsdottir, M. K., Snaedal, J., & Petersen, P. H. (2021). Cerebrospinal Fluid C18 Ceramide Associates with Markers of Alzheimer's Disease and Inflammation at the Pre- and Early Stages of Dementia. J Alzheimers Dis, 81(1), 231-244. doi:10.3233/jad-200964

III. Teitsdottir, U. D., Darreh-Shori, T., M. K., Lund, Jonsdottir, S. H., Snaedal, J., & Petersen, P. H. (2021). Phenotypic displays of cholinergic enzymes associate with markers of inflammation and neurodegeneration in a memory clinic cohort.

In addition, some unpublished data is presented.

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Declaration of contribution

I, Unnur Diljá Teitsdóttir, planned the research in co-operation with my supervisor Pétur Henry Petersen and Jón Snædal, the director of The Icelandic MCI study. I also took part in writing ethical approvals and grant applications. I carried out the enzyme activity assays at Taher Darreh-Shori's lab at KI, Sweden, under his supervision. I performed all ELISAs for measurements of protein levels, except for the core AD markers, $A\beta_{42}$, T-tau and P-tau. The medical laboratory MVZ Labor P.D. Dr. Volkmann und Kollegen GbR, located in Karlsruhe, Germany, carried out those measurements. The identification and quantification of lipids were performed by members of Center for Systems Biology, UI (Skarphéðinn Halldórsson and Hulda Soffía Jónasdóttir). Neuropsychological tests were administrated by licensed psychologists under the supervision of María Kristín Jónsdóttir. I analyzed all the data and performed the statistical analysis with the guidance of Sigrún Helga Lund. I drafted the manuscripts for all papers, responded to comments from reviewers and co-authors and prepared the final manuscripts for submission. I wrote this thesis with the guidance of my supervisor and the doctoral committee.

1 Introduction

1.1 Overview of Alzheimer's disease (AD) and other dementias

Dementia is a term that covers different medical conditions, most commonly found among older people. It is characterized by cognitive decline (e.g. loss of memory, language, reasoning, visual and spatial abilities) to the extent of impacting daily life and activities. Types of dementia include Alzheimer's disease (AD), vascular dementia (VD), Lewy body dementia (LBD) and frontotemporal dementia (FTD). Mixed dementia is also common, which is a combination of two or more types of dementias (Arvanitakis et al., 2019).

Approximately 50 million people worldwide were living with dementia in 2015. Furthermore, that number is predicted to double every 20 years to 130 million by 2050 (Prince et al., 2015). AD is the most common neurological cause of dementia, accounting for 50-70% of total cases (Prince et al., 2014). A key symptom of AD is a gradual decline in memory. Early signs include difficulties remembering recent conversations, names or events. As the disease progresses, other symptoms like impaired communication skills, poor judgment, confusion, disorientation, mood changes and, finally, difficulty speaking, eating and walking may emerge (Levenson et al., 2014).

Despite extensive research into the pathogenesis of AD, no attempts of developing pharmacologic treatments for cure have been successful to date. It is believed that for future treatments to be effective in haltering disease progression, it will be crucial for administration to begin early in the disease continuum. Until recently, only four drugs were approved by The U.S. Food and Drug Administration (FDA) - rivastigmine, galantamine, donepezil and memantine (Qaseem et al., 2008). The first three are cholinesterase inhibitors (Birks, 2006) and the last one is a N-methyl D-aspartate (NMDA) antagonist (Kishi et al., 2017). These drugs temporarily improve cognitive symptoms but do not reverse the pathological damage. In June 2021, the FDA approved for the first time in 18 years a new drug, Aduhelm (aducanumab), which targets amyloid beta (Aβ) plaques. It is the first approved drug with the purpose of treating the possible cause of the disease, rather than only the symptoms. Although the drug has the ability to remove Aβ plaques from the brain, its clinical efficacy has been questioned as it does

not appear to effectively slow down cognitive decline among patients with mild AD dementia (Musiek & Bennett, 2021).

1.2 Risk factors of AD

AD is caused by the interplay between genetics, lifestyle and environment. The most common risk factors for AD are age, APOE genotype, gender, educational level, cardiovascular disease and diabetes mellitus type 2 ("2016 Alzheimer's disease facts and figures," 2016).

Advanced age is the strongest risk factor for AD and is classified into two types based on time of onset; early-onset AD (EOAD) and late-onset AD (LOAD). The age of 65 is used as an arbitrary cut-off point for division of the two categories (Ayodele et al., 2021).

1.3 Genetics of AD

Familiar EOAD cases have been associated with mutations inherited in an autosomal-dominant manner. LOAD is a more complex disease and most cases are sporadic, with no clear pattern of inheritance within families (Van Cauwenberghe et al., 2016).

EOAD is associated with rare and dominantly inherited mutations in three genes involved in Aβ generation - APP (Goate et al., 1991; St George-Hyslop et al., 1987), PSEN1 (Sherrington et al., 1995; St George-Hyslop et al., 1992; Van Broeckhoven et al., 1992) and PSEN2 (Sherrington et al., 1996). These mutations correspond to less than 1% of all cases of AD and usually show complete penetrance (Van Cauwenberghe et al., 2016). Symptoms typically develop between 30 and 50 years of age (Bateman et al., 2011), much earlier compared to sporadic LOAD. Mutations in PSEN1 are the most common genetic cause of EOAD and are more frequent than APP and PSEN2 mutations (Theuns et al., 2000).

A complex interaction between genetics and environment is thought to play a key role in the onset, progression and severity of LOAD. LOAD has a strong genetic component and up to 60-80% of cases are estimated to be attributable to genetic factors (Gatz et al., 2006). The APOE gene is the major risk factor for the pathogenesis of LOAD. The gene encodes apolipoprotein E (ApoE), the primary cholesterol carrier in the brain, and is located on chromosome 19q13.2. APOE has three common alleles; APOE ε2 (Cys112, Cys158), APOE ε3 (Cys112, Arg158), and APOE ε4 (Arg112, Arg158) (Zannis et al., 1982). The APOE ε4 allele is associated with an increased LOAD risk. Individuals carrying one copy of the ε4 allele are about

three times more likely to develop AD compared to those with two copies of the ε3 allele, while the risk is eight-to 12-fold higher for those with two copies of the ε4 allele compared to the same group (Verghese et al., 2011).

The ε4 allele of the APOE gene is the most prevalent genetic risk factor for LOAD and was, before the era of large-scale genome-wide association studies (GWAS), the only one. With technological advances, a number of regions within the genome that contribute to the risk of developing LOAD have been discovered over the past decade. More than 20 genetic risk genes, associated with inflammatory, lipid metabolism and endosomal vesicle recycling pathways have been identified with GWAS (Karch & Goate, 2015). The genes each contribute only minimally to increased risk, but can almost double the chances of developing the disease when combined into a polygenic risk score (Escott-Price et al., 2015). Many of these genes are predominantly expressed by microglia, including the triggering receptor expressed on myeloid cells 2 (TREM2), Phospholipase C-gamma 2 (PLCG2) and CD33 genes. Those findings indicate that microglial activation plays a critical part in the pathogenesis of the disease (Hansen et al., 2017; Jonsson et al., 2013; Sims et al., 2017).

1.4 Signature AD pathology

The neuropathological features of AD were first described by the German neuropathologist Alois Alzheimer at the beginning of the twentieth century (Hippius & Neundörfer, 2003). Neuropathologically, AD is characterized by the accumulation of Aβ protein into senile plaques (Gouras et al., 2010) and intraneuronal tangles of hyperphosphorylated tau protein (Wang & Mandelkow, 2016). Other pathological features include neuropil threads, dystrophic neurites and the activation of astrocytes and microglial cells. Cerebral amyloid angiopathy (CAA) frequently coexists with AD as well (Greenberg et al., 2020). The downstream consequences of Aβ aggregation include neurodegeneration such as synaptic dysfunction, neuronal loss and brain atrophy (Serrano-Pozo et al., 2011) (Figure 1).

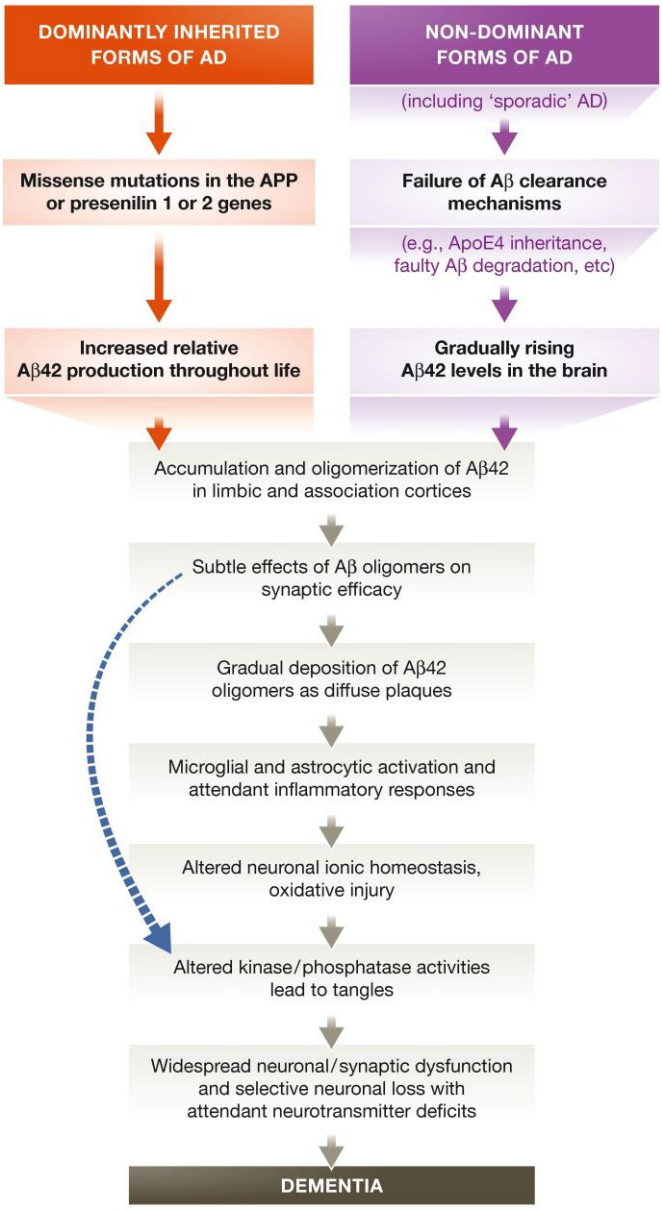

Figure 1. The sequence of major pathogenic events leading to AD proposed by the amyloid cascade hypothesis. The curved blue arrow indicates that Aβ oligomers may directly injure the synapses and neurites of brain neurons, in addition to activating microglia and astrocytes. Figure and figure text reprinted from (Selkoe & Hardy, 2016) with permission from EMBO Molecular Medicine.

The amyloid hypothesis, the most accepted theory of AD pathogenesis, indicates that aggregation of pathological forms of Aβ in the brain is the

fundamental pathological process, caused by an imbalance between the production and clearance of Aβ. Aβ is generated from the amyloid precursor protein (APP), a type I transmembrane protein, through two major steps of cleavage by aspartic proteases. The amyloidogenic processing is initiated by beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), which cuts of the bulk of the APP extracellular domain. This is followed by a cleavage within the membrane by y-secretase, releasing the protein into the extracellular space (Chen et al., 2017). As the site of y-secretase cleavage can vary, alloforms of different lengths can be formed. Typically, they have between 39 to 43 amino acids. The most common alloform is $A\beta_{40}$ (90%), followed by $A\beta_{42}$ (10%) (Olubiyi & Strodel, 2012). Monomeric Aβ spontaneously self-aggregates into various types of assemblies, including soluble oligomers (2 to 6 peptides), which can cluster together into insoluble fibrils, and eventually form plaques. Within plaques, $A\beta_{42}$ is more abundant than $A\beta_{40}$, due to its higher rate of fibrillization and insolubility (Kayed et al., 2003).

Strong support for a central role of the amyloid hypothesis of AD comes from genetics. All known familial AD mutations (APP, PSEN1 and PSEN2) are involved in Aβ generation. The APP gene encodes the APP protein (Yoshikai et al., 1990). A mutation in the APP gene, discovered in the Icelandic population, showed a strong protective effect against AD, further illustrating the central role of APP in the disease (Jonsson et al., 2012). The missense mutation (A673T) results in a decrease of Aβ accumulation. Presenilins (PSENs), the proteins encoded by PSEN1 and PSEN2, constitute the catalytic subunits of γ-secretases (Vetrivel et al., 2006).

According to the amyloid hypothesis, Aβ aggregation triggers a chain of events, including the formation of neurofibrillary tangles (NFTs) within neurons, oxidation and inflammation, which eventually lead to neuronal dysfunction and death. Tau is predominantly expressed in neurons, where it is primarily localized in axons for the purpose of microtubule stabilization and axonal transport. Functions of tau can be regulated by post-translational modification. Tau binding to microtubules is, for example, regulated mainly by phosphorylation. In AD, tau is hyperphosphorylated at numerous sites (including serines, threonines and tyrosines), which results in reduced ability to bind microtubules and enhanced tau aggregation. Under those conditions, tau starts to self-assemble into oligomers, paired helical filaments (PHP) and finally, NFTs (Wang & Mandelkow, 2016).

The spatiotemporal progression of Aβ plaques and NFTs within the AD brain is considerably consistent. The accumulation of Aβ starts in the association cortices and spreads from the neocortex to the allocortex, from there to the brainstem and finally, to the cerebellum (van der Kant et al., 2020). The progression pattern of NFTs is different from Aβ plaques and is typically divided into six stages, first described by Braak (1991). Accumulation of tau initially develops within the (trans)entorhinal cortex (I-II), spreads into the limbic areas (e.g. hippocampus, III-IV) and eventually into the isocortex (V-VI).

1.5 Inflammation in AD

The discovery of AD risk genes linked to innate immune functions has resulted in neuroinflammation being central to the research of AD. The leading players of the innate immune system in the central nervous system (CNS) are microglia and astrocytes. Under physiological conditions, microglia serve various roles, including immune surveillance, control of synaptic homeostasis, plasticity and trophic support. The processes of microglia constantly survey their microenvironment, making contact with synapses and axons, sensing the presence of pathogens and cellular debris (Ousman & Kubes, 2012; Salter & Stevens, 2017). Similarly, the roles of astrocytes under physiological conditions include maintenance of synaptic homeostasis, trophic support, recycling of neurotransmitters and regulation of cerebral blood flood. Astrocytic processes support the blood-brain barrier (BBB) and synapses (Arranz & De Strooper, 2019).

Microglia are essentially involved in the pathogenesis of AD. Soluble Aβ oligomers and Aβ fibrils act on microglial surface receptors, including Toll-like receptors (TLR1, TLR2, TLR4 and TLR6), CD14, CD47, α6β1 integrin and scavenger receptors (including CD36). The recognition of pathological triggers activates phagocytic abilities of the microglia and enables the internalization and degradation of Aβ. It also activates the nuclear factor-κB (NF-κB) pathway, inducing to the secretion of pro-inflammatory cytokines. This, in turn, promotes further the activation of glial cells, neuronal dysfunction and upregulation of BACE1, the APP cleaving enzyme generating pathogenic Aβ (Leng & Edison, 2021). Smaller oligomers of Aβ have been found to be more toxic compared to fibrils, inducing stronger microglial immune response (Yang et al., 2017).

Research suggests that astrocytes are pivotally involved in AD neurodegeneration rather than just being innocent bystanders (González-

Reyes et al., 2017). Figure 2 depicts a theoretical model of astrocyte activation in AD (Arranz & De Strooper, 2019). The combination of secretion of cytokines by activated microglia and Aβ activating the NF-κB pathway, can possibly induce the A1 reactive astrocyte phenotype and related neurotoxic reactive cell states of astrocytes. Neurotoxic A1 reactive astrocytes secrete an unknown neurotoxin, leading to the loss of neurons and oligodendrocytes. Astrocytic NF-κB activation results in the extracellular release of the complement protein C3. The binding of C3 to the neuronal receptor C3aR disrupts dendritic morphology and network function while binding to microglial C3aR changes Aβ phagocytosis (Lian et al., 2015; Liddelow et al., 2017).

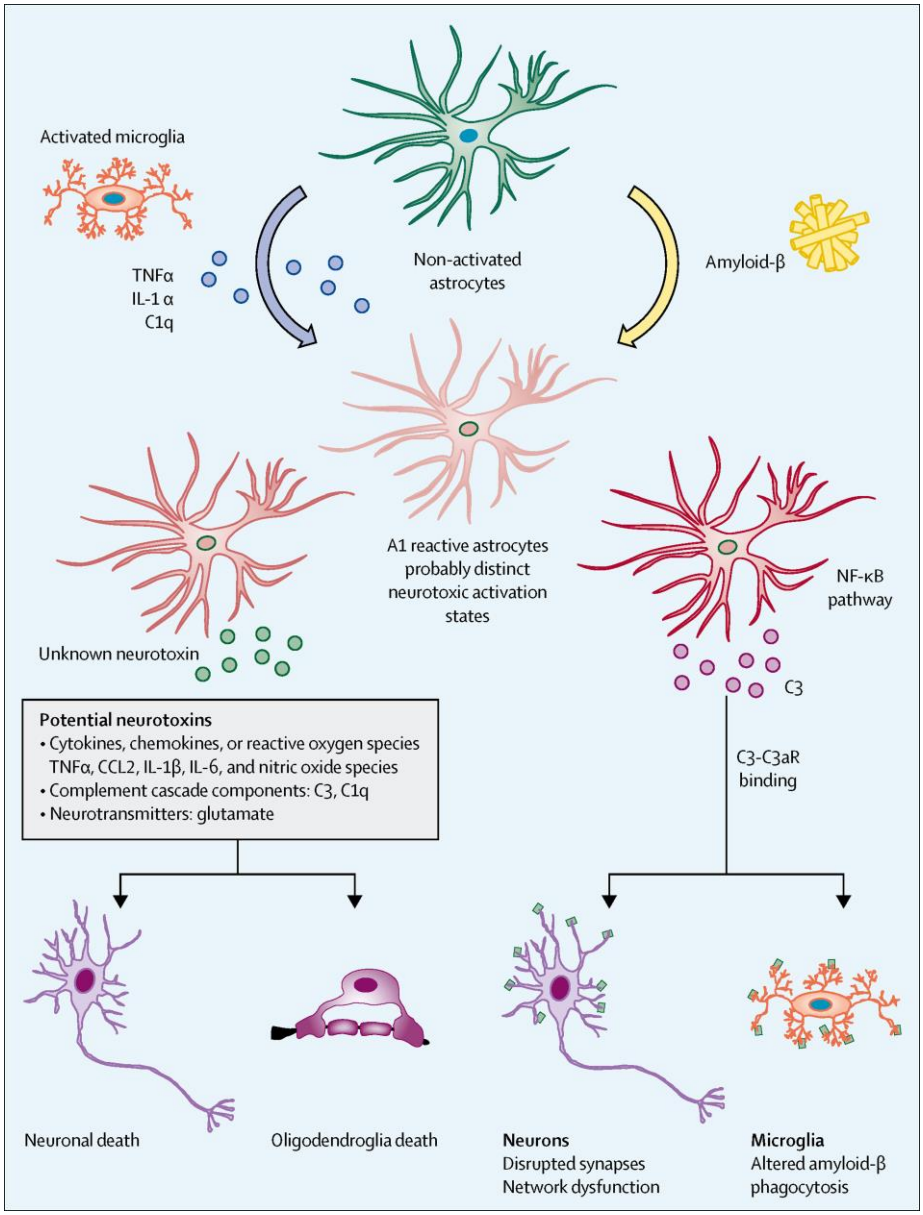

Figure 2. Model of astrocyte activation in AD. Reprinted from (Arranz & De Strooper, 2019) with permission from The Lancet Neurology.

1.6 Lipid metabolism in AD

Lipids are among the principal classes of biomolecules and are involved in various biological functions such as energy storage and signaling, as well as being the key component of membranes (van Meer et al., 2008). In general, lipids have been defined as compounds soluble in organic solvents but hydrophobic in nature (Currie, 1998). The LIPID MAPS classification system was established in 2005 (Fahy et al., 2005) and has since become internationally accepted. According to this system, lipids are divided into eight categories which are comprised of fatty acyls, sphingolipids, saccharolipids, glycerolipids, glycerophospholipids, polyketides, sterol lipids and prenol lipids. Furthermore, each of these categories can be divided into subcategories such as lipid class (e.g. ceramide) and lipid species (lipids expressed as lipid class, total number of carbon atoms and total number of double bonds) (Liebisch et al., 2013).

Lipid metabolism has been associated with AD and could possibly contribute to the pathogenesis of the disease. Membrane lipids, for example, are thought to play a role in the production of Aβ. As previously mentioned, Aβ is generated by cleavage of the APP protein by secretases (Walter & van Echten-Deckert, 2013). Lipids, as the main components of membranes, can alter the cleavage of these secretases and subsequently the processing of APP (Hartmann et al., 2007). Aβ peptides, implicated in AD, interact with the cellular membrane and induce amyloid toxicity. The enzymes and APP can, in turn, modify the lipid structure of membranes. Lipids are therefore likely to be implicated in the pathogenesis and progression of AD (Walter & van Echten-Deckert, 2013).

Ceramides are the core components of sphingolipid metabolism, consisting of a sphingosine backbone tethered to a fatty acid chain varying in length of carbon atoms (C14-C26) (Fanani & Maggio, 2017). They play significant roles in maintaining the function and integrity of membranes as well as affecting cellular processes including differentiation, proliferation, inflammation and apoptosis (Cuvillier, 2002; Gomez-Munoz et al., 2016). Changes in sphingolipid metabolism have been associated with normal aging and neurodegenerative diseases such as AD (Stranahan et al., 2011). Cellular and animal studies indicate that ceramides can increase Aβ production and contribute to AD pathogenesis (Jazvinšćak Jembrek et al., 2015). Ceramides promote Aβ production by post-translational stabilization of BACE1, thereby extending its half-life (Kalvodova et al., 2005; Puglielli et al., 2003). Aβ (both soluble and insoluble assemblies) can also incite the

hydrolysis of sphingomyelin (SM) into ceramide by sphingomyelinases (SMases) (Grimm et al., 2005; Lee et al., 2004) through oxidative stress-mediated mechanisms (Cutler et al., 2004). This creates a vicious cycle resulting in increased production of ceramide, which in turn leads to elevated immune response and, eventually, loss of neurons in AD. Ceramides of longer chain lengths, particularly C18 ceramide, have been associated with increased phosphorylation of tau via Protein phosphatase 2A (PP2A) activity (Chalfant et al., 1999; Dobrowsky et al., 1993; Goedert et al., 1995; Gong et al., 1994; Mukhopadhyay et al., 2009). Elevated levels of ceramide have been observed in brain tissues of AD patients compared to a healthy control group according to several post-mortem studies (Cutler et al., 2004; Filippov et al., 2012; Han et al., 2002; He et al., 2010; Stranahan et al., 2011). Although post-mortem studies are important, in vivo studies are critical for further evaluating the roles of lipids in pathogenesis and progression of AD. For example, very few studies have been performed for the evaluation of ceramide levels with AD in cerebrospinal fluid (CSF).

1.7 The cholinergic system in AD

Cholinergic dysfunction is an early hallmark of AD, usually ascribed to an early breakdown of cholinergic neurons. Cholinergic neurons are widely distributed in the CNS, with almost all regions of the brain being innervated by them (Armstrong et al., 1983; Woolf & Butcher, 2011). Studies have shown a prominent loss of this type of neurons in the basal forebrain of AD patients, particularly the nucleus basalis of Meynert (NBM). Other functions outside of NBM appear to be spared, pointing to a preferential vulnerability of the NBM in relation to AD.

The cholinergic hypothesis of AD originated from observations made by Davies & Maloney (1976). They were the first to report the loss of cholinergic neurons in brain tissue of AD patients at post-mortem. It was proposed that a shortage of the neurotransmitter acetylcholine (ACh) in the brain and the following reduction in neurotransmission at central cholinergic synapses caused some of the cognitive impairment experienced by AD patients. Although other components of AD pathology have received more attention in recent years, treatments that target the cholinergic system still remain an essential part of AD patient management. This has further validated the cholinergic system as an essential biological target in the treatment of the disease.

The classical view of cholinergic neurotransmission (Fisher & Wonnacott, 2012) is presented in Figure 3. The neurotransmitter ACh is synthesized from choline and acetyl coenzyme A (acetyl-CoA) by the enzyme choline acetyltransferase (ChAT) in the cytosol of cholinergic presynaptic neurons. ChAT is then loaded into synaptic vesicles by the vesicular acetylcholine transporter (VAT). Depolarization of the presynaptic neuron triggers the release of ACh into the synaptic cleft, where it can bind to nicotinic (nAchRs) or muscarinic (mAchRs) ACh receptors, propagating neurotransmission. The transmission is subsequently terminated by the cleavage of ACh into choline (Ch) and acetate by acetylcholinesterase (AChE). Choline is then transported back to the presynaptic neuron through choline transporters (CHT).

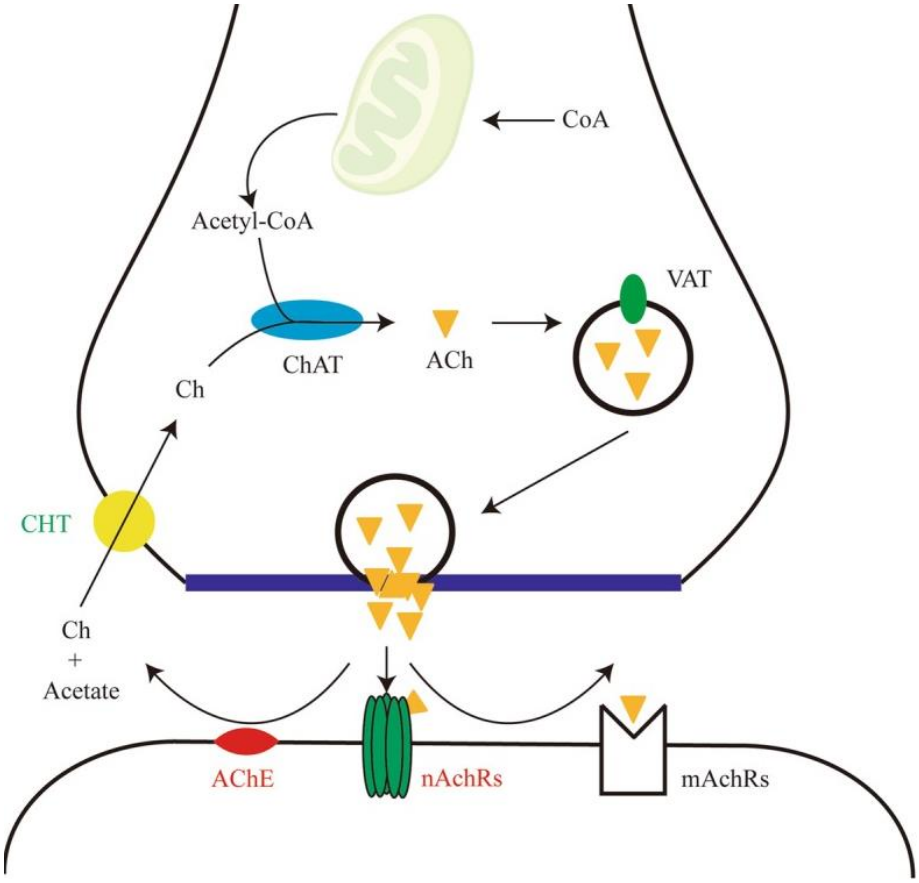

Figure 3. Schematic presentation of cholinergic neurotransmission. Reprinted from (Wang et al., 2019) with permission from BMC Genomics in accordance with the terms of Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/). No changes were made to the original figure.

Butyrylcholinesterase (BuChE), like the related AChE, is a serine hydrolase that rapidly affects the hydrolysis of the neurotransmitter (Darvesh et al., 2003). At the time when the cholinergic hypothesis was first proposed in 1976, the enzyme considered to be mainly involved in the regulation of ACh was AChE, with BuChE thought to only have minor effect on the regulation of ACh. However, more recent studies have found that BuChE can also play a critical role in the regulation of ACh in the human brain (Darvesh et al., 1998; Mesulam et al., 2002). Synaptic membrane-bound AChE is more critical in regulating cholinergic neurotransmission, while BuChE plays a more important role in the control of extracellular ACh levels that modulate the functional properties of glial cells (Darreh-Shori et al., 2013; Darvesh et al., 2003; Lane & Darreh-Shori, 2015). BuChE is primarily localized to glial cells but can also be found near neurons and endothelial cells (Darvesh et al., 1998; Darvesh & Hopkins, 2003). It has been hypothezised that the close proximity of synaptic AChE and glial BuChE facilitates the degradation of ACh by BuChE when AChE becomes substrate-inhibited (Greig et al., 2001). The excess substrate binding to the peripheral anionic site causes the hydrolytic activity of AChE to decrease while it causes the hydrolytic activity of BuChE to increase (Masson et al., 2002; Masson et al., 1999). Studies have also indicated that both the enzymes may possibly be involved in functions other than hydrolysis of ACh, such as neuroinflammation and in the formation of Aβ plaques and NFTs (Lane et al., 2006).

A relationship between the dysfunction of the cholinergic system and Aβ pathology has long been established. Perry et al. (1978) found the diminishing activity of ChAT to be correlated with increasing numbers of neuritic plaques in AD brains at post-mortem. Previous studies have reported short-term oligomeric Aβ exposure to affect both ACh synthesis and release of rat brain hippocampal slices and cultured neurons, thereby contributing to cholinergic dysfunction (Hoshi et al., 1997; Kar et al., 1998; Kar et al., 1996; Nunes-Tavares et al., 2012; Pedersen & Blusztajn, 1997; Pedersen et al., 1996; Satoh et al., 2001). Interestingly, recent studies have suggested that Aβ peptides can form highly stable and soluble complexes with ApoE and cholinesterases, named BaβACs (Darreh-Shori et al., 2011a; Darreh-Shori et al., 2011b; Kumar et al., 2016; Vijayaraghavan et al., 2013). BaβACs possibly act as direct modulators of cholinergic signaling, promoting hyper-activation of cholinesterases which leads to increased degradation of AD.

The cholinergic signaling system has also been associated with inflammation, both in general and in neurodegenerative diseases like AD. ACh is hypothesized to act as a suppressor on non-excitable cholinoceptive

cells, including astrocytes and microglia, exerting long distant regulatory effects through activation of nicotinic α7-ACh receptors (Pavlov et al., 2009; van Westerloo et al., 2005). This is called the cholinergic anti-inflammatory pathway (CAP). However, it has been difficult in the past to explain how ACh molecules can possibly reach and act on the distantly located non-excitable cells since extracellular ACh is very short-lived due to the abundant presence of the very efficient ACh-degrading enzymes AChE and BuChE in CSF. A previous study (Vijayaraghavan et al., 2013) offered a plausible explanation for this dilemma. It showed compelling evidence that ChAT is not solely a cytosolic enzyme but is also plentifully present in CSF and is secreted by cultured human astrocytes (both under resting and stimulatory conditions). Their hypothesis is that long-distance action of ACh is regulated through maintenance of steady-state equilibrium between ACh hydrolysis and synthesis, which in turn could regulate glial activation status.

1.8 Cognitive manifestation of AD

Deficits in episodic memory are the most prominent and salient early symptoms of AD (Hodges, 2000; Tromp et al., 2015). This cognitive domain refers to the recollection of previously experienced events (Tulving, 1983), and is typically measured by verbal list learning tests involving subjects recalling or recognizing words from a verbally presented list (Dubois et al., 2014). Measures of delayed recall (the ability to recall information after a period of time), have specifically been shown to be sensitive to early memory changes in AD (Estévez-González et al., 2003). Neuropathological studies have reported the medial temporal lobe (MTL) structures (e.g. entorhinal cortex and the hippocampus) to be critical for memory functions (Squire & Zola-Morgan, 1991; Weissberger et al., 2017) as well as being susceptible to early changes in AD (Braak & Braak, 1991; Schöll et al., 2016). The entorhinal cortex and hippocampus, in particular, are important for the encoding and the storage of new information (Du et al., 2001). It has also been widely confirmed that MTL structures are mostly unaffected in the normal aging brain, while significant neuronal loss can be found within these structures at the earliest stages of diagnosed AD (Gallagher & Koh, 2011).

Other cognitive domains are also affected in AD as the neurodegenerative changes, specifically characterized by NFTs, spread beyond MTL structures to the association cortices of temporal, frontal and parietal lobes (Salmon & Bondi, 2009). Impairment in executive functions (Guarino et al., 2019; Perry & Hodges, 1999) and language abilities (Hodges & Patterson, 1995; Verma & Howard, 2012) can occur relatively early in AD. Executive functions present a

wide range of higher-level cognitive processes essential for goal-directed behavior. Those include planning, problem-solving, inhibitory control and multitasking (Diamond, 2013; Grafman & Litvan, 1999). Impairment in language abilities are frequently measured by tests reflecting verbal fluency (the ability to find and express words), confrontation naming (connecting a label to a corresponding viewed stimulus) and semantic categorization (classification of items based on meaning) (Henry et al., 2004; Nebes, 1989). Deficits in other cognitive domains are also likely to appear during the course of AD, for example in non-verbal memory (Smith & Bondi, 2013) and processing speed (van Deursen et al., 2009; Warkentin et al., 2008). Visuoconstruction (the ability to reproduce objects or pictures previously viewed) is a part of non-verbal memory and is frequently affected. Processing speed refers to the time it takes to process and respond to information (Salthouse, 2000). The tests measuring this cognitive domain are commonly simple and do not require higher-level thinking.

A small portion (6-14%) of AD patients do not initially express the amnestic phenotype of the disease (Villain & Dubois, 2019). These cases are categorized as atypical AD and are more frequently found among younger patients under 65 years of age. Pathological changes are initiated in different regions of the brain compared to typical AD, although pathological hallmarks (Aβ plaques and NFTs) are the same (Graff-Radford et al., 2021). The most common variants of atypical AD are posterior cortical atrophy (PCA) and logopenic progressive aphasia (LPA). PCA is a syndrome characterized by a progressive decline in visual and spatial processing, with atrophy most prominently found in the occipital cortex and the parietal cortex (Crutch et al., 2012). LPA, a type of primary progressive aphasia (PPA), is a syndrome characterized by language impairment (e.g. difficulties finding words) caused by atrophy in the left posterior temporal cortex and inferior parietal lobule (Henry & Gorno-Tempini, 2010).

1.9 Clinical diagnosis of AD

The process of AD diagnosis differs between centers in clinical practice but is generally followed by a certain set of criteria. One of the most used criteria was published in 1984 by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) in 1984 (McKhann et al., 1984). In this criteria, AD was essentially defined as a clinical-pathological entity. An individual with a gradual onset of cognitive impairment, typically involving memory, which substantially affected daily activities, would be

diagnosed as having dementia. After ruling out other potential contributing factors, a degenerative disorder was confirmed and the label possible or probable AD given. The criteria was mostly based on clinical assessment, with laboratory tests only used to exclude other causes of cognitive decline. Diagnosis of definite AD could only be made after death through post-mortem identification of Aβ plaques and NFTs in brain tissue.

Between 1984 and 2011, considerable progress was made in understanding AD-related pathological changes. In 2011, the National Institute on Aging and the Alzheimer's Association (NIA-AA) (McKhann et al., 2011) redefined the criteria intended for routine diagnosis in clinical practice. The major difference between the old and the new criteria was in regards to the application of disease stages. The previous guidelines recognized only the dementia stage of AD. In contrast, the 2011 criteria identified three distinct stages - preclinical AD, mild cognitive impairment (MCI) and AD dementia. MCI is considered a transitional state between normal aging and dementia (Petersen, 2004). The condition is characterized by cognitive decline beyond what is expected from normal aging, without interfering with the ability to carry out daily activites. In addition, the term subjective cognitive impairment (SCI) was introduced in 2014 by the Subjective Cognitive Decline Initiative (SCD-I) international working group (Jessen et al., 2014). SCI is a condition defined as a self-perceived decline in cognitive functions without being verified by neuropsychological tests and commonly precedes MCI.

Like dementia, MCI is heterogeneous in its underlying pathology as well as in clinical expressions. Approximately 10% of MCI cases convert annually to AD on average (Mitchell & Shiri-Feshki, 2009). A proportion of individuals with MCI will remain stable however and do not convert to AD or other types of dementia (Winblad et al., 2004).

Numerous studies have demonstrated a discordance between the clinical manifestation of AD and its pathological hallmarks. Aβ plaques and NFTs have been detected in the brains of elderly cognitively unimpaired individuals, and conversely, individuals with clinical symptoms of AD do not always have the defining pathology on post-mortem examination. This is not surprising as symptoms are clinical consequences rather than the etiology of a disease. It is therefore of importance to base the diagnosis of AD not only on symptoms, but also on biomarkers indicative of specific biological processes. It is necessary for disease-modifying treatments to be successful, as they target specific biological pathways. It is also essential to find biomarkers qualified to monitor disease progression and treatment response (Jack et al., 2018).

1.10 Types of biomarkers

The Biomarkers Definitions Working Group of the National Institutes of Health (NIH) define a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” ("Biomarkers and surrogate endpoints: preferred definitions and conceptual framework," 2001, p. 91).

Biomarkers can be categorized into different types ("Biomarkers and surrogate endpoints: preferred definitions and conceptual framework," 2001), according to their intended use. A diagnostic biomarker, for example, is used to determine the presence of a disease, its subtype or a condition of interest. Predictive biomarker identifies individuals who are more likely to respond in a positive or negative way to a particular intervention. This type of marker can be of importance in selecting the proper treatment for a patient. A prognostic biomarker indicates the likelihood of a health outcome or disease progression, irrespective of the treatment. Monitoring biomarker is repeatedly measured for assessment of the status of a disease or a particular condition. They can be of value to measure therapeutic effect or disease progression while on treatment. A pharmacodynamic or response biomarker measures changes in levels in response to an intervention.

1.11 Development of pathological and clinical events leading to AD

Since the publication of the NIA-AA 2011 guidelines, more emphasis has been on in vivo biomarker research. Studies have strengthen the idea that imaging and CSF biomarkers are valid measurements for pathological change in AD (Jack et al., 2018). The disease is now considered as a continuum rather than three separated entities (Dubois et al., 2016). It has become evident that cognitive impairment and change in biomarker values gradually evolve (Fagan et al., 2014; Resnick et al., 2010), and the appearance of clinical symptoms and pathology varies in time (Bateman et al., 2012; Villemagne et al., 2013).

With the buildup of data regarding biomarkers for AD, Jack et al. (2010) proposed a theoretical model describing the temporal course of the pathological and clinical events leading to the disease. The model and the biomarker staging are depicted in Figure 4. The model focuses on the five most extensively researched biomarkers of AD pathology; decreased CSF $A\beta_{42}$, positron emission tomography (PET) amyloid imaging, increased CSF

tau, decreased uptake on fluorodeoxyglucose-PET (FDG-PET), and structural magnetic resonance imaging (MRI) measures of cerebral atrophy.

Both CSF $A\beta_{42}$ and amyloid PET imaging reflect Aβ plaque load in the brain. The radioligands utilized in amyloid PET imaging, such as carbon 11 labeled Pittsburgh compound B (PiB) and the various F^{18} ligands, bind explicitly to fibrillar Aβ (Bacskai et al., 2007; Ikonomovic et al., 2008). A high correlation has been found between PiB binding and fibrillar Aβ deposition, both at antemortem and post-mortem examination (Bacskai et al., 2007; Ikonomovic et al., 2008). Similarly, a high correlation has been found between low concentrations of CSF Aβ with Aβ neuropathology at autopsy (Clark et al., 2003; Strozyk et al., 2003). The validation of both CSF $A\beta_{42}$ and amyloid PET as biomarkers for Aβ plaque load have further been established by the very high concordance found between the two measurements (Fagan et al., 2006; Grimmer et al., 2009; Jagust et al., 2009; Tolboom et al., 2009).

CSF tau, FDG-PET and structural MRI are all measurements of neurodegeneration. Different forms of tau can be measured in CSF, such as total tau (T-tau) and phosphorylated tau (P-tau). T-tau presents both phosphorylated and non-phosphorylated tau. CSF tau is an indicator of neuronal injury (T-tau) and NFTs (P-tau), with increased CSF levels believed to be a direct consequence of tau accumulation in neurons. Decreased FDG-PET uptake serves as a proxy for synaptic dysfunction. FDG-PET measures brain glucose metabolism, with glucose mainly being utilized for synaptic transmission in order to restore membrane potentials (Kadekaro et al., 1985). The validation of the marker has further been confirmed by high correlations with post-mortem measurements of the synaptic structural protein synaptophysin (Rocher et al., 2003). Structural MRI uses strong magnetic fields and radio waves for the production of detailed organs and tissues images. It can provide measures of atrophy, which in the brain describes a loss of volume within neurons, extracellular space, or glia. Atrophy on MRI is not considered a good diagnostic marker for AD, but the degree of atrophy correlates well with Braak staging at autopsy (Jack et al., 2002).

The highly accepted hypothetical model of AD (Figure 4) postulates that the biomarkers become abnormal in a temporally ordered manner as the disease progresses. Biomarker magnitude is depicted with sigmoidal curves, where biomarker change happens first rapidly, then more slowly as the disease progresses. Biomarkers of Aβ deposition (CSF $A\beta_{42}$ or PET amyloid imaging) become abnormal first, prior to neurodegeneration, and have mostly reached a plateau by the time clinical symptoms appear. This is followed by

changes in biomarkers of tau-mediated neuronal injury (CSF tau) and dysfunction (FDG-PET), which also associate with the severity of clinical symptoms. Structural MRI, measuring the rate of atrophy, is the last biomarker to become abnormal. It though retains a closer relationship with cognitive performance later into the disease than other biomarkers. All of the biomarkers precede the development memory deficits and other clinical symptoms.

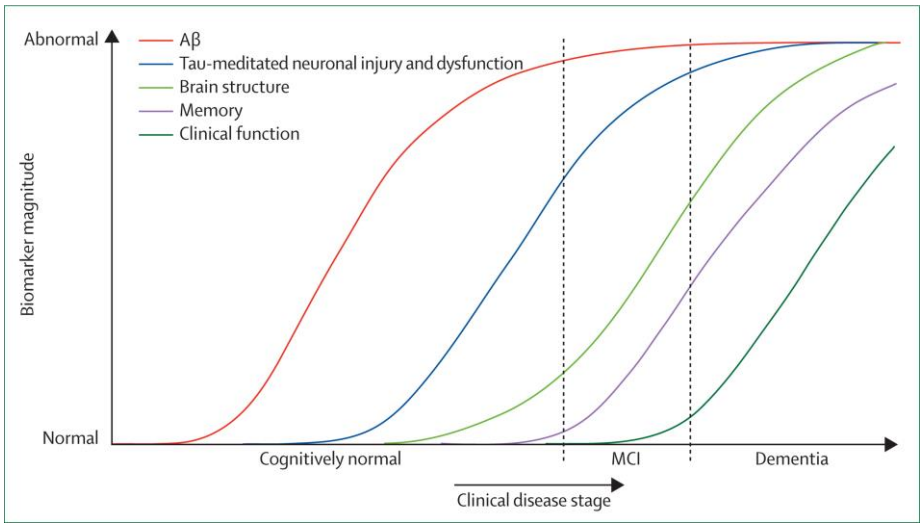

Figure 4. Dynamic biomarkers of the AD pathological cascade. Aβ is identified by CSF $A\beta_{42}$ or PET amyloid imaging. Tau-mediated neuronal injury and dysfunction is identified by CSF tau or FDG-PET. Brain structure is measured by use of structural MRI. Figure and figure text reprinted from (Jack et al., 2010) with permission from The Lancet Neurology.

1.12 Research guidelines for the diagnosis of AD

The NIA-AA working group updated their research guidelines for the diagnosis of AD in 2018 (Jack et al., 2018) from the previous one set in 2011 (McKhann et al., 2011). Within this framework, AD is defined as a pathological process identified primarily by biomarkers. This is important as it shifts the definition of AD from the clinical outcomes of the disease to the biological construct. Emphasis on the biological construct will facilitate a more precise understanding of the etiology of the disease and the series of events leading to cognitive decline. This approach will most likely also be beneficial for clinical trials as specific pathways can be targeted in selected patient groups. In 2018, NIA-AA working group proposed the biomarker based AT(N) framework. Biomarkers were divided into three classes, with each having a positive or a negative outcome. The letter “A” stands for Aβ

deposition (CSF $A\beta_{42}$, CSF $A\beta_{42}$/ $A\beta_{40}$ or PET amyloid imaging), "T" for tau pathology (CSF P-tau or PET tau imaging) and "N" for neurodegeneration (CSF T-tau, FDG-PET or structural MRI). Negative outcome within each class ($A^-B^-N^-$) defines a normal biomarker profile. $A^+T^-N^-$ indicates AD pathological change while A^+T^+, with or without N^+, presents AD. Biomarker profiles with A^- and T^+/N^+, indicate non-AD pathology. Cognitive symptoms are only used for staging purposes. An important aspect of the framework is the flexibility to incorporate newly validated biomarkers.

Another widely known research criteria for AD comes from the International Working Group (IWG), published in 2014 (Dubois et al., 2014). Like the 2018 NIA-AA research framework, it emphasizes the use of biomarkers. The difference lies in the dependence of clinical manifestation. The diagnosis of AD depends on both the presence of cognitive decline and signature AD biomarker profile (increased amyloid PET deposition or lowered CSF $amyloid_{42}$ and elevated CSF T-tau or P-tau). Although biomarkers are only part of the AD research criteria for as of now, it is expected that they will be integrated into clinical criteria in the future.

1.13 Biomarkers in CSF

CSF is a clear bodily fluid that fills the subarachnoid space and the ventricles in the brain. CSF acts as a liquid buffer for the protection of the CNS and can be obtained via lumbar puncture. CSF is in direct contact with the brain and is tightly separated from blood by the BBB. It is, therefore, a very informative fluid in biomarkers discovery for neurodegenerative diseases (Blennow et al., 2010) and can provide specific insight into disease pathogenesis (Blennow et al., 2015).

1.13.1 Core AD biomarkers

The core CSF biomarkers reflecting signature AD pathology, Aβ, T-tau and P-tau, have been extensively studied (Ittner & Götz, 2011). Although the diagnostic accuracies of these markers are generally satisfactory (Ferreira et al., 2014), their levels reach a plateau during the symptomatic stages of the disease and do not strongly follow the progression of cognitive decline (Jack et al., 2010; Perrin et al., 2009; Zhou et al., 2009). This requires the discovery of novel biomarkers for a better understanding of other AD-related pathological processes which could aid diagnosis and better monitor the progression of the disease.

1.13.1.1 Aβ

Under normal conditions, Aβ peptides are either cleared from interstitial fluid (ISF) to the CSF or transported from ISF through the BBB (Spies et al., 2012). In AD, overproduction or dysfunction in clearance of Aβ results in the formation of neuritic plaques in the brain parenchyma (Ovod et al., 2017). The plaques primarily consist of $A\beta_{42}$ and work like a “sink” for the peptide, reducing the levels of $A\beta_{42}$ in both CSF and blood.

A decrease in CSF $A\beta_{42}$ levels in AD patients was first described by Motter et al. (1995) and has been confirmed in numerous studies (Olsson et al., 2016). An inverse relationship of CSF $A\beta_{42}$ levels with both amyloid PET imaging (Fagan et al., 2006; Grimmer et al., 2009) and plaque load in AD brains at autopsy (Strozyk et al., 2003; Tapiola et al., 2009) has also been established.

Aβ species other than $A\beta_{42}$ are present in human CSF, with $A\beta_{40}$ having the highest concentration (Portelius et al., 2007; Portelius et al., 2006). The CSF $A\beta_{42}/A\beta_{40}$ ratio has proven to be a more accurate biomarker for AD in comparison to CSF $A\beta_{42}$ (Hansson et al., 2007; Lewczuk et al., 2004; Wiltfang et al., 2007). Multiple studies have though reported no or minor CSF $A\beta_{40}$ concentration changes in AD (Olsson et al., 2016). It is not entirely clear why the ratio improves the performance, but a likely hypothesis is that $A\beta_{40}$ can be used as a measurement of total Aβ levels and the ratio corrects for individual differences in the production of Aβ (Lewczuk et al., 2015).

1.13.1.2 P-tau

Studies have revealed a correlation between P-tau levels measured in antemortem CSF samples and the amount of neocortical NFTs at autopsy (Buerger et al., 2006; Tapiola et al., 2009). This indicates that P-tau reflects the hyperphosphorylation of tau and its formation into tangles in the brain.

CSF levels of P-tau, unlike T-tau, do not alter in relation to acute brain injury such as acute ischaemic stroke (Hesse et al., 2001). The levels are also normal or only marginally increased in neurodegenerative disorders such as Creutzfeldt–Jakob disease (CJD), which is characterized with neurodegeneration but no tangles (Riemenschneider et al., 2003; Skillbäck et al., 2014b). Those findings support the idea that CSF levels of P-tau probably reflect the state of tau phosphorylation and not only neurodegeneration. Furthermore, CSF P-tau appears to be specific for AD, as higher levels are mainly observed in AD and not in other tauopathies (neurodegenerative diseases characterized by aggregation of tau proteins into NFTs in the brain)

(Skillbäck et al., 2015). One possible explanation could be that the tau release from neurons in AD is due to Aβ exposure (Maia et al., 2013; Sato et al., 2018).

1.13.1.3 T-tau

Like P-tau, higher levels of CSF T-tau are not detected among patients with non-AD tauopathies (Skillbäck et al., 2015). CSF T-tau is though regarded as a general neurodegeneration marker, serving as a proxy for the intensity of neurodegeneration or acute neuronal damage (Blennow & Hampel, 2003). Indeed, CSF T-tau levels have shown to increase rapidly within days following acute brain damage, before reaching normal levels weeks later (Hesse et al., 2001; Zetterberg et al., 2006). The highest T-tau levels can be found in diseases characterized by severe neurodegeneration. CJD, for example, has 10- to 20-fold higher T-tau levels compared to AD (Riemenschneider et al., 2003; Skillbäck et al., 2014b). Other neurodegenerative disease reported to have elevated CSF levels of T-tau is dementia with Lewy bodies (DLB) (Arai et al., 1997; Parnetti et al., 2008). CSF T-tau is therefore considered a less specific biomarker for AD compared to P-tau.

1.13.2 Neurodegeneration markers

1.13.2.1 NFL

Neurofilaments are major components of the neural cytoskeleton and are classified into three types of subunits based on their molecular mass; Neurofilament light (NFL), neurofilament medium (NFM), and neurofilament heavy (NFH) (Lépinoux-Chambaud & Eyer, 2013). Although the precise functions of neurofilaments are unknown, they are believed to be critical in maintaining neuronal structure and enabling high-velocity nerve conductions (Barry et al., 2012; Rao et al., 2003). NFL, the lightest chain of 68 kD (Petzold, 2005), is mainly located in the axoplasm of large myelinated neurons.

Axonal damage leads to cytoskeletal proteins, including neurofilaments, to be released into the extracellular space and subsequently into bodily fluids (CSF and blood) (Zetterberg, 2016). Increased CSF concentrations of NFL have been associated with age (Vågberg et al., 2015) and neurodegenerative diseases (Skillbäck et al., 2014a) including AD, FTD (Landqvist Waldö et al., 2013; Pijnenburg et al., 2007; Sjögren et al., 2000) and amyotrophic lateral sclerosis (ALS) (Zetterberg et al., 2007).

Several studies have consistently found higher CSF levels of NFL in AD patients compared to controls (Molinuevo et al., 2018). A large meta-analysis by Olsson et al. (Olsson et al., 2016) found a large effect size for differentiating between the two groups. One study also found higher CSF NFL levels in AD compared to stable MCI as well as higher levels in both study groups compared to controls (Zetterberg et al., 2016).

NFL appears not to be an AD-specific marker as levels of the protein is also elevated in other neurodegenerative diseases. Higher CSF NFL concentrations have, for example, been found in FTD compared to AD (Skillbäck et al., 2014a). CSF NFL levels also correlate with changes in white matter, brain atrophy and cognitive decline independently of Aβ pathology (Mattsson et al., 2016; Zetterberg et al., 2016). The marker therefore has a potential to be used as a non-specific biomarker for disease progression and severity and as an aid in differential diagnosis of neurodegenerative diseases.

A strong correlation can be found between levels of NFL in CSF and blood, as demonstrated in numerous studies across different neurological diseases (Bacioglu et al., 2016; Kuhle et al., 2016; Wilke et al., 2016). This gives hope that plasma or serum NFL could be utilized as a non-invasive biomarker, avoiding the risk involved in obtaining CSF samples by lumbar puncture.

1.13.3 Inflammation markers

Increasing evidence reveal that inflammation plays a critical role in the pathogenesis and progression of AD and other neurodegenerative diseases (Ardura-Fabregat et al., 2017; Calsolaro & Edison, 2016). Studies have shown toxic forms of Aβ to elicit an immune response through activation of microglia and astrocytes (El Khoury et al., 2003; Medeiros & LaFerla, 2013; Steardo et al., 2015). Furthermore, inflammation is also believed to contribute to the formation of NFTs, affecting dysfunction and loss of neurons (Heppner et al., 2015). It is therefore of interest to explore glial activation markers in relation to the diagnosis and monitoring of AD.

1.13.3.1 YKL-40

YKL-40, also known as chitinase 3-like protein 1, is one of four inactive chitinases that bind to chitin but has no chitinase activity (Hakala et al., 1993). It belongs to the glycosyl hydrolase family 18 and consists of 383 amino acids (Rehli et al., 2003). The protein is encoded by the CHI3L1 gene in humans and is located on chromosome 1q31-q32 (Rehli et al., 1997; Rehli

et al., 2003). Although the physiological role of YKL-40 remains unknown, it is thought to play a role in tissue remodeling during inflammation (Craig-Schapiro et al., 2010).

At the cellular level, YKL-40 is secreted by various types of cells including neutrophils, synovial fibroblasts, chondrocytes and macrophages (Kirkpatrick et al., 1997). Although YKL-40 is widely used as a glial activation marker, the cellular source of expression in the brain remains uncertain. Some studies have revealed an increased expression of YKL-40 in astrocytes in neuroinflammatory conditions, such as multiple sclerosis (MS) and traumatic brain injury (TBS) (Bonneh-Barkay et al., 2010; Cantó et al., 2015). On the other hand, other studies have reported increased expression of the protein in macrophages/microglia in the same conditions (Cantó et al., 2015; Hinsinger et al., 2015). For AD specifically, the expression of YKL-40 has been related to both microglia and astrocytes as well as, occasionally, neurons (Bonneh-Barkay et al., 2012; Craig-Schapiro et al., 2010; Mattsson et al., 2011).

YKL-40 is upregulated in the brain in multiple pathological conditions such as cardiovascular disorders (Rathcke & Vestergaard, 2009), diabetes mellitus, inflammatory diseases, cancer (Johansen, 2006) and neurological disorders including AD, ALS, MS (Comabella et al., 2010) and TBS (Bonneh-Barkay et al., 2010). YKL-40 has frequently been found surrounding amyloid plaques in AD brains (Craig-Schapiro et al., 2010). Increased YKL-40 expression has also been found to associate with higher tau pathology burden, linking glial activation to neurodegeneration (Querol-Vilaseca et al., 2017).

Higher CSF YKL-40 levels have been reported to be higher in AD compared to controls (Molinuevo et al., 2018) as well as in preclinical late AD vs. early AD stages (Alcolea et al., 2017). The degree of increase was though found to be modest compared to neurodegeneration markers NFL and T-tau in a large meta-analysis performed by Olsson et al. (Olsson et al., 2016). Difference in CSF YKL-40 levels have been observed in AD compared to DLB, Parkinson's disease (PD) (Wennström et al., 2015) and FTLD (Baldacci et al., 2017), albeit an early study did not find difference between disease groups (Mattsson et al., 2011). Studies have found a strong association of CSF YKL-40 with tau protein levels, but not with $A\beta_{42}$ (Alcolea et al., 2014; Alcolea et al., 2015b; Antonell et al., 2014; Craig-Schapiro et al., 2010). A strong association has also been found between CSF levels of YKL-40 and

NFL (Melah et al., 2016), further establishing a connection between the activation between glial cells and neurodegeneration.

1.13.3.2 S100B

S100 calcium-binding protein B (S100B) belongs to a highly homologous family of calcium-binding proteins. The human gene encoding the S100B protein, maps to chromosome 21q22.3 (Allore et al., 1988). S100B carries two Ca^{2+}-binding sites of the EF-hand (helix-loop-helix) type (Donato, 2001; Heizmann et al., 2002), with the binding of calcium triggering conformational changes allowing interaction with tharget proteins (Donato, 2003). In CNS, S100B is primarily expressed in astrocytes but also in other types of glial cells such as oligodendrocytes, Schwann cells, ependymal cells, retinal Muller cells, enteric glial cells, as well as in definite neuron subpopulations (Brockes et al., 1979; Didier et al., 1986; Ferri et al., 1982; Ludwin et al., 1976; Rickmann & Wolff, 1995; Yang et al., 1995).

S100B exerts both intracellular and extracellular functions. As an intracellular regulator, it has been implicated as having roles in the regulation of many diverse processes such as calcium homeostasis, interaction with elements of the cytoskeleton, protein phosphorylation and degradation. S100B can also act as a cytokine by secretion into the extracellular space. Its release can be triggered by various molecules including glutamate, serotonin, TNF, IL-1β, Aβ and lysophosphatidic acid. Extracellular S100B mediates both paracrine and autocrine effects on neurons and glia by interaction with receptor for advanced glycation end products (RAGE) and likely other receptors (Donato, 2003). The effect of extracellular S100B can be either trophic or toxic, depending on its concentration. In nanomolar concentrations, the protein is neuroprotective by inducing neurite outgrowth and triggers proliferation, while in micromolar concentrations, it has neurotoxic effects (Huttunen et al., 2000; Selinfreund et al., 1991; Tramontina et al., 2002). High levels of extracellular S100B have been detected in various conditions including brain trauma, ischemia and neurodegenerative, inflammatory and psychiatric diseases (Rothermundt et al., 2003).

Very few studies have evaluated CSF S100B levels in relation to AD. Studies by Nooijen et al. (Nooijen et al., 1997) and Jesse et al. (2009) did not find any significant differences in levels of the protein between AD patients and non-demented controls. In both studies, higher levels of the protein were though detected in patients with CJD compared to both the study groups. Interestingly, Peskind et al. (Peskind et al., 2001) did find higher levels of CSF S100B in patients with mild to moderate AD compared to healthy older

controls and patients with advanced AD. Few studies have explored the association between S100B and core AD markers in CSF. Hov et al. (Hov et al., 2017) detected a relationship between S100B and P-tau, but not $A\beta_{42}$, among elective surgery patients free from dementia and delirium.

1.13.3.3 GFAP

Intermediate filaments (IF), along with microtubules and microfilaments, make up the cytoskeleton of most eukaryotic cells. Glial fibrillary acidic protein (GFAP), a class-III IF, is a key component of the astrocyte's cytoskeleton in addition to vimentin, nestin and synemin (Yang & Wang, 2015). The protein is encoded by the GFAP gene, located on chromosome 17q21 in humans (Bongcam-Rudloff et al., 1991). GFAP is almost exclusively present in astrocytes and is highly expressed in the CNS (Yang & Wang, 2015). IFs play pivotal roles in providing support for the plasma membrane and the cytoskeleton. The exact function of GFAP is though poorly understood. Astrocytes react to toxic and traumatic insults by a change in morphology and function (reactive astrogliosis) (Burda & Sofroniew, 2014). Those changes include hypertrophy, increased proliferation and production of IFs such as GFAP (Sofroniew & Vinters, 2010). GFAP is therefore commonly used as a marker of reactive astrocytes. Diseases that express increased GFAP messenger RNA (mRNA) and protein levels include AD, scrapies and CJD (Middeldorp & Hol, 2011).

Expression of GFAP has been associated with amyloid plaque load in AD (Hanzel et al., 1999). A strong association between GFAP mRNA levels and the density of neuritic plaques was found in the frontal neocortex of the AD brain (Le Prince et al., 1993). Another study (Pike et al., 1995) demonstrated a significant increase in GFAP mRNA levels in relation to the elevation of Aβ deposits density. GFAP expression has also, to a lesser extent, been associated with the number of NFTs in the entorhinal cortex (Beach et al., 1989; Muramori et al., 1998; Porchet et al., 2003).

Studies exploring the relationship between levels of GFAP and AD in CSF are few, with results being inconsistent. Andreasen et al. (2001) and Rosén et al. (2011) did not find a significant difference in levels between AD and controls. In contrast, other studies (Abu-Rumeileh et al., 2019; Fukuyama et al., 2001; Ishiki et al., 2016; Jesse et al., 2009) have found higher levels in patients with AD compared to controls. Ishiki et al. (2016) also found higher levels of CSF GFAP in FTD compared to AD. GFAP has been recently measured in plasma and serum, where increased levels were found in different neurological conditions, including AD (Chen et al., 2020; Elahi et al., 2020; Heller et al., 2020).

2 Aims

The general aim of this thesis was to examine the relationships of selected CSF novel biomarkers with a CSF profile reflecting signature AD pathology, AD-related biological processes (brain amyloid status, neurodegeneration, inflammation) and cognitive functions among subjects at the symptomatic pre- or early dementia stages.

Specific aims were:

I. to evaluate the association of CSF glial and neurodegeneration markers with a CSF AD profile, AD-related biological processes and different cognitive domains (**Paper I**).

II. to identify CSF lipid species associating with CSF AD profile (**Paper II**).

III. to explore the relationships between selected lipid species and measures reflecting AD-related biological processes and impairment in verbal episodic memory (**Paper II**).

IV. to examine the association between the activity of cholinergic enzymes and measures reflecting AD-related biological processes and impairment in verbal episodic memory (**Paper III**).

3 Materials and methods

3.1 Study design

Individuals, who were referred to the Landspitali University Hospital (LUH) Memory Clinic over a four-year period, were potential candidates for The Icelandic MCI study. The design of the study is presented in Figure 5. At first visit, each candidate´s cognitive status was evaluated. All those who scored 24-30 points on the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) and 4.0 points or less on the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) (Jorm, 2004), were invited to join the study. The criteria for excluding candiates included i) cognitive impairment as a consequence of a pre-existing condition, ii) complications due to issues regarding health or social status, and iii) residency outside the Reykjavík Metropolitan Area.

In entering the study, each subject underwent standard clinical and neuropsychological assessment, brain imaging with MRI for the estimation of medial temporal lobe atrophy (MTA) and white matter lesions, and an electroencephalography (EEG) recording (Johannsson et al., 2015; Snaedal et al., 2012). Furthermore, lumbar puncture for collection of CSF was carried out, although optional by the requirement of the National Bioethics Committee.

These investigations, except for the EEG recordings, formed the basis for the diagnosis of each subject. Only subjects with SCI or MCI diagnosis at baseline had an annual re-evaluation of diagnosis based on neuropsychological assessment of their cognitive status. If the diagnosis at first follow-up was stable (SCI/MCI), the subject had a second follow-up planned a year later.

The clinical diagnosis of AD was both dependent on the NIA-AA criteria for probable AD dementia (McKhann et al., 2011), as well as evidence of AD pathology based on MTA score and core CSF markers, if available. Subjects with LBD required the consensus criteria of McKeith (McKeith et al., 2017). Diagnosis of MCI was based on the Winblad criteria (Winblad et al., 2004). None of the subjects had been treated with cholinesterase inhibitors, such as donepezil, galantamine and rivastigmine, before joining the study.

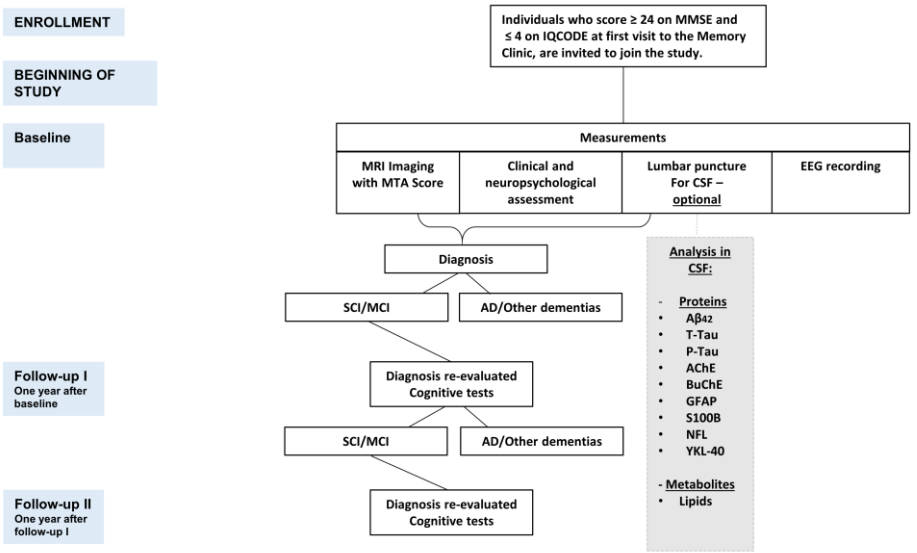

Figure 5. Design of The Icelandic MCI study.

3.2 Study cohort

A total of 218 subjects were recruited into The Icelandic MCI study. Recruitment of subjects took place over a four-year period, from April 2014 to June 2018 (Figure 6). Only subjects who had undergone lumbar puncture for the collection of CSF, were eligible for selection for this PhD project (n=76). Final sample selection was slightly different between **Paper I** (n=52), **Paper II** (n=60) and **Paper III** (n=46). The reason was mainly twofold. First, CSF analytes were measured at different time points, during or after the enrolment period. Cholinergic enzyme activity assays were performed first (exclusively used for **Paper III**) and lipidomics analysis second (exclusively used for **Paper II**), before the end of enrolment. Protein levels were measured last, after the end of enrolment. Second, 16 subjects were excluded for **Paper I** because of incomplete neuropsychological assessment across different cognitive domains. Only in **Paper I** were data from more than one cognitive domain (verbal episodic memory) used.

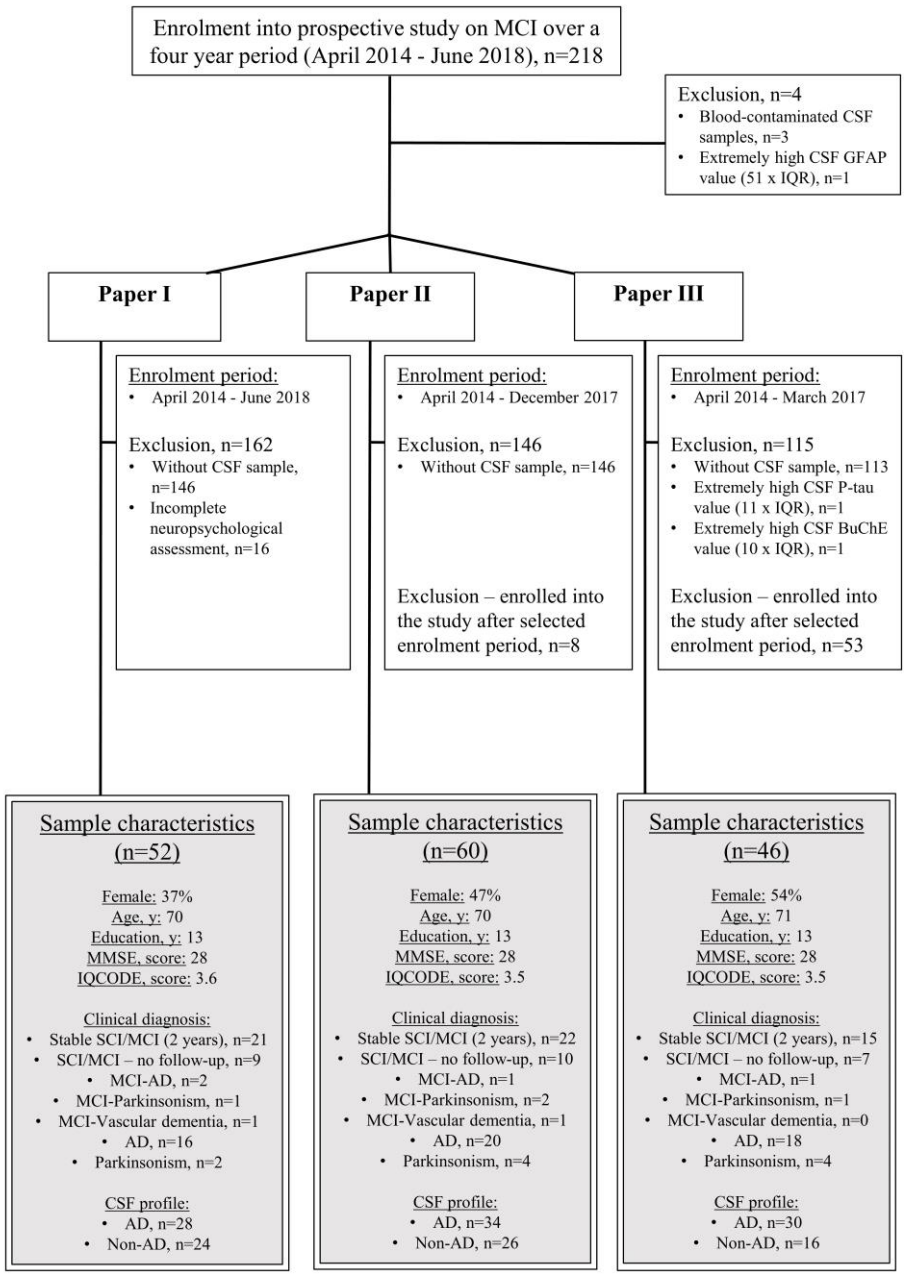

Figure 6. Overview of sample selection and sample characteristics for each paper. Numbers for age, education, MMSE and IQCODE present a median value. Parkinsonism in this project included LBD, PD and corticobasal degeneration (CBD). Abbreviations: *IQR* Interquartile range.

3.3 Measurements in CSF

3.3.1 Collection and storage

Lumbar puncture was performed in the morning for the collection of CSF. A 22-gauge spinal needle was inserted into the L3/4 or the L4/5 interspace. Samples, uncentrifuged, were stored at -80 °C in 2 ml polypropylene tubes (Sarstedt 72.694.006) within one hour after collection. Part of the samples for each subject was sent to a medical laboratory in Germany for the measurement of the core CSF AD markers $A\beta_{42}$, P-tau and T-tau. For analysis of other proteins, the 2 ml tubes were thawed (first freeze-thaw cycle) and the CSF aliquoted into 0.5 ml tubes (Sarstedt 72.730.105), before stored again. Proteins NFL, S100B and AChE were measured following two freeze-thaw cycles and YKL-40, GFAP and BuChE following three freeze-thaw cycles.

3.3.2 ELISAs

Commercially available sandwich enzyme-linked immunosorbent assays (ELISAs) for measuring levels of proteins, were performed according to manufacturer‘s instructions. Levels of T-tau (IBL International, Hamburg, Germany), P-tau181 (INNOTEST, Gent, Belgium) and $A\beta_{42}$ (IBL International, Hamburg, Germany), were measured in the ISO 15189 accredited medical laboratory MVZ Labor P.D. Dr. Volkmann und Kollegen GbR (Karlsruhe, Germany). Levels of NFL (Uman Diagnostics, Umeå, Sweden), YKL-40 (Quantikine ELISA Human Chitinase-3–like 1; R&D systems, MN, USA), S100B (BioVendor GmbH, Heidelberg, Germany) and GFAP (BioVendor GmbH, Heidelberg, Germany) were measured in a laboratory at the University of Iceland (UI). Samples were run in dublicates.

Intra-assay coefficient of variation (CV) is a measure of variance between sample replicates within the same batch (plate) while inter-assay CV is a measure of variance between sample replicates run on different batches. The core CSF AD markers measured at the medical laboratory in Germany have intra-assay and inter-assay CVs generally below 10%. All protein assays performed at the UI had mean intra-assay CV <10% and inter-assay CV <15% (Table 1).

Table 1. Intra-assay and inter-assay CV (%) calculations for measurements in CSF

Protein	Intra-assay CV (%)*	Inter-assay CV (%)	Number of batches
S100B (pg/ml)	4.3	13.6[a]	2
GFAP (ng/ml)	3.0	1.5[a]	2
NFL (ng/ml)	1.6	6.5[b]	3
YKL-40 (ng/ml)	3.3	9.6[b]	3
AChE (nmol/min/ml)	2.1	-	1
BuChE (nmol/min/ml)	2.2	-	1
$A\beta_{42}$ (pg/ml)[†]	<10.0	<10.0	N/A
P-tau (pg/ml)[†]	<10.0	<10.0	N/A
T-tau (pg/ml)[†]	<10.0	<10.0	N/A

Abbreviations: *CV* Coefficient of variation, *N/A* Not available

[†]$A\beta_{42}$, P-tau and T-tau proteins were measured by the medical laboratory MVZ Labor P.D. Dr. Volkmann und Kollegen GbR (Karlsruhe, Germany). *CV (%) for each set of replicates was calculated by dividing the standard deviation of the set by its mean and multiple by 100 to produce a percentage. Intra-assay CV (%) was then calculated by averaging the sample CVs (%). [a]Both kits for S100B and GFAP included two quality controls (QCs), with a known concentration of the protein of interest (high and low). CV (%) for each QC was calculated by first finding the mean of replicates within both batches. Next, the standard deviation between means of each batch was divided by the mean between batches and multiplied by 100. Finally, inter-assay CV (%) was calculated by averaging the CVs (%) for the high and low QCs. [b]No QCs were provided with the kits for NFL and YKL-40. Instead, the calculations of inter-assay CV was based on three different subject samples. Sample nr. 36 was run on batches 1 and 2, sample nr. 152 on batches 1 and 3, and sample nr. 175 on batches 2 and 3. For each subject sample, mean of replicates were calculated between batches. Subsequently, the standard deviation between batches was divided by the mean of the batch means multiplied by 100. For inter-assay CV(%), the average of the subject sample CVs (%) were calculated.

3.3.3 Enzyme activity assays

AChE and BuChE activities were measured for **Paper III**, using a modified Ellman's colorimetric method (Darreh-Shori et al., 2002; Ellman et al., 1961) at Karolinska Institutet (KI), Sweden. The assays were performed in 384-well plates (Thermo Scientific Nunc Maxisorb, Cat no 464718). For both measurements, the CSF samples were diluted 4X with dilution buffer, pH 7.4 (Tris-buffered saline (TBS; 10mM Tris–HCl); 0.1% Bovine serum albumin [BSA]; 1mM Ethylenediaminetetraacetic acid [EDTA] and 0.05%Triton X-100). All samples were run in triplicate (50 µl per well of diluted sample). The reaction was started by adding 25 µl of reagent mixture into each well. The mixture contained the substrates Acetylthiocholine iodide (ATC; final concentration 0.5 mM, Sigma 01480) or Butyrylthiocholine iodide (BTC; final concentration 5.0 mM, Sigma-Aldrich B3253), the selective inhibitors 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide (BW284C51; final concentration 1.0 µM, Sigma) or Ethopropazine (EPP; final concentration 0.1 mM, Sigma), Ellman's reagent 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB; final concentration 0.4 mM, Sigma-Aldrich D8130) and sodium/potassium phosphate buffer (50 mM, pH 7.4). The AChE substrate ATC and BuChE inhibitor EPP were used as a part of the reagent mixture for measurement of AChE activity, while BuChE substrate BTC and AChE inhibitor BW284C51 were used for measurement of BuChE activity. A microplate spectrophotometer reader was used for monitoring the reaction for at least 10 minutes at 412-nm wavelength, using SOFTmax PRO software (version 2.6.1 for PC; Molecular Devices Corp.). Reaction rates were calculated based on the linear portion of the reaction curves as changes of absorbance per minute. The intra-assay CV (%) for both assays were below 3%, with all samples measured in one batch (Table 1).

3.3.4 Subject grouping based on CSF measures

Subjects were classified as having a CSF AD or a non-AD profile, based on the ratio of T-tau and $A\beta_{42}$ values. The cut-off point of 0.52 was choosen based on results from a large memory clinic cohort study (Duits et al., 2014), where different methods of classification were compared. CSF AD profile, reflecting signature AD pathology, was defined as T-tau/$A\beta_{42}$ ratio > 0.52. This method of calculation is very similar to the Hulstaert regresson formula, (1240 + 1.18*T-tau)/ $A\beta_{42}$, which was recommended for classification by the medical laboratory measuring the core CSF AD markers. The Hulstaert formula gives approximately the same results as the ratio between T-tau and $A\beta_{42}$ with a cut-off point of 0.52. Identical categorization of subjects into CSF

profile groups resulted from the use of both of the methods. The classification into CSF profiles was independent of clinical diagnosis, but the clinical diagnosis of AD was partly based on CSF AD profile. Therefore, a full concordance existed between CSF AD profile and AD clinical diagnosis.

For **Paper III**, three different CSF biomarker profiles were created, one for each core CSF AD marker ($A\beta_{42}$, T-tau and P-tau). For each profile, subjects were dichotomized into two categories based on protein levels; abnormal (positive) or normal (negative). The specific cut-off points were established by the medical laboratory performing the assays, with abnormal values being defined as $A\beta_{42}$ < 375 pg/ml, T-tau > 445 pg/ml and P-tau > 61 pg/ml.

3.4 Lipidomic profiling

Lipidomic profiling was performed for **Paper II.**

3.4.1 Preparation of samples and UPLC-MS analysis

CSF sample extraction was performed as described in Bird et al. (Bird et al., 2011). C12 ceramide and C17 sphingomyelin (SM d18:1/17:0), purchased from Avanti Polar Lipids (Alabaster, AL, USA), were used as internal standards and added to 30 µl of CSF prior to lipid extraction. Dried lipid extracts were resuspended in 300 µL of ACN/IPA/H2O (65:30:5 v/v/v) and stored at –80 °C befure further analyses. Reference samples C18 SM (d18:1/18:0) and C18 ceramide (d18:1/18:0) were run alongside other samples as both SMs and ceramides have been related to AD in the literature (Haughey et al., 2010). Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS) lipidomic analysis were performed as described in Kotronoulas et al. (2020). The analytical instrumentation used was an ACQUITY UPLC system (UPLC ACQUITY, Waters Corporation, Milford, MA) coupled to a traveling-wave ion mobility (IMS) qTOF mass spectrometer (Synapt G2 HDMS, Waters Corporation, Manchester, U.K.). The chromatographic gradient separation was performed on an ACQUITY CSH C18 column (2.1 mm × 150 mm, 1.7 µm particle size, Waters Corporation) at 60 °C. Mobile phase A was H2O/2-isopropanol (80:20) and mobile phase B 2-isopropanol/ACN/H2O (90:9.1), both with 0.05% of formic acid and 5 mM ammonium formate. Injection volume was 10 µL, flow rate was 0.4 mL/min, and the run time was 17 min. The following gradient pattern (solvent B) was used: 0 min, 40%B; 1 min, 40%B; 3 min, 60%B; 10 min, 100%B; 13.5 min, 100%B; 14 min, 40%B; 17 min, 40 %B. Both positive (+) and negative (-) electrospray ionization (ESI) modes were acquired. The capillary and cone voltage were 2.5 kV and 30 V, respectively. The source and desolvation temperature were 120 and 500 °C and the desolvation gas flow was 800 L/h. During High Definition MSE (HDMSE) experiments, the

collision energy in the trap cell was off. It ranged from 20 to 30 eV for the positive mode and from 25 to 40 eV for the negative mode in the transfer cell. The MS-Dial program was used for further analysis of the resulting MS data.

3.4.2 Spectral processing

The Reifycs Abf Converter tool was applied for convertion of Waters RAW files to ABF format. ABF files from all subjects and pooled samples (either positive or negative controls) were loaded into the MS-Dial application (Tsugawa et al., 2015). Sample alignment and peak detection was performed in MS-Dial. Selected mass-to-charge ratio (m/z) peaks, hereafter referred to as m/z features, were manually curated to minimize the effect of sample drift. All m/z features were normalized by internal standards for reduction of variations between samples due to sample handling and processing. Annotations of m/z features were performed with MS-Dial and compared to MS/MS spectra in the LipidBlast Library (Kind et al., 2013). A total of 1013 m/z features were identified based on their peak mass. Five of the m/z features had more than 5% missing values and were therefore dropped from the analysis. The final dataset included 1008 features, with remaining missing values (5% or less) imputed by the smallest number of that particular feature.

Annotations of lipids are commonly reported in a specific manner and at various levels (Liebisch et al., 2013). At the lipid species level the lipid class is reported first, followed by the total number of carbon atoms and lastly by the number of double bonds (e.g. ceramide 36:1). The specific structure of a lipid species can also be analyzed in more detail. For example, one possible structure of ceramide 36:1 is C18 ceramide (d18:1/18:0). The first part of the parenthese (d18:1) refers to a sphingosine base of 18 carbons with one double bond, and the second part (18:0) to a fatty acid chain with 18 carbons and no double bond.

3.5 Neuropsychological tests

A detailed neuropsychological assessment was performed by licensed psychologists under the supervision of a clinical neuropsychologist. Specific tests were selected from the assessment for the evaluation of five cognitive domains frequently associated with aging and AD (Figure 2). It should though be emphasized that there is an overlap between tests across domains and that domains are not entirely independent from one another.

Two tests were used for the assessment of verbal episodic memory. The first, Rey Auditory Verbal Learning Test (RAVLT) (Lezak et al., 2004), was composed of 15 nouns read to the subject for five consecutive trials. Each

trial was followed by a free-recall test. After a 30 min delay, subjects were asked to recall the words without hearing the list again. Finally, a list of the 15 nouns previously presented to the subject as well as 30 new ones, were read again (recognition). The subject was then required to identify the previously heard nouns from the list. The second test was an orally presented story (Wechsler, 1987), which included 25 ideas. The story was read aloud and the subject asked to recall it without any hints (immediate free recall). The subjects were then asked to recall the story again 30 min later (delayed free recall).

The Rey–Osterrieth complex figure (ROCF) is a test widely used for the assessment of non-verbal episodic memory (Lezak et al., 2004) and requires the subject to reproduce a complex geometrical figure. First, the figure is copied free-hand and second, from memory (both immediately and after a 30 min delay).

Verbal fluency tests typically evaluate either semantic or phonemic fluency (Shao et al., 2014). For the former category, subjects were asked to generate as many animal names as possible within 60 seconds. For the latter, subjects were asked to name as many words starting with the letters H and S within same time limits.

Two subtests were utilized for the assessment of processing speed. Part A of The Trail Making Test (TMT-A) (Tombaugh, 2004) consists of 25 numbered circles randomly positioned on a piece of paper. The task of the subject is to draw a line between the circles in ascending order. Word reading, the most simple part of the Stroop test, was also used for the evaluation of processing speed (Stroop, 1935). The task requires subjects to read colored names out loud, written in black ink, in the fastest time possible.

The Digit Symbol Substitution Test (DSST), part B of TMT and Stroop 4th/3rd parts were administrated for the evaluation of executive functions. DSST (Wechsler, 1955) is a test consisting of digit-symbol pairs. A key containing digits from 1 to 9 is shown to the subject. Under each digit is a corresponding geometric symbol. The task of the subject is to copy the symbols into spaces below rows of digits. The number of correct symbols within 120 seconds is counted. TMT-B includes both numbered circles (1-13), just as in TMT-A, as well as circles containing letters (A-L). The subject is required to draw lines between the circles, alternating between numbers and letters in an ascending pattern (1-A-2-B-3-C, etc.). Part 3 of the Stroop test requires subjects to name colors of squares, while part 4 requires naming color of words when colors and words don’t match. The ratio between part 3 and part 4 was calculated for the purpose of controlling for speed.

Table 2. List of neuropsychological tests administrated. Reprinted from (Teitsdottir et al., 2020) with permission from Alzheimer's Research & Therapy.

Cognitive domain	Neuropsychological test	Scores (range)
Verbal episodic memory	RAVLT immediate recall	Free recall - the sum of the number of words recalled from trials 1 through 5 (0 to 75)
	RAVLT delayed recall	Delayed free recall –number of words recalled after 30 minutes delay (0 to 15)
	RAVLT recognition – false positives	Recognition - number of words recognized from a list of 45 words. Number of false positives subtracted from the score (-30 to 15)
	Story immediate recall	Recall of a story containing 25 ideas (0 to 25)
	Story delayed recall	Recall of a story containing 25 ideas again after 30 minutes delay (0 to 25)
Non-verbal episodic memory	ROCF immediate recall	Complicated drawing reproduced (0 to 36)
	ROCF delayed recall	Complicated drawing reproduced again after 30 minutes delay (0 to 36)
Language	Verbal fluency animals	Number of animal names produced in 60 seconds
	Verbal fluency H+S	Number of words that begin with H/S in 60 seconds
Processing speed	TMT-A	Time in seconds to connect a set of 25 numbered dots in sequential order
	Stroop test, part I	Time in seconds to read a set of color words written in black
Executive functions	DSST	Number of symbols correctly produced in 120 seconds
	TMT-B	Time in seconds to connect 25 targets, alternating between numbers and letters
	Stroop $4^{tn}/3^{rd}$ part	Part 3 – Time in seconds it takes to name squares of given colors Part 4 – Time in seconds it takes to name the color of a word

Abbreviations: *RAVLT* Rey Auditory Verbal Learning Test, *ROCF* Rey–Osterrieth complex figure, *DSST* Digit symbol substitution test, *TMT* Trail Making Test

3.6 Statistical analysis

Mann-Whitney U and Kruskal-Wallis tests were used for comparisons between independent groups when the dependent variable was continuous and chi-square tests when it was categorical. Raw values of CSF protein levels (**Paper I** and **Paper III**) were naturally log-transformed to make distribution conform to normality. Raw values of CSF measures in **Paper II** (m/z features and proteins) were both log2-transformed and autoscaled. Autoscaling (value subtracted by mean and divided by standard deviation) lead to each measure having average and standard deviation of 0 and 1, respectively. In **Paper I**, calculations for composite scores were done by converting the scores of each neuropsychological tests into z-scores, averaging them for each cognitive domain and subsequently converting them again into z-scores. Z-scores for tests based on time of reaction were reversed (TMT, Stroop test, DSST) before the calculations of composite scores for the purpose of score consistency (higher scores equalizing better performance).

In **Paper II**, the sample was divided into discovery and validation sets for the purpose of selecting the m/z features best distinguishing between CSF profiles (AD and non-AD). A bootstrap sampling method (Efron & Gong, 1983) was utilized for creating the sets. For the creation of a discovery set, a random sample was drawn with replacement (n=60) from the original one (n=60). On average, a bootstrap sample includes approximately 63% of the original observations (Chernick & Labudde, 2011). The discovery set, therefore, included on average 38 subjects (63%), while the rest of the subjects (n=22) made up the validation set (37%). Comparisons of the levels of each m/z feature between the two profile groups were done by the use of Mann-Whitney U tests. The comparison

s were performed for both discovery and validation sets, with the significance level set at 0.05. The whole procedure (creation of sets and comparisons of feature levels between groups) was repeated 1000 times with the goal of increasing the stability of feature selection. The m/z features most often distinguishing between profile groups within both sets at each repetition, were selected for a more precise identification. The selection of the optimal percentage as a cut-off point was based on the distribution of significant m/z features after 1000 bootstrap replications. The cut-off point of 20% was chosen, as frequencies of m/z features started to slowly increase below that point.

The area under curve (AUC) method (DeLong et al., 1988) is used in **Paper I** for evaluating the ability of CSF protein levels and outcomes on neuropsychological tests to discriminate between the two CSF profiles. AUC values range from 0 to 1, where 0.5 corresponds to distinguishability being equal to random chance and 1 to perfect.

Pearson's correlations and linear regression models were applied for estimation of relationships between continuous variables. In **Papers I** and **III**, least absolute shrinkage and selection operator (LASSO) regression (Tibshirani, 1996) was utilized as it is especially useful when dealing with highly correlated predictors (multicollinearity). Large variance due to multicollinearity is reduced by introducing a penalty, in exhange for a bias of a tolerable amount. This regression method also performs a variable selection as some coefficients shrink towards zero. Stable selection was performed in combination with LASSO regression, by fitting the model on subsamples drawn from it instead of the whole sample. This approach is advantageous, as resampling the data reduces the danger of overfitting when handling small sample size. The R package glmnet and stabs were used to perform LASSO model fitting and stable selection (Hofner et al., 2015; Meinshausen & Bühlmann, 2010). A cut-off value of 75% (the frequency of a variable selected into a model) and a per-family error rate (PFER) of 1 were set for stable selection. Number of subsamples were drawn from the original sample (n=100), each containing half the size of the original one. Scatter plots were used for the display of relationships between continuous variables. Before the calculations of Pearson's r coefficients in **Paper I**, composite scores for cognitive domains were adjusted for age and education. A linear regression model was created for each cognitive domain, with age and education as independent variables and composite z-score as the dependent variable. Subsequently, the residual (observed minus predicted score) for each subject was calculated.

The aim of the study was on discovering relationships, rather than confirmation. Therefore, values of significance were not adjusted for multiple comparisons. The statistical sofware R was used for all statistical analyses (version 3.6.1, The R Foundation for Statistical Computing).

3.7 Ethical approval

All procedures performed in The Icelandic MCI study were approved by the National Research Ethics Committee of Iceland (VSN-14-028). Written, informed consent was obtained from all subjects. The study was conducted in accordance with the current version of Helsinki Declaration (published in 2013).

4 Results and discussion

4.1 Accuracy of inflammatory and neurodegeneration CSF markers in distinguishing between CSF profiles

The first objective of **Paper I** was to evaluate the ability of CSF inflammatory (YKL-40, S100B, GFAP) and neurodegeneration (NFL) markers to distinguish between CSF AD (n=28) and non-AD (n=24) profiles. Accuracies for differentiating between the two CSF profiles were based on univariable ROC analyses. Results showed discrimination abilities for the novel CSF markers GFAP (AUC=0.64, CI=0.48-0.79), YKL-40 (AUC=0.63, CI=0.47-0.78), NFL (AUC=0.62, CI=0.45-0.78) and S100B (AUC=0.61, CI=0.46-0.77) to be poor (AUC < 0.70). For comparison, the discrimination ability of neuropsychological tests reflecting five different cognitive domains (verbal episodic memory, non-verbal episodic memory, language, processing speed and executive functions), were also calculated. Out of all measurements (CSF and tests), the tests reflecting verbal episodic memory (composite score, RAVLT immediate recall, delayed recall and recognition, Story immediate and delayed recall) distinguished the best between the profiles (AUC > 0.70). The highest AUCs were detected for the composite score (AUC=0.80, CI=0.69-0.92) and RAVLT delayed recall (AUC=0.80, CI=0.68-0.93).

LASSO logistic regression was also performed for the selection of a set of variables best distinguishing between the two CSF profiles. The four novel CSF markers and the five composite tests reflecting each cognitive domain were eligible for the selection. Stability selection was applied, by fitting the model on 100 subsamples drawn from the whole sample. The cut-off value for selecting a predictor was set at 75% (number of times a variable is selected into the model). Only the composite test for verbal episodic memory reached this criteria, selected 96 times out of 100 (96%) into the model as a predictor. The selection frequency for all other possible predictors was much lower (≤ 20%).

Our results indicated that the inflammatory (YKL-40, S100B, GFAP) and neurodegeneration (NFL) markers performed poorly in differentiating between the CSF AD and non-AD profiles. In comparison to both CSF markers and all cognitive measures, neuropsychological tests reflecting verbal episodic memory did show the highest accuracy in distinguishing

between the different CSF profiles. These results are in accordance with previous studies. A meta-analysis (Weissberger et al., 2017) reported that tests reflecting immediate and delayed memory did consistently differentiate with high accuracy (above 80%) between AD and healthy control groups, especially those that were based on list recall. It is likely that the slighty higher accuracy is due to these studies being based on clinical diagnosis of AD, while our study focused on a CSF profile reflecting AD pathology, independent of diagnosis.

4.2 Associations between CSF markers reflecting different AD related processess

The second objective of **Paper I** was to examine the relationships between CSF markers and measures reflecting different AD related processes. Figure 7 presents a Pearson's r correlation matrix between the CSF markers, age and length of education. Biomarkers NFL, T-tau, YKL-40 and S100B all positively correlated with each other. Of the CSF markers, GFAP only correlated significantly with S100B ($r = 0.53$, $p < 0.001$). None of the variables correlated significantly ($p < 0.05$) with $A\beta_{42}$. Positive, significant correlations were found between age and CSF markers, with the exception of $A\beta_{42}$.

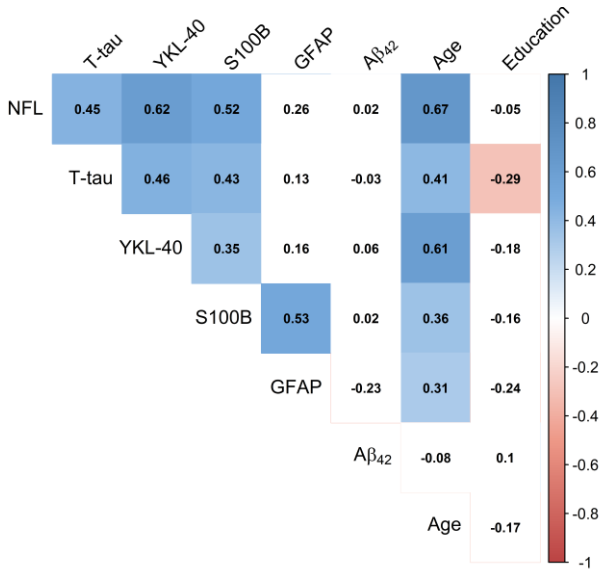

Figure 7. Correlation matrix of CSF markers and demographic variables. The numbers inside the squares present Pearson's r correlation coefficient. A colored square presents a statistical significance ($p < 0.05$). Values of CSF markers were natural log-transformed. Reprinted from (Teitsdottir et al., 2020) with permission from Alzheimer's Research & Therapy.

In our study, both NFL and YKL-40 levels correlated with T-tau, a neurodegeneration marker, but not with $A\beta_{42}$, an indicator of amyloid plaques in the brain. These results further point to these proteins reflecting biological processes independent of Aβ pathology (Alcolea et al., 2015a; Gangishetti et al., 2018; Mattsson et al., 2016; Olsson et al., 2019; Pereira et al., 2020). In comparison to NFL and YKL-40, CSF markers S100B and GFAP have much less been studied in relation to core AD markers. Our results showed S100B strongly associating with CSF levels of NFL and T-tau, but not with $A\beta_{42}$. Two previous studies (Christl et al., 2019; Hov et al., 2017) have also reported no relationship between S100B levels and $A\beta_{42}$ levels. Results regarding the association between S100B and neurodegeneration markers have though not been as consistent. One of the studies (Hov et al., 2017), conducted among elective surgery patients free from dementia and delirium, reported association with P-tau, while the other (Christl et al., 2019), conducted among AD patients and healthy controls, did not find any relationship with tau. In contrast with our results for the other novel markers, GFAP did neither correlate with the CSF levels of T-tau nor $A\beta_{42}$. This is in accordance with the results from another study (Ishiki et al., 2016) based on a sample comprised of dementia cases and healthy controls.

Overall, CSF NFL, YKL-40, S100B, and GFAP all performed poorly in differentiating between the CSF AD and non-AD profiles and did not correlate with CSF $A\beta_{42}$ levels. These results are in line with previous findings that have indicated markers NFL, YKL-40, S100B, and GFAP to be non-AD specific.

4.3 Selection of CSF markers best predicting outcomes on tests reflecting cognitive domains

The third objective of **Paper I** was to evaluate the association between the novel CSF markers with the composite scores reflecting each of the five cognitive domains. LASSO linear regression with a stability selection was applied for identifying a set of measures (CSF markers and demographic variables) most reliably predicting composite scores of each domain. Two LASSO regression analyses were performed for each cognitive domain. The first analysis included all subjects (n=52), and the second only those with a CSF AD profile (n=28). Selection criteria was set at 75%. The variables reaching that criteria were considered reliable predictors. The results for each cognitive domain are presented in Figure 8. Only GFAP (78%) reached the selection criteria as a reliable predictor for executive functions, when all subjects were part of the analysis (Figure 8a). Similarly, only age (95%) was selected as a predictor for non-verbal memory (Figure 8b). For both cognitive domains, no variables reached the criteria when only those with CSF AD profile were included. In contrast, GFAP (87%) and age (81%) were selected as predictors for processing speed (Figure 8c) and NFL (80%) for verbal episodic memory (Figure 8d) only among those with a CSF AD profile. No potential predictors reached the selection criteria for predictions of score reflecting language (Figure 8e).

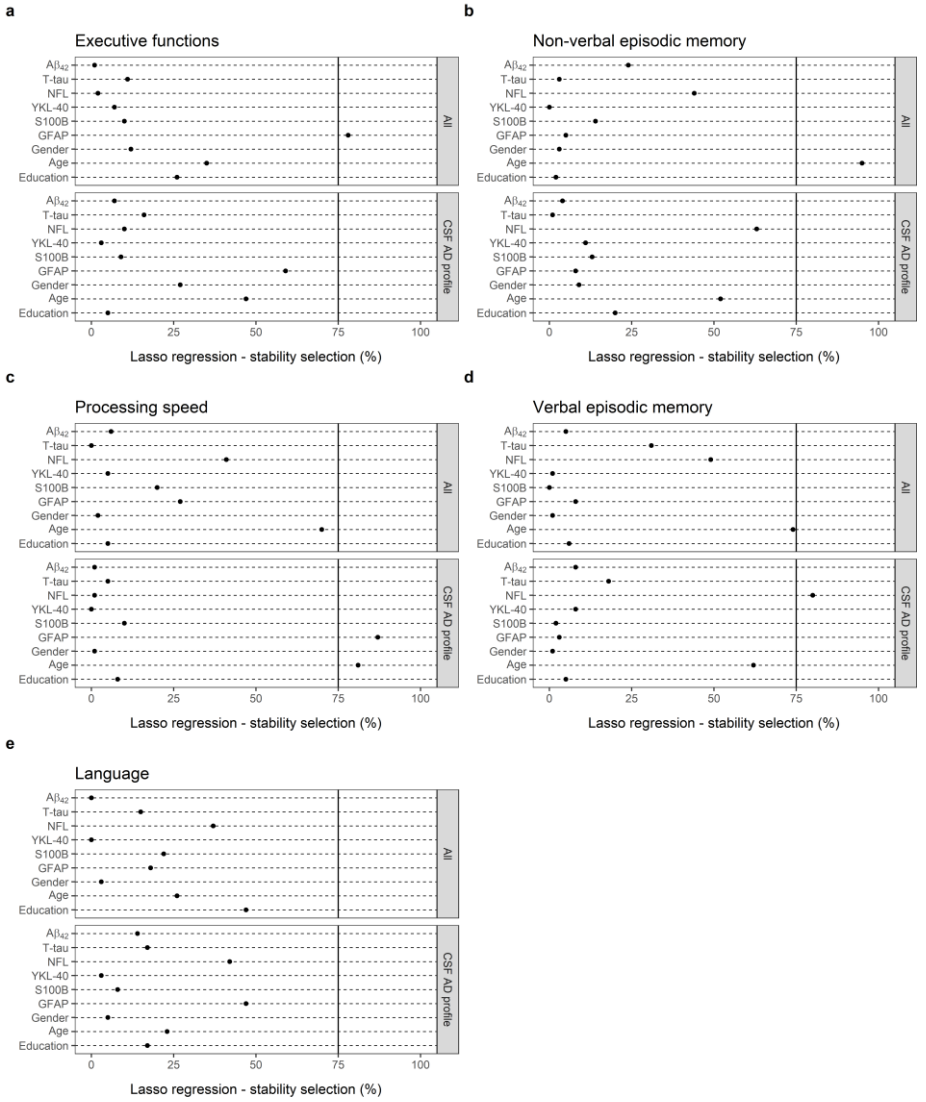

Figure 8. LASSO linear regression for the stable selection of predictors best predicting composite z-scores reflecting a) executive functions, b) non-verbal episodic memory, c) processing speed, d) verbal episodic memory, and e) language. Reprinted from (Teitsdottir et al., 2020) with permission from Alzheimer's Research & Therapy.

The associations between the CSF measures and the cognitive domains selected by the LASSO regression-stability selection method, were further explored. Pearson's r correlation analysis was performed among subjects within each profile group. It is well known that age and level of education can affect performance on cognitive tests (Ganguli et al., 2010). The composite z-scores of each cognitive domain were, therefore, adjusted for these two covariates prior to the correlation analysis. A significant correlation was detected between the levels of CSF NFL and verbal episodic memory performance among subjects with a CSF AD profile ($r = -0.43$, $p = 0.02$), but not among those with a non-AD profile ($r = -0.05$, $p = 0.82$). Similarly, a correlation was found between CSF GFAP levels and performance on processing speed among subjects with CSF AD profile ($r = -0.68$, $p < 0.001$), but not those without ($r=0.02$, $p=0.94$). Weak correlation was observed between GFAP and executive functions performance within the whole cohort ($r = -0.37$, $p = 0.01$).

Overall, the results indicate that the novel CSF markers exhibited different patterns of association with certain cognitive domains. These patterns were primarily detected among subjects with a CSF AD profile. Within that profile, higher levels of the neurodegeneration marker NFL related to worse verbal episodic memory performance while higher levels of inflammatory marker GFAP related to worse performance on processing speed. In addition, a relationship between higher levels of GFAP and worse executive functions performance was observed within the whole cohort. These results are particularly interesting as neither NFL nor GFAP did distinguish between CSF AD and non-AD profiles.

Our results, regarding the relationship between NFL and cognitive decline are in line with results from a recent review (Ramani et al., 2021). The review, based on 37 research studies, reported that higher levels of NFL associates in general with worse cognitive performance across various neurological diseases. Out of 37 articles, 16 included AD or FTD patients with different types of biofluid samples (serum, plasma, CSF). NFL consistently associated with worse cognitive functions, regardless of the type of biofluid collected. In majority of the studies, the relationship between the protein and global cognition was estimated, by the use of MMSE. Only four of the studies evaluated NFL levels in relation to different cognitive domains in AD. The results of these studies indicated associations between the protein with various domains including memory, language, speed processing and executive functions. As well as correlating with cognitive decline, studies have shown NFL to associate with brain atrophy, strengthening the case for the protein as a progression marker. Higher CSF levels of NFL correlated

with lower hippocampal and whole-brain volume as well as larger ventricular volume over time in individuals with MCI (Zetterberg et al., 2016). Association of NFL with hippocampal and ventricular volumes has also been reported in a cohort (controls, MCI and AD), regardless of Aβ load status in the brain (Mattsson et al., 2016).

T-tau, a neurodegeneration marker like NFL, did not reach criteria as a reliable predictor for any of the cognitive domains. This is not surprising, considering that T-Tau levels reach plateau in the early stages of the disease and is therefore not a great marker of disease progression (Jack et al., 2010). A recent in vivo study by Pereira et al. (Pereira et al., 2020) reported that amyloid-related synaptic changes occurred first in AD, followed by tau-related axonal degeneration. Indeed, their results suggested tau to be contributor to increasing NfL levels. This further implicates that axonal degeneration, indirectly measured by CSF NFL levels, is a downstream event of tau pathology. This hypothetical cascade of events is also supported by findings from animal studies (Koffie et al., 2009; Spires-Jones & Hyman, 2014; Zempel et al., 2010). Aβ plaques are thought to induce reduction in number of postsynaptic spines and presynaptic vesicles. As synaptic alterations become more prominent, tau becomes hyperphosphorylated, dissociates from microtubules and assembles into NFTs. Furthermore, this structural change is followed by neurofilament dissociation from microtubules, axonal degeneration and finally, cognitive decline.

Very few studies have evaluated the relationship between GFAP levels and cognitive decline in CSF. Two studies tested the relationship between CSF GFAP levels and MMSE scores, one among AD patients (Darreh-Shori et al., 2013) and the other among dementia patients and healthy controls (Ishiki et al., 2016). Unlike our study, neither observed a relationship between the protein and cognitive decline. A possible explanation is that we evaluated different cognitive domains, not only global cognition. In comparison to CSF, the relationship between GFAP and AD has been better explored in blood. The relationships between the protein and cognitive domains have, for example, been examined in serum. Verberk et al. (Verberk et al., 2021) found baseline serum GFAP levels to be related to greater rate of decline in memory, processing speed and executive functions in a memory clinic cohort presenting cognitively normal individuals. These results are in accordance with our results, as we found an association between higher levels og GFAP and processing speed. Higher serum baseline GFAP levels have also been associated with faster decline in hippocampal volume and cortical thickness in a cohort of older individuals (Rajan et al., 2020), further indicating the potential of the protein as a progression marker.

4.4 Selection of lipid species best distinguishing between CSF profiles

An untargeted lipidomic analysis was performed on CSF samples from subjects in **Paper II**, with the purpose of selecting those lipid species best differentiating between CSF AD and non-AD profiles. Mann-Whitney U non-parametric tests were used for the selection of the m/z features best discriminating between the two CSF profiles (AD and non-AD). Eight out of 1008 features did significantly differ in levels between the two profiles within both the discovery and validation sets in 20% (200 of 1000) of the boostrap replicates. Vast majority of the features (n=969) reached significance in less than 2% of the replicates. Each of the eight features was assigned a lipid species annotation by the use of the LipidBlast spectral library. The chemical structure of seven out of the eight annotated lipid species could not clearly be confirmed based on comparison of MS/MS fragmentation spectra to the LipidBlast reference spectra. In contrast, the structure of C18 ceramide was confirmed by a comparison to a reference standard. Two reference standards were run, with one being C18 ceramide (d18:1/18:0). Comparison of CSF C18 ceramide levels between different CSF profiles are presented in Figure 9. Levels were elevated among those with a CSF AD profile compared to those with a non-AD profile (p=0.002).

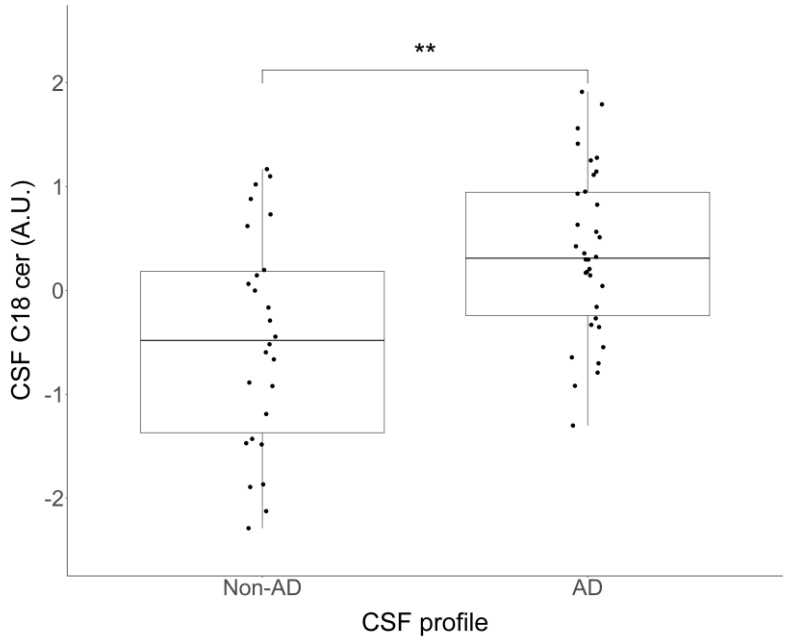

Figure 9. Levels of CSF C18 ceramide compared between different CSF profiles (non-AD and AD). **p<0.01 significance by use of Mann-Whitney U non-parametric test. Values were converted to arbitrary units (A.U.), log2-transformed and autoscaled. Reprinted from (Teitsdottir et al., 2021) with permission from Journal of Alzheimer's Disease.

This is, to the best of my knowledge, the first time higher levels of C18 ceramide have been associated with CSF profile reflecting signature AD pathology. Very few studies have been conducted comparing CSF ceramide levels between AD patients and healthy controls or other disease groups. A study by Satoi et al. (Satoi et al., 2005) found a significantly higher levels of ceramide among AD patients compared to patients with other neurological diseases. Fonteh et al. (Fonteh et al., 2015) also found a slightly higher levels of ceramides in supernatant CSF fluid among AD compared to MCI and controls, although the difference did not reach significance.

4.5 Association between selected lipid species and AD related CSF markers

The second aim of **Paper II** was to examine the relationship of CSF markers reflecting signature AD pathology ($A\beta_{42}$, T-tau, P-tau), inflammation (YKL-40, S100B, GFAP) and neurodegeneration (NFL) with CSF C18 ceramide. The estimations were performed using linear regression (Table 3). Unadjusted

standardized beta (st. β) coefficients were calculated as well as adjusted for covariates age, gender and education. After adjustment of covariates, a negative relationship was found between $A\beta_{42}$ and C18 ceramide levels (st. β=-0.36, *p*=0.007) while a positive relationship was found between levels of T-tau and the same lipid species (st. β=0.41, *p*=0.005). No statistically significant association was found between either levels of P-tau or NFL and C18 ceramide. Of the inflammatory markers, C18 ceramide only positively related to S100B (st. β=0.51, *p*=0.001) after adjustment of covariates. The same CSF markers that significantly associated with C18 ceramide, also did so when linear regression was not adjusted for covariates, although the associations were not as strong. Test scores reflecting verbal episodic memory (RAVLT, Story) did not significantly relate to levels of C18 ceramide. As one subject did not take the RAVLT test, the linear regression analyses for the subtests were only based on 59 subjects. This did though not skew results as the outcome was approximately the same when calculated without that particular subject.

Table 3. Linear regression models, unadjusted and adjusted for covariates, examining the association of CSF markers and cognitive scores with levels of CSF C18 ceramide. Reprinted and modified from (Teitsdottir et al., 2021) with permission from Journal of Alzheimer's Disease.

	CSF C18 ceramide (A.U.)[a]			
	Unadjusted model		Adjusted model*	
	St. β	*p*	St. β	*p*
Demographics				
Age, yrs	-0.02	0.902	-0.05	0.765
Education, yrs	-0.05	0.688	-0.07	0.596
CSF proteins[a]				
$A\beta_{42}$ (pg/ml)	-0.35	0.006	-0.36	0.007
T-tau (pg/ml)	0.30	0.018	0.41	0.005
P-tau (pg/ml)	0.17	0.188	0.21	0.129
T-tau/$A\beta_{42}$	0.44	<0.001	0.52	<0.001
NFL (ng/ml)	0.03	0.835	0.05	0.782
YKL-40 (ng/ml)	0.11	0.411	0.25	0.194
S100B (pg/ml)	0.37	0.004	0.51	0.001
GFAP (ng/ml)	0.22	0.087	0.26	0.087
Verbal episodic memory				
Composite z-score[b]	-0.18	0.172	-0.28	0.120
RAVLT immediate recall, score[b]	-0.02	0.893	-0.01	0.928
RAVLT delayed recall, score[b]	-0.18	0.339	-0.29	0.095
RAVLT recognition-fp, score[b]	-0.16	0.240	-0.20	0.223
Story immediate recall, score	-0.13	0.356	-0.14	0.345
Story delayed recall, score	-0.13	0.305	-0.15	0.335

Abbreviations: *A.U.* Arbitrary units, *RAVLT* Rey Auditory Verbal Learning Test, *St.β* Standardized beta coefficients. *Adjusted for age, gender and years of education. [a]Values log2-transformed and autoscaled. [b]Analysis was only based 59 subjects, with one subject not having a score

The statistically significant relationships between CSF markers and C18 ceramide are presented in Figure 10. Relationships between the CSF markers with C18 ceramide were also evaluated within each CSF profile group by calculations of Pearson's coefficients, but none reached significance ($p>0.05$).

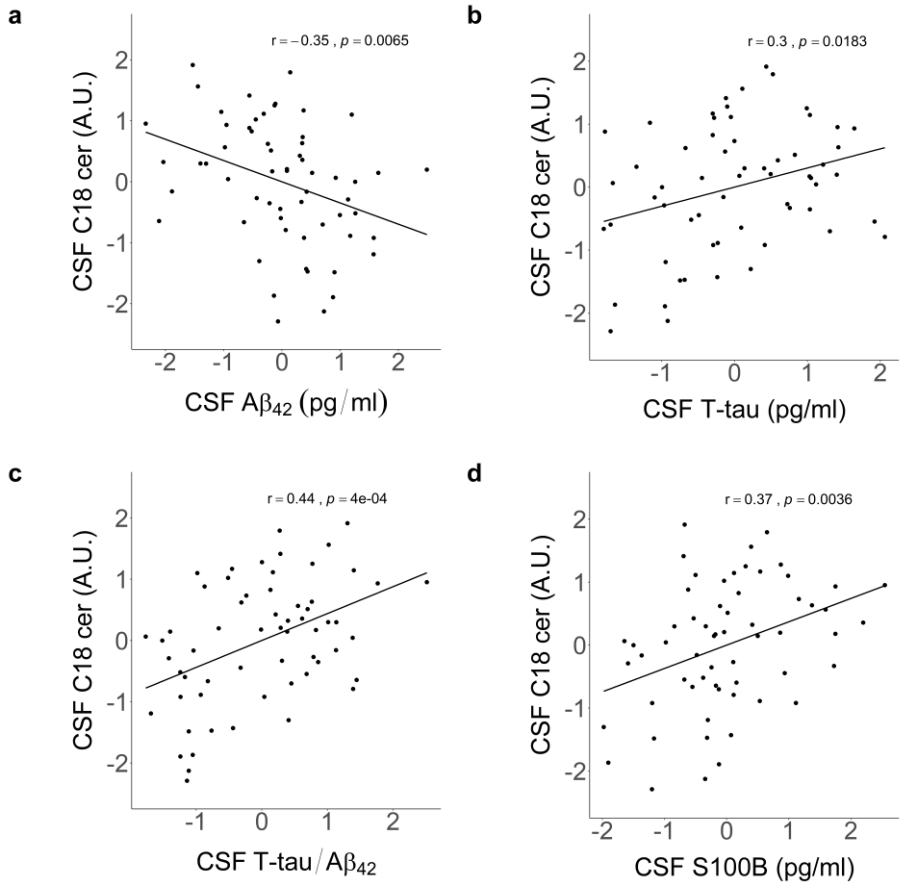

Figure 10. Scatter plots presenting Pearson's correlations between CSF levels of a) $A\beta_{42}$, b) T-tau, c) T-tau/$A\beta_{42}$, d) S100B and CSF C18 ceramide. All CSF values were log2-transformed and autoscaled. Reprinted from (Teitsdottir et al., 2021) with permission from Journal of Alzheimer's Disease.

The potential of ceramide species as CSF biomarkers in AD has not been much researched, including their association with core markers. One study by Mielke et al. (Mielke et al., 2014), examined those relationships in a cohort of cognitively healthy individuals aged 36–69 years (n=91) with a parental history of AD. In that study, C18 ceramide correlated positively with all measured Aβ species, except for $A\beta_{42}$. In contrast, our study showed that higher levels of C18 ceramide associated with lower levels of $A\beta_{42}$. This could possibly be explained by difference in sample composition. Our sample included subjects at the symptomatic pre- or early stages of dementia and the higher correlation between $A\beta_{42}$ and C18 ceramide could be due to progression of disease severity. Our results also showed higher levels of the

lipid species associating with higher levels of T-tau, but not with P-tau. This was in accordance with the results from the study by Mielke et al (2014). Interestingly, these collective results are in contrast with results from cellular studies, which have found a relationship between C18 ceramide and P-tau (Chalfant et al., 1999; Dobrowsky et al., 1993; Goedert et al., 1995; Gong et al., 1994; Mukhopadhyay et al., 2009).

Surprisingly, we did not detect a relationship between C18 ceramide and NFL levels. NFL is a marker of both axonal degeneration and white matter changes, while the precursor of ceramide, SM, is abundant in the myelin sheath of neurons. Our hypothesis is that C18 ceramide could be a marker of apoptosis, rather than axonal degeneration. Previous studies suggest that ceramides could play a role in neuronal apoptosis, acting as second messenger in activating the apoptotic cascade, ultimately leading to neuronal death (Jazvinšćak Jembrek et al., 2015).

We found a positive relationship between the levels of the lipid species and S100B. In comparison, we did not detect association between C18 ceramide and YKL-40 or GFAP, which are also inflammatory markers. One possible explanation could be that both ceramides and S100B have been associated with the induction of neuronal apoptosis (Donato et al., 2009; Jazvinšćak Jembrek et al., 2015). At high concentrations, extracellular S100B can contribute to neuronal death through activation of RAGE. Excessive stimulation of neurons expressing RAGE causes hyperactivation of the Ras/MEK/ERK pathway, which subsequently can lead to apoptosis (Fanò et al., 1993; Huttunen et al., 2000). S100B can also contribute to neuronal death through activation of RAGE as it can lead to release of nitric oxide by microglia and astrocytes (Adami et al., 2001; Hu et al., 1997).

The relationship between ceramides and cognitive functions have not been much researched, especially not in CSF. We did not observe an association between C18 ceramide and verbal memory performance, which is in accordance with results from the previously mentioned study by Mielke et al. (2014). They, on the other hand, found a relationship between longer carbon chain species (C20, C22, and C26) and the same cognitive domain. The relationship between ceramide species and cognitive functions have been better explored in blood. Previous studies have suggested that higher levels of long ceramide species (C14 – C24) in plasma or serum relate to worse memory in health (Mielke et al., 2010a), MCI (Mielke et al., 2010b) and in diseases including Parkinson's disease dementia (PDD) (Xing et al., 2016) and coronary artery disease (CAD) (Chan et al., 2018; Saleem et al., 2013). Only the research by Chan et al. (Chan et al., 2018), observed an association between plasma C18 ceramide and memory.

4.6 Associations between activity of cholinergic enzymes and AD related CSF markers

The first objective of **Paper III** was to evaluate the relationships between the activity of the ACh-degrading enzymes of the cholinergic system (AChE and BuChE) and CSF markers reflecting the state of brain amyloidosis, neurodegeneration and inflammation. Three different biomarker profiles were created for each core CSF AD marker ($A\beta_{42}$, T-tau, P-tau). Subjects were divided into two categories, reflecting either abnormal (positive; +) or normal (negative; -) protein levels.

No differences were observed in AChE (p=0.35) or BuChE activity (p=0.92) between the $A\beta_{42}$ based profile groups. In contrast, AChE activity was higher among subjects with T-tau+ (p=0.008) or P-tau+ profiles compared to those without. No differences were detected between T-tau (p=0.11) or P-tau profiles regarding BuChE activity. When subjects were divided into CSF AD and non-AD profile groups, a slightly higher levels were observed amond the AD group (p=0.03). No statistical difference was found between the profile groups regarding BuChE levels (p=0.52). Pearsons‘r correlations were also calculated for the estimation of associations between the CSF markers of amyloidosis, neurodegeneration, inflammation and activity of the cholinergic enzymes (Figure 11). T-tau (r=0.46, p=0.001) and P-tau (r=0.45, p=0.002) levels positively corelated with AChE activity. Inflammatory markers S100B (r=0.43, p=0.003) and YKL-40 (r=0.32, p=0.03) showed a postive association with activity of AChE. Similarly, both S100B (r=0.47, p<0.001) and YKL-40 (r=0.38, p=0.009) positively related to BuChE activity.

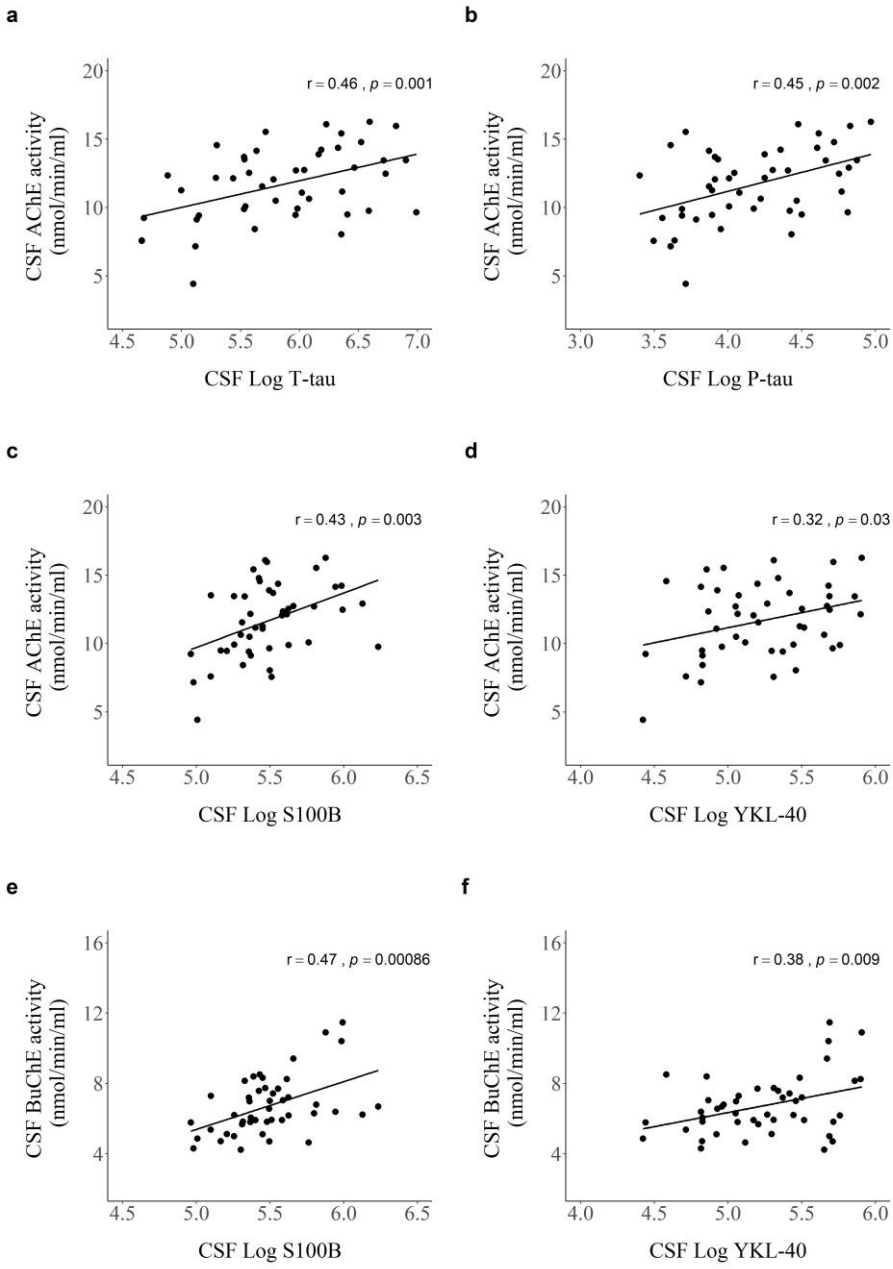

Figure 11. Scatter plots presenting Pearson's correlations between levels of AChE activity and a) T-tau, b) P-tau, c) S100B, d) YKL-40 and BuChE activity and levels of e) S100B and f) YKL-40 in CSF. Values of CSF proteins (T-tau, P-tau, YKL-40 and S100B) were natural log-transformed.

LASSO linear regression with a stability selection was employed for the selection of variables best predicting the activity of each cholinergic enzyme (Figure 12). Variables which were selected more than 75% of the time into the regression model, were considered reliable as predictors. The potential predictors included the seven CSF markers ($A\beta_{42}$, T-tau, P-tau, NFL, YKL-40, S100B, GFAP) as well as gender, age and length of education. None of the variables reached the selection criteria for either AChE (Figure 12a) nor BuChE activity (Figure 12b). Nonetheless, S100B was the CSF marker with the highest selection frequency, both for prediction of AChE (68%) and BuChE (73%) activity. The variables with the next highest selection frequency for the prediction of AChE activity, were education (68%), T-tau (45%) and P-tau (40%). All other variables had frequency below 25%. For BuChE, the next potential predictors were gender (64%), YKL-40 (61%) and education (38%). All other variables had a selection frequency below 15%.

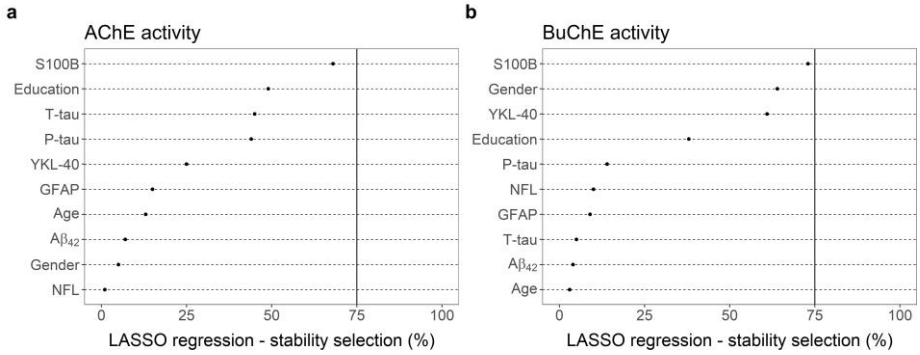

Figure 12. LASSO linear regression—stability selection analyses for prediction of CSF enzyme activities of a) AChE and b) BuChE. The cut-off selection value was set at 75% and the per-family error rate (PFER) at 1.

Results from previous studies examining the relationship between $A\beta_{42}$ levels and AChE activity in CSF have been inconsistent. An association between $A\beta_{42}$ levels and AChE activity was not observed here, which is in accordance with some (Darreh-Shori et al., 2006; Johansson et al., 2013), but not all (García-Ayllón et al., 2008) studies. In comparison, a clearer relationship has been established between Aβ pathology and cholinergic abnormalities through in vitro and post-mortem studies. AChE activity has been found to be decreased in the AD brain, although the activity is frequently increased in regions around Aβ plaques and NFTs (Mesulam & Asuncion Morán, 1987; Perry et al., 1980; Ulrich et al., 1990). Furthermore, studies have revealed that ACh synthesis is reduced by 40-50% in cultured

cholinergic neurons when exposed to high nanomolar concentration of $A\beta_{42}$ (Hoshi et al., 1997; Kar et al., 1998; Nunes-Tavares et al., 2012; Pedersen et al., 1996). As with AChE, we did not detect relationship between BuChE activity and $A\beta_{42}$ levels. Those results are in accordance with previous studies (Darreh-Shori et al., 2006; Gabriel et al., 2017). In summary, more research is needed for a better understanding the interactions between Aβ and the cholinergic system, especially in vivo as the biochemical environment is very complex.

Very few studies have evaluated the relationship between tau levels and cholinesterase activity in CSF. Our study did find an association between either T-tau or P-tau and AChE activity. The results are in line with findings from a previous study (Johansson et al., 2013), where a positive relationship was also found between levels of tau and AChE activity in CSF among dementia patients and healthy controls. Furthermore, these results are in line with post-mortem and animal studies. NFTs in the NBM of the basal forebrain have been associated with cholinergic neuronal loss (Braak & Del Tredici, 2013; Geula & Mesulam, 1995; Mesulam, 2013). P-tau is also thought to play a role in the regulation of AChE expression (Silveyra et al., 2012). For example, over-expression of mutant P-tau in the brains in transgenic mice led to increased AChE activity, suggesting that abnormally high tau phosphorylation was the cause of increased AChE expression around NFTs. In contrast, we did not find a relationship between tau and BuChE activity. That is in accordance with a study by Gabriel et al. (Gabriel et al., 2017), which did not detect a relationship within a group of AD patients. The difference in our results between the two enzymes could possibly be due to difference in localizations. Tau is mainly expressed in neurons and AChE is predominantly anchored to neuronal synapses, while BuChE is mainly secreted by glial cells as a soluble enzyme (Giacobini, 2003; Wright et al., 1993). Surprisingly, an association was not found between NFL, a marker of axonal degeneration, and the cholinergic enzymes. NFL has, to the best of my knowledge, not been studied in regards to cholinergic activity in CSF samples from a memory cohort.

The neurotransmitter ACh can also affect regions more distal from the synaptic cleft and is therefore not only important to neurons (Nizri et al., 2006). Through the activation of α7 nicotinic ACh receptors, expressed on glial cells like astrocytes and microglia, the neurotransmitter inhibits pro-inflammatory responses (Nizri et al., 2006). Although both AChE and BuChE are ACh-degrading enzymes, BuChE plays specifically important role in function of glial cells (Lane & He, 2013). CSF BuChE activity has been found

to associate positively with CSF S100B and GFAP in AD (Darreh-Shori et al., 2013). This could possibly be explained by lowering levels of extracellular ACh associating with higher BuChE activity, enhancing the reactivity of glia. This process could have a protective role in the beginning, but with a prolonged activation, could gradually lead to neuronal dysfunction and loss, further eliciting inflammatory responses (Lane & Darreh-Shori, 2015). In the same study, no association was found between activity of AChE and levels of S100B and GFAP. In our study, higher levels of S100B, and to a lesser extent YKL-40, related to higher activity of both cholinergic enzymes. YKL-40, as to the best of my knowledge, has not been evaluted in relation to cholinergic enzymes. Interestingly, in this study no relationship was detected between GFAP levels and the cholinergic enzymes. The different relationships between the enzymes and the inflammatory markers could possibly be explained by different cellular functions. GFAP (Yang & Wang, 2015) is an intracellular protein while both YKL-40 (Bonneh-Barkay et al., 2012) and S100B (Donato et al., 2009) can be secreted into the extracellular matrix in response to glial activation. Increase in levels of CSF GFAP could possibly take place at later stages in the pathogenic cascade.

4.7 Associations between CSF cholinergic enzymes and verbal episodic memory

The second objective of **Paper III** was to evaluate the relationship between the activity of the cholinergic enzymes with a composite z-score reflecting verbal episodic memory functions. As two subjects did not take the RAVLT test, analysis was based on 44 instead of 46 subjects.

A weak, negative correlation was detected between CSF AChE activity and the verbal episodic memory score (r=-0.34, p=0.02). No significant relationship was found between BuChE activity and the same score (r=-0.19, p=0.21).

LASSO linear regression with a stability selection was performed for the identification of a set of variables predicting the performance on verbal episodic memory (Figure 13). The potential predictors included the CSF markers (AChE, BuChE, $A\beta_{42}$, P-tau, T-tau, NFL, YKL-40, S100B, GFAP) and demographic measures (gender, age, length of education). Age was the only variable to reach the selection criteria, with frequency of 91%. Neurodegeneration markers NFL (61%) and P-tau (55%) were selected into the model more than half of the time. All other measures were selected much less frequently (<30%), with both AChE (8%) and BuChE (0%) having selection frequency below 10%.

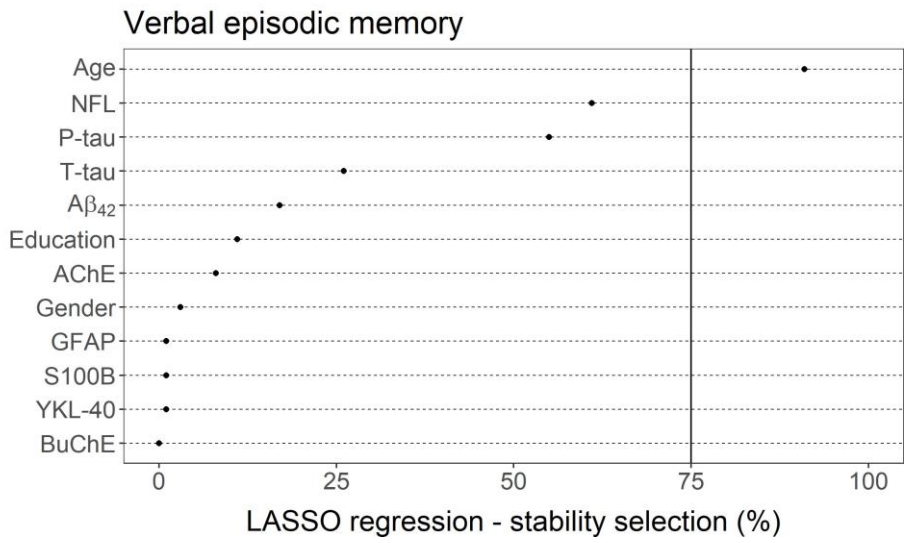

Figure 13. LASSO linear regression—stability selection analysis for the assocation between various measures, including cholinergic enzymes, and verbal episodic memory performance. The cut-off selection value was set at 75% and the per-family error rate (PFER) at 1.

It is unknown how structural and functional changes in the cholinergic system relates to memory function at the symptomatic pre-dementia stages (SCI or MCI) (Peter et al., 2016). At the stages of moderate to advanced AD, severe neurofibrillary degeneration and loss takes place within the basal forebrain, particularly in the NBM. In contrast, more subtle changes have been observed at the MCI stage, characterized by alterations in function rather than loss of cholinergic neurons (Schliebs & Arendt, 2011). In line with results from this study, histological studies have found that at least 30% of cholinergic neurons in the basal forebrain have deteriorated before cognitive impairment is apparent (Schliebs & Arendt, 2006) and at the MCI stage only around 15% loss in volume is detected compared to healthy individuals (Peter et al., 2016). The results of our study are in accordance with those findings as our sample included subjects at the pre- or early stages of dementia and AChE activity only weakly associated with score on verbal episodic memory. When other factors were simultaenously considered, neither AChE nor BuChE proved to be a reliable predictor for the function of verbal episodic memory. Previous research has shown higher levels in NFL to be associated with worse cognitive functions in both AD and other neurodegenerative disease (Olsson et al., 2019; Zetterberg et al., 2016). In this study, NFL did not correlate with cholinergic enzymes, showcasing further the lack of the cholinergic system in contributing to memory impairment in the early stages of dementia.

4.8 Strengths and limitations

A strong aspect of this study was the use of biomarkers for the definition of AD. Clinical diagnosis was partly based on neuropsychological battery, meaning that an intrinsic relationship existed between the two factors. The use of biomarker-based profiles was therefore essential in exploring the relationships of novel biomarkers with AD pathology and cognitive functions separately. Another strength was the cognitive evaluation of each subject by the use of an extensive neuropsychological battery. Cognitive impairment is commonly evaluated by brief, cognitive screening tools like the MMSE test. In contrast, the focus of this project was on the evaluations of different cognitive domains. Each cognitive domain was based on scores from two or more subtests, increasing the range of possible scores and decreasing the likelihood of skewed results based on ceiling or floor effects. Third, various analytes, reflecting different biological processes, were measured in the same CSF samples. Finally, the subjects all came from a clinical setting cohort. This gave realistic information based on a relatively heterogeneous memory clinic population.

There are several limitations to the project. The sample size was relatively small for all studies, and hence, the present findings need to be validated in a larger study. This was specifically a limitation for **Paper II** as a vast number of features were compared to the number of subjects, resulting in decreased statistical power and a higher risk of type-II errors. Multiple comparisons corrections were, therefore, not performed for comparing m/z feature levels between profile groups. Instead, the procedure of creating discovery and validation sets was repeated 1000 times, for the purpose of enhancing the robustness of feature selection. Furthermore, a univariable analysis (Mann-Whitney U test) for the selection of features was used as the sample was relatively small. The validation sets only included about 22 subjects, and therefore it was not possible to have more than one independent variable (lipid species) in the statistical model. With a bigger sample, it would be possible to use more advanced statistical methods (e.g. multivariable LASSO regression) for the detection of disease-specific lipid patterns.

The small sample size also affected the inclusion of data regarding cognitive domains. The main aim of **Paper II** was feature selection, and with features outnumbering subjects, it was of utmost importance to utilize all available data. Almost a full record of scores for subtests reflecting verbal episodic memory existed, but subtests reflecting other cognitive domains contained more missing values. Therefore, information about those cognitive

domains was not included in the analyses, as it would have decreased the number of available subjects. The same limitation also applied for **Paper III**. The sample size was smaller compared to the two other papers, due to cholinergic enzyme activity not being measured in samples of all enrolled subjects (Figure 6). For the purpose of not losing more subjects, only information about verbal episodic memory was included.

This cross-sectional analysis would certainly have benefited from having a healthy control group. Inclusion of healthy individuals could have increased the range of values for each CSF marker, and in turn, lead to a better estimation between variables. The lack of a healthy control group in this study is though, in my view, not a major limitation as the primary aims were to explore relationships between different variables in a clinical setting. Second, the project did not include subjects with moderate to severe AD as well as having very few subjects with other types of dementias. A more diverse cohort would have given the opportunity to explore better how the CSF markers related to disease progression, both in AD and other dementias.

No genetic data exists for The Icelandic MCI study. This type of data would have given the chance to explore relationships between different measures (e.g. different biomarkers or biomarkers and cognitive domains) by genotype. Information about APOE and BCHE genotypes would have been of particular interest. Studies have consistently found the APOE Ɛ4 allele to be associated with lower CSF levels of $A\beta_{42}$ in a dosage-dependent manner (Lautner et al., 2017; Mehrabian et al., 2015). Studies have also revealed reduction in BuChE activity among individuals carrying both the BCHE-K and the APOE Ɛ4 alleles (Lane & Darreh-Shori, 2015).

The CSF samples were not centrifuged. According to a recent consensus update from The World Federation of Societies of Biological Psychiatry (WFSP) (Lewczuk et al., 2018), centrifugation of CSF (at 2,000 × g) for 10 min at room temperature (Reijs et al., 2015) can be considered before analysis or storage but is not essential, except when CSF sample is blood-contaminated. None of the CSF samples used for this project were visibly blood contaminated.

5 Summary and conclusion

A shift from clinical towards biological definition of AD has occurred in recent years. This is due to symptoms (e.g. memory impairment) neither being sensitive nor specific for neuropathological changes that characterize the disease. It is therefore of importance to examine the relationships between novel biomarkers with signature AD pathology and symptoms separately. Such an approach can both strengthen understanding of the underlying pathological processes of AD and the sequence of events leading to clinical expression.

The general aim of the project was to examine the relationships of selected CSF novel biomarkers with a CSF profile reflecting signature AD pathology and cognitive functions, independent of diagnosis, among subjects at the symptomatic pre- or early dementia stages. In **Paper I**, CSF biomarkers reflecting neurodegeneration (NFL) and inflammation (YKL-40, S100B and GFAP) were compared in relation to core CSF AD markers and cognitive functions. While these findings suggest that these CSF markers did not accurately differentiate between CSF profiles (AD and non-AD), they exhibited different patterns of association with tests reflecting certain cognitive domains. This pattern was mainly observed among subjects with a CSF AD profile. Within that profile, higher levels of the neurodegeneration marker NFL associated with worse performance on verbal episodic memory while higher levels of inflammatory marker GFAP associated with worse performance on processing speed. Furthermore, higher levels of GFAP associated weakly with worse performance on executive functions within the whole cohort. In **Paper II**, untargeted lipidomic analysis was applied for the identification of CSF lipid species related to signature AD profile. Of those 1008 m/z features detected, eight were selected as possible markers. Out of those eight, only one feature was fully confirmed as the lipid species C18 ceramide. The results indicated that levels of C18 ceramide were higher among subjects with a CSF AD profile compared to those with a non-AD profile. A positive association was also observed between the levels of inflammatory marker S100B and C18 ceramide. No relationship was detected between C18 ceramide and verbal episodic memory. In **Paper III**, the relationships of ACh-degrading enzymes AChE and BuChE with CSF markers reflecting different pathological processes and verbal episodic

memory were explored. The results indicated no difference in enzyme activity when subjects with abnormally low CSF $A\beta_{42}$ levels were compared to those with normal levels. In contrast, higher activity of AChE was measured amongst subjects with abnormally high T-tau or P-tau levels compared to those with normal levels. Furthermore, higher levels of inflammatory markers S100B and GFAP both related to higher AChE and BuChE activity. When other factors (CSF markers and demographic measures) were simultaneously entered into the model, S100B emerged as the most reliable predictor of the activity of both enzymes. Although AChE activity slightly correlated with worse episodic memory score, the enzyme did not prove to be a good predictor when other factors had been accounted for.

Overall, our results indicate how CSF markers, reflecting different biological processes, relate in different ways to AD pathology and cognitive functions at the pre- or early stages of dementia. First, C18 ceramide associated with both signature AD pathology and inflammation. These results suggest that ceramide metabolism could potentially contribute to AD pathology during the early symptomatic stages. Furthermore, ceramides could possibly act as therapeutic targets, with treatments aiming at decreasing ceramide synthesis to prevent or slow down Aβ pathology, neuronal loss and neuroinflammation. Second, our results showed the activity of ACh-degrading cholinergic enzymes to be related to neurodegenerative and inflammatory processes. As the cholinergic system is the main therapeautic target in the treatment of symptomatic AD, a better understanding of its key players and how they relate to both pathology and cognitive functions, is essential. Interestingly, neither C18 ceramide nor the cholinergic enzymes associated with verbal episodic memory. A possible explanation could be that changes in ceramide levels and cholinergic activity occur at the earlier stages of the disease. Finally, higher levels of NFL associated with lower score on verbal episodic memory while higher levels of GFAP associated with lower score on processing speed, among individuals with a signature AD pathology. These results indicate that the proteins could possibly be utilized as progression markers, monitoring subtle cognitive changes among individuals with AD pathology. NFL has particularly shown a promise as a robust marker of progression, both in CSF and blood, as its levels correlate with both brain atrophy and cognitive decline.

References

2016 Alzheimer's disease facts and figures. (2016). *Alzheimers Dement, 12*(4), 459-509. doi:10.1016/j.jalz.2016.03.001

Abu-Rumeileh, S., Steinacker, P., Polischi, B., Mammana, A., Bartoletti-Stella, A., Oeckl, P., Baiardi, S., Zenesini, C., Huss, A., Cortelli, P., Capellari, S., Otto, M., & Parchi, P. (2019). CSF biomarkers of neuroinflammation in distinct forms and subtypes of neurodegenerative dementia. *Alzheimer's research & therapy, 12*(1), 2. doi:10.1186/s13195-019-0562-4

Adami, C., Sorci, G., Blasi, E., Agneletti, A. L., Bistoni, F., & Donato, R. (2001). S100B expression in and effects on microglia. *Glia, 33*(2), 131-142.

Alcolea, D., Carmona-Iragui, M., Suárez-Calvet, M., Sánchez-Saudinós, M. B., Sala, I., Antón-Aguirre, S., Blesa, R., Clarimón, J., Fortea, J., & Lleó, A. (2014). Relationship between β-Secretase, inflammation and core cerebrospinal fluid biomarkers for Alzheimer's disease. *J Alzheimers Dis, 42*(1), 157-167. doi:10.3233/jad-140240

Alcolea, D., Martínez-Lage, P., Sánchez-Juan, P., Olazarán, J., Antúnez, C., Izagirre, A., Ecay-Torres, M., Estanga, A., Clerigué, M., Guisasola, M. C., Sánchez Ruiz, D., Marín Muñoz, J., Calero, M., Blesa, R., Clarimón, J., Carmona-Iragui, M., Morenas-Rodríguez, E., Rodríguez-Rodríguez, E., Vázquez Higuera, J. L., Fortea, J., & Lleó, A. (2015a). Amyloid precursor protein metabolism and inflammation markers in preclinical Alzheimer disease. *Neurology, 85*(7), 626-633. doi:10.1212/wnl.0000000000001859

Alcolea, D., Vilaplana, E., Pegueroles, J., Montal, V., Sánchez-Juan, P., González-Suárez, A., Pozueta, A., Rodríguez-Rodríguez, E., Bartrés-Faz, D., Vidal-Piñeiro, D., González-Ortiz, S., Medrano, S., Carmona-Iragui, M., Sánchez-Saudinós, M., Sala, I., Anton-Aguirre, S., Sampedro, F., Morenas-Rodríguez, E., Clarimón, J., Blesa, R., Lleó, A., & Fortea, J. (2015b). Relationship between cortical thickness and cerebrospinal fluid YKL-40 in predementia stages of Alzheimer's disease. *Neurobiol Aging, 36*(6), 2018-2023. doi:10.1016/j.neurobiolaging.2015.03.001

Alcolea, D., Vilaplana, E., Suárez-Calvet, M., Illán-Gala, I., Blesa, R., Clarimón, J., Lladó, A., Sánchez-Valle, R., Molinuevo, J. L., García-Ribas, G., Compta, Y., Martí, M. J., Piñol-Ripoll, G., Amer-Ferrer, G., Noguera, A., García-Martín, A., Fortea, J., & Lleó, A. (2017). CSF sAPPβ, YKL-40, and neurofilament light in frontotemporal lobar degeneration. *Neurology, 89*(2), 178-188. doi:10.1212/wnl.0000000000004088

Allore, R., O'Hanlon, D., Price, R., Neilson, K., Willard, H. F., Cox, D. R., Marks, A., & Dunn, R. J. (1988). Gene encoding the beta subunit of S100 protein is on chromosome 21: implications for Down syndrome. *Science, 239*(4845), 1311-1313. doi:10.1126/science.2964086

Andreasen, N., Gottfries, J., Vanmechelen, E., Vanderstichele, H., Davidson, P., Blennow, K., Rosengren, L., & Blennow, K. (2001). Evaluation of CSF biomarkers for axonal and neuronal degeneration, gliosis, and beta-amyloid metabolism in Alzheimer's disease. *J Neurol Neurosurg Psychiatry, 71*(4), 557-558. doi:10.1136/jnnp.71.4.557

Antonell, A., Mansilla, A., Rami, L., Lladó, A., Iranzo, A., Olives, J., Balasa, M., Sánchez-Valle, R., & Molinuevo, J. L. (2014). Cerebrospinal fluid level of YKL-40 protein in preclinical and prodromal Alzheimer's disease. *J Alzheimers Dis, 42*(3), 901-908. doi:10.3233/jad-140624

Arai, H., Morikawa, Y., Higuchi, M., Matsui, T., Clark, C. M., Miura, M., Machida, N., Lee, V. M., Trojanowski, J. Q., & Sasaki, H. (1997). Cerebrospinal fluid tau levels in neurodegenerative diseases with distinct tau-related pathology. *Biochem Biophys Res Commun, 236*(2), 262-264. doi:10.1006/bbrc.1997.6908

Ardura-Fabregat, A., Boddeke, E., Boza-Serrano, A., Brioschi, S., Castro-Gomez, S., Ceyzériat, K., Dansokho, C., Dierkes, T., Gelders, G., Heneka, M. T., Hoeijmakers, L., Hoffmann, A., Iaccarino, L., Jahnert, S., Kuhbandner, K., Landreth, G., Lonnemann, N., Löschmann, P. A., McManus, R. M., Paulus, A., Reemst, K., Sanchez-Caro, J. M., Tiberi, A., Van der Perren, A., Vautheny, A., Venegas, C., Webers, A., Weydt, P., Wijasa, T. S., Xiang, X., & Yang, Y. (2017). Targeting Neuroinflammation to Treat Alzheimer's Disease. *CNS Drugs, 31*(12), 1057-1082. doi:10.1007/s40263-017-0483-3

Armstrong, D. M., Saper, C. B., Levey, A. I., Wainer, B. H., & Terry, R. D. (1983). Distribution of cholinergic neurons in rat brain: demonstrated by the immunocytochemical localization of choline acetyltransferase. *J Comp Neurol, 216*(1), 53-68. doi:10.1002/cne.902160106

Arranz, A. M., & De Strooper, B. (2019). The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications. *The Lancet Neurology, 18*(4), 406-414. doi:https://doi.org/10.1016/S1474-4422(18)30490-3

Arvanitakis, Z., Shah, R. C., & Bennett, D. A. (2019). Diagnosis and Management of Dementia: Review. *JAMA, 322*(16), 1589-1599. doi:10.1001/jama.2019.4782

Ayodele, T., Rogaeva, E., Kurup, J. T., Beecham, G., & Reitz, C. (2021). Early-Onset Alzheimer's Disease: What Is Missing in Research? *Current Neurology and Neuroscience Reports, 21*(2), 4. doi:10.1007/s11910-020-01090-y

Bacioglu, M., Maia, L. F., Preische, O., Schelle, J., Apel, A., Kaeser, S. A., Schweighauser, M., Eninger, T., Lambert, M., Pilotto, A., Shimshek, D. R., Neumann, U., Kahle, P. J., Staufenbiel, M., Neumann, M., Maetzler, W., Kuhle, J., & Jucker, M. (2016). Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases. *Neuron, 91*(1), 56-66. doi:10.1016/j.neuron.2016.05.018

Bacskai, B. J., Frosch, M. P., Freeman, S. H., Raymond, S. B., Augustinack, J. C., Johnson, K. A., Irizarry, M. C., Klunk, W. E., Mathis, C. A., Dekosky, S. T., Greenberg, S. M., Hyman, B. T., & Growdon, J. H. (2007). Molecular imaging with Pittsburgh Compound B confirmed at autopsy: a case report. *Arch Neurol, 64*(3), 431-434. doi:10.1001/archneur.64.3.431

Baldacci, F., Toschi, N., Lista, S., Zetterberg, H., Blennow, K., Kilimann, I., Teipel, S., Cavedo, E., Dos Santos, A. M., Epelbaum, S., Lamari, F., Dubois, B., Floris, R., Garaci, F., Bonuccelli, U., & Hampel, H. (2017). Two-level diagnostic classification using cerebrospinal fluid YKL-40 in Alzheimer's disease. *Alzheimers Dement, 13*(9), 993-1003. doi:10.1016/j.jalz.2017.01.021

Barry, D. M., Stevenson, W., Bober, B. G., Wiese, P. J., Dale, J. M., Barry, G. S., Byers, N. S., Strope, J. D., Chang, R., Schulz, D. J., Shah, S., Calcutt, N. A., Gebremichael, Y., & Garcia, M. L. (2012). Expansion of neurofilament medium C terminus increases axonal diameter independent of increases in conduction velocity or myelin thickness. *J Neurosci, 32*(18), 6209-6219. doi:10.1523/jneurosci.0647-12.2012

Bateman, R. J., Aisen, P. S., De Strooper, B., Fox, N. C., Lemere, C. A., Ringman, J. M., Salloway, S., Sperling, R. A., Windisch, M., & Xiong, C. (2011). Autosomal-dominant Alzheimer's disease: a review and proposal for the prevention of Alzheimer's disease. *Alzheimer's research & therapy, 3*(1), 1-1. doi:10.1186/alzrt59

Bateman, R. J., Xiong, C., Benzinger, T. L. S., Fagan, A. M., Goate, A., Fox, N. C., Marcus, D. S., Cairns, N. J., Xie, X., Blazey, T. M., Holtzman, D. M., Santacruz, A., Buckles, V., Oliver, A., Moulder, K., Aisen, P. S., Ghetti, B., Klunk, W. E., McDade, E., Martins, R. N., Masters, C. L., Mayeux, R., Ringman, J. M., Rossor, M. N., Schofield, P. R., Sperling, R. A., Salloway, S., Morris, J. C., & Dominantly Inherited Alzheimer, N. (2012). Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *The New England journal of medicine, 367*(9), 795-804. doi:10.1056/NEJMoa1202753

Beach, T. G., Walker, R., & McGeer, E. G. (1989). Patterns of gliosis in Alzheimer's disease and aging cerebrum. *Glia, 2*(6), 420-436. doi:10.1002/glia.440020605

Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. (2001). *Clin Pharmacol Ther, 69*(3), 89-95. doi:10.1067/mcp.2001.113989

Bird, S. S., Marur, V. R., Sniatynski, M. J., Greenberg, H. K., & Kristal, B. S. (2011). Serum lipidomics profiling using LC-MS and high-energy collisional dissociation fragmentation: focus on triglyceride detection and characterization. *Anal Chem, 83*(17), 6648-6657. doi:10.1021/ac201195d

Birks, J. (2006). Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database Syst Rev*(1), Cd005593. doi:10.1002/14651858.cd005593

Blennow, K., Dubois, B., Fagan, A. M., Lewczuk, P., de Leon, M. J., & Hampel, H. (2015). Clinical utility of cerebrospinal fluid biomarkers in the diagnosis of early Alzheimer's disease. *Alzheimers Dement, 11*(1), 58-69. doi:10.1016/j.jalz.2014.02.004

Blennow, K., & Hampel, H. (2003). CSF markers for incipient Alzheimer's disease. *The Lancet. Neurology, 2*(10), 605-613. doi:10.1016/s1474-4422(03)00530-1

Blennow, K., Hampel, H., Weiner, M., & Zetterberg, H. (2010). Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol, 6*(3), 131-144. doi:10.1038/nrneurol.2010.4

Bongcam-Rudloff, E., Nistér, M., Betsholtz, C., Wang, J. L., Stenman, G., Huebner, K., Croce, C. M., & Westermark, B. (1991). Human glial fibrillary acidic protein: complementary DNA cloning, chromosome localization, and messenger RNA expression in human glioma cell lines of various phenotypes. *Cancer Res, 51*(5), 1553-1560.

Bonneh-Barkay, D., Bissel, S. J., Kofler, J., Starkey, A., Wang, G., & Wiley, C. A. (2012). Astrocyte and macrophage regulation of YKL-40 expression and cellular response in neuroinflammation. *Brain Pathol, 22*(4), 530-546. doi:10.1111/j.1750-3639.2011.00550.x

Bonneh-Barkay, D., Zagadailov, P., Zou, H., Niyonkuru, C., Figley, M., Starkey, A., Wang, G., Bissel, S. J., Wiley, C. A., & Wagner, A. K. (2010). YKL-40 expression in traumatic brain injury: an initial analysis. *J Neurotrauma, 27*(7), 1215-1223. doi:10.1089/neu.2010.1310

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta neuropathologica, 82*(4), 239-259. doi:10.1007/bf00308809

Braak, H., & Del Tredici, K. (2013). Reply: the early pathological process in sporadic Alzheimer's disease. *Acta neuropathologica, 126*(4), 615-618. doi:10.1007/s00401-013-1170-1

Brockes, J. P., Fields, K. L., & Raff, M. C. (1979). Studies on cultured rat Schwann cells. I. Establishment of purified populations from cultures of peripheral nerve. *Brain Res, 165*(1), 105-118. doi:10.1016/0006-8993(79)90048-9

Buerger, K., Ewers, M., Pirttilä, T., Zinkowski, R., Alafuzoff, I., Teipel, S. J., DeBernardis, J., Kerkman, D., McCulloch, C., Soininen, H., & Hampel, H. (2006). CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain, 129*(Pt 11), 3035-3041. doi:10.1093/brain/awl269

Burda, J. E., & Sofroniew, M. V. (2014). Reactive gliosis and the multicellular response to CNS damage and disease. *Neuron, 81*(2), 229-248. doi:10.1016/j.neuron.2013.12.034

Calsolaro, V., & Edison, P. (2016). Neuroinflammation in Alzheimer's disease: Current evidence and future directions. *Alzheimers Dement, 12*(6), 719-732. doi:10.1016/j.jalz.2016.02.010

Cantó, E., Tintoré, M., Villar, L. M., Costa, C., Nurtdinov, R., Álvarez-Cermeño, J. C., Arrambide, G., Reverter, F., Deisenhammer, F., Hegen, H., Khademi, M., Olsson, T., Tumani, H., Rodríguez-Martín, E., Piehl, F., Bartos, A., Zimova, D., Kotoucova, J., Kuhle, J., Kappos, L., García-Merino, J. A., Sánchez, A. J., Saiz, A., Blanco, Y., Hintzen, R., Jafari, N., Brassat, D., Lauda, F., Roesler, R., Rejdak, K., Papuc, E., de Andrés, C., Rauch, S., Khalil, M., Enzinger, C., Galimberti, D., Scarpini, E., Teunissen, C., Sánchez, A., Rovira, A., Montalban, X., & Comabella, M. (2015). Chitinase 3-like 1: prognostic biomarker in clinically isolated syndromes. *Brain, 138*(Pt 4), 918-931. doi:10.1093/brain/awv017

Chalfant, C. E., Kishikawa, K., Mumby, M. C., Kamibayashi, C., Bielawska, A., & Hannun, Y. A. (1999). Long chain ceramides activate protein phosphatase-1 and protein phosphatase-2A. Activation is stereospecific and regulated by phosphatidic acid. *J Biol Chem, 274*(29), 20313-20317. doi:10.1074/jbc.274.29.20313

Chan, P., Saleem, M., Herrmann, N., Mielke, M. M., Haughey, N. J., Oh, P. I., Kiss, A., & Lanctôt, K. L. (2018). Ceramide Accumulation Is Associated with Declining Verbal Memory in Coronary Artery Disease Patients: An Observational Study. *J Alzheimers Dis, 64*(4), 1235-1246. doi:10.3233/jad-180030

Chen, C.-H., Cheng, Y.-W., Chen, Y.-F., Tang, S.-C., & Jeng, J.-S. (2020). Plasma neurofilament light chain and glial fibrillary acidic protein predict stroke in CADASIL. *J Neuroinflammation, 17*(1), 124. doi:10.1186/s12974-020-01813-5

Chen, G. F., Xu, T. H., Yan, Y., Zhou, Y. R., Jiang, Y., Melcher, K., & Xu, H. E. (2017). Amyloid beta: structure, biology and structure-based therapeutic development. *Acta Pharmacol Sin, 38*(9), 1205-1235. doi:10.1038/aps.2017.28

Chernick, M., & Labudde, R. (2011). *An Introduction to Bootstrap Methods with Applications to R*.

Christl, J., Verhülsdonk, S., Pessanha, F., Menge, T., Seitz, R. J., Kujovic, M., Höft, B., Supprian, T., & Lange-Asschenfeldt, C. (2019). Association of Cerebrospinal Fluid S100B Protein with Core Biomarkers and Cognitive Deficits in Prodromal and Mild Alzheimer's Disease. *Journal of Alzheimer's Disease, 72*, 1119-1127. doi:10.3233/JAD-190550

Clark, C. M., Xie, S., Chittams, J., Ewbank, D., Peskind, E., Galasko, D., Morris, J. C., McKeel, D. W., Jr., Farlow, M., Weitlauf, S. L., Quinn, J., Kaye, J., Knopman, D., Arai, H., Doody, R. S., DeCarli, C., Leight, S., Lee, V. M., & Trojanowski, J. Q. (2003). Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? *Arch Neurol, 60*(12), 1696-1702. doi:10.1001/archneur.60.12.1696

Comabella, M., Fernández, M., Martin, R., Rivera-Vallvé, S., Borrás, E., Chiva, C., Julià, E., Rovira, A., Cantó, E., Alvarez-Cermeño, J. C., Villar, L. M., Tintoré, M., & Montalban, X. (2010). Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. *Brain, 133*(Pt 4), 1082-1093. doi:10.1093/brain/awq035

Craig-Schapiro, R., Perrin, R. J., Roe, C. M., Xiong, C., Carter, D., Cairns, N. J., Mintun, M. A., Peskind, E. R., Li, G., Galasko, D. R., Clark, C. M., Quinn, J. F., D'Angelo, G., Malone, J. P., Townsend, R. R., Morris, J. C., Fagan, A. M., & Holtzman, D. M. (2010). YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol Psychiatry, 68*(10), 903-912. doi:10.1016/j.biopsych.2010.08.025

Crutch, S. J., Lehmann, M., Schott, J. M., Rabinovici, G. D., Rossor, M. N., & Fox, N. C. (2012). Posterior cortical atrophy. *The Lancet. Neurology, 11*(2), 170-178. doi:10.1016/S1474-4422(11)70289-7

Currie, B. L. (1998). Oxford Dictionary of Biochemistry and Molecular Biology Edited by A. D. Smith, S. P. Datta, G. H. Smith, P. N. Campbell, R. Bentley, and H. A. McKenzie. Oxford University Press, Oxford. 1997. xi + pp. 20.5 x 26 cm. ISBN 0-19-854768-4. $60.00. *Journal of Medicinal Chemistry, 41*(14), 2661-2661. doi:10.1021/jm980247v

Cutler, R. G., Kelly, J., Storie, K., Pedersen, W. A., Tammara, A., Hatanpaa, K., Troncoso, J. C., & Mattson, M. P. (2004). Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proc Natl Acad Sci U S A, 101*(7), 2070-2075. doi:10.1073/pnas.0305799101

Cuvillier, O. (2002). Sphingosine in apoptosis signaling. *Biochim Biophys Acta, 1585*(2-3), 153-162. doi:10.1016/s1388-1981(02)00336-0

Darreh-Shori, T., Almkvist, O., Guan, Z. Z., Garlind, A., Strandberg, B., Svensson, A. L., Soreq, H., Hellström-Lindahl, E., & Nordberg, A. (2002). Sustained cholinesterase inhibition in AD patients receiving rivastigmine for 12 months. *Neurology, 59*(4), 563-572. doi:10.1212/wnl.59.4.563

Darreh-Shori, T., Brimijoin, S., Kadir, A., Almkvist, O., & Nordberg, A. (2006). Differential CSF butyrylcholinesterase levels in Alzheimer's disease patients with the ApoE epsilon4 allele, in relation to cognitive function and cerebral glucose metabolism. *Neurobiol Dis, 24*(2), 326-333. doi:10.1016/j.nbd.2006.07.013

Darreh-Shori, T., Forsberg, A., Modiri, N., Andreasen, N., Blennow, K., Kamil, C., Ahmed, H., Almkvist, O., Långström, B., & Nordberg, A. (2011a). Differential levels of apolipoprotein E and butyrylcholinesterase show strong association with pathological signs of Alzheimer's disease in the brain in vivo. *Neurobiol Aging, 32*(12), 2320.e2315-2332. doi:10.1016/j.neurobiolaging.2010.04.028

Darreh-Shori, T., Modiri, N., Blennow, K., Baza, S., Kamil, C., Ahmed, H., Andreasen, N., & Nordberg, A. (2011b). The apolipoprotein E ε4 allele plays pathological roles in AD through high protein expression and interaction with butyrylcholinesterase. *Neurobiol Aging, 32*(7), 1236-1248. doi:10.1016/j.neurobiolaging.2009.07.015

Darreh-Shori, T., Vijayaraghavan, S., Aeinehband, S., Piehl, F., Lindblom, R. P., Nilsson, B., Ekdahl, K. N., Långström, B., Almkvist, O., & Nordberg, A. (2013). Functional variability in butyrylcholinesterase activity regulates intrathecal cytokine and astroglial biomarker profiles in patients with Alzheimer's disease. *Neurobiol Aging, 34*(11), 2465-2481. doi:10.1016/j.neurobiolaging.2013.04.027

Darvesh, S., Grantham, D. L., & Hopkins, D. A. (1998). Distribution of butyrylcholinesterase in the human amygdala and hippocampal formation. *J Comp Neurol, 393*(3), 374-390.

Darvesh, S., & Hopkins, D. A. (2003). Differential distribution of butyrylcholinesterase and acetylcholinesterase in the human thalamus. *J Comp Neurol, 463*(1), 25-43. doi:10.1002/cne.10751

Darvesh, S., Hopkins, D. A., & Geula, C. (2003). Neurobiology of butyrylcholinesterase. *Nat Rev Neurosci, 4*(2), 131-138. doi:10.1038/nrn1035

Davies, P., & Maloney, A. J. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet, 2*(8000), 1403. doi:10.1016/s0140-6736(76)91936-x

DeLong, E. R., DeLong, D. M., & Clarke-Pearson, D. L. (1988). Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics, 44*(3), 837-845.

Diamond, A. (2013). Executive functions. *Annual review of psychology, 64*, 135-168. doi:10.1146/annurev-psych-113011-143750

Didier, M., Harandi, M., Aguera, M., Bancel, B., Tardy, M., Fages, C., Calas, A., Stagaard, M., Møllgård, K., & Belin, M. F. (1986). Differential immunocytochemical staining for glial fibrillary acidic (GFA) protein, S-100 protein and glutamine synthetase in the rat subcommissural organ, nonspecialized ventricular ependyma and adjacent neuropil. *Cell Tissue Res, 245*(2), 343-351. doi:10.1007/bf00213941

Dobrowsky, R. T., Kamibayashi, C., Mumby, M. C., & Hannun, Y. A. (1993). Ceramide activates heterotrimeric protein phosphatase 2A. *J Biol Chem, 268*(21), 15523-15530.

Donato, R. (2001). S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int J Biochem Cell Biol, 33*(7), 637-668. doi:10.1016/s1357-2725(01)00046-2

Donato, R. (2003). Intracellular and extracellular roles of S100 proteins. *Microsc Res Tech, 60*(6), 540-551. doi:10.1002/jemt.10296

Donato, R., Sorci, G., Riuzzi, F., Arcuri, C., Bianchi, R., Brozzi, F., Tubaro, C., & Giambanco, I. (2009). S100B's double life: intracellular regulator and extracellular signal. *Biochim Biophys Acta, 1793*(6), 1008-1022. doi:10.1016/j.bbamcr.2008.11.009

Du, A. T., Schuff, N., Amend, D., Laakso, M. P., Hsu, Y. Y., Jagust, W. J., Yaffe, K., Kramer, J. H., Reed, B., Norman, D., Chui, H. C., & Weiner, M. W. (2001). Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. *J Neurol Neurosurg Psychiatry, 71*(4), 441-447. doi:10.1136/jnnp.71.4.441

Dubois, B., Feldman, H. H., Jacova, C., Hampel, H., Molinuevo, J. L., Blennow, K., DeKosky, S. T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G. B., Fox, N. C., Galasko, D., Habert, M. O., Jicha, G. A., Nordberg, A., Pasquier, F., Rabinovici, G., Robert, P., Rowe, C., Salloway, S., Sarazin, M., Epelbaum, S., de Souza, L. C., Vellas, B., Visser, P. J., Schneider, L., Stern, Y., Scheltens, P., & Cummings, J. L. (2014). Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *The Lancet. Neurology, 13*(6), 614-629. doi:10.1016/s1474-4422(14)70090-0

Dubois, B., Hampel, H., Feldman, H. H., Scheltens, P., Aisen, P., Andrieu, S., Bakardjian, H., Benali, H., Bertram, L., Blennow, K., Broich, K., Cavedo, E., Crutch, S., Dartigues, J. F., Duyckaerts, C., Epelbaum, S., Frisoni, G. B., Gauthier, S., Genthon, R., Gouw, A. A., Habert, M. O., Holtzman, D. M., Kivipelto, M., Lista, S., Molinuevo, J. L., O'Bryant, S. E., Rabinovici, G. D., Rowe, C., Salloway, S., Schneider, L. S., Sperling, R., Teichmann, M., Carrillo, M. C., Cummings, J., & Jack, C. R., Jr. (2016). Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement, 12*(3), 292-323. doi:10.1016/j.jalz.2016.02.002

Duits, F. H., Teunissen, C. E., Bouwman, F. H., Visser, P. J., Mattsson, N., Zetterberg, H., Blennow, K., Hansson, O., Minthon, L., Andreasen, N., Marcusson, J., Wallin, A., Rikkert, M. O., Tsolaki, M., Parnetti, L., Herukka, S. K., Hampel, H., De Leon, M. J., Schröder, J., Aarsland, D., Blankenstein, M. A., Scheltens, P., & van der Flier, W. M. (2014). The cerebrospinal fluid "Alzheimer profile": easily said, but what does it mean? *Alzheimers Dement, 10*(6), 713-723.e712. doi:10.1016/j.jalz.2013.12.023

Efron, B., & Gong, G. (1983). A Leisurely Look at the Bootstrap, the Jackknife, and Cross-Validation. *The American Statistician, 37*(1), 36-48. doi:10.2307/2685844

El Khoury, J. B., Moore, K. J., Means, T. K., Leung, J., Terada, K., Toft, M., Freeman, M. W., & Luster, A. D. (2003). CD36 mediates the innate host response to beta-amyloid. *J Exp Med, 197*(12), 1657-1666. doi:10.1084/jem.20021546

Elahi, F. M., Casaletto, K. B., La Joie, R., Walters, S. M., Harvey, D., Wolf, A., Edwards, L., Rivera-Contreras, W., Karydas, A., Cobigo, Y., Rosen, H. J., DeCarli, C., Miller, B. L., Rabinovici, G. D., & Kramer, J. H. (2020). Plasma biomarkers of astrocytic and neuronal dysfunction in early- and late-onset Alzheimer's disease. *Alzheimers Dement, 16*(4), 681-695. doi:10.1016/j.jalz.2019.09.004

Ellman, G. L., Courtney, K. D., Andres, V., Jr., & Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol, 7*, 88-95. doi:10.1016/0006-2952(61)90145-9

Escott-Price, V., Sims, R., Bannister, C., Harold, D., Vronskaya, M., Majounie, E., Badarinarayan, N., Morgan, K., Passmore, P., Holmes, C., Powell, J., Brayne, C., Gill, M., Mead, S., Goate, A., Cruchaga, C., Lambert, J. C., van Duijn, C., Maier, W., Ramirez, A., Holmans, P., Jones, L., Hardy, J., Seshadri, S., Schellenberg, G. D., Amouyel, P., & Williams, J. (2015). Common polygenic variation enhances risk prediction for Alzheimer's disease. *Brain, 138*(Pt 12), 3673-3684. doi:10.1093/brain/awv268

Estévez-González, A., Kulisevsky, J., Boltes, A., Otermín, P., & García-Sánchez, C. (2003). Rey verbal learning test is a useful tool for differential diagnosis in the preclinical phase of Alzheimer's disease: comparison with mild cognitive impairment and normal aging. *Int J Geriatr Psychiatry, 18*(11), 1021-1028. doi:https://doi.org/10.1002/gps.1010

Fagan, A. M., Mintun, M. A., Mach, R. H., Lee, S. Y., Dence, C. S., Shah, A. R., LaRossa, G. N., Spinner, M. L., Klunk, W. E., Mathis, C. A., DeKosky, S. T., Morris, J. C., & Holtzman, D. M. (2006). Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. *Ann Neurol, 59*(3), 512-519. doi:10.1002/ana.20730

Fagan, A. M., Xiong, C., Jasielec, M. S., Bateman, R. J., Goate, A. M., Benzinger, T. L. S., Ghetti, B., Martins, R. N., Masters, C. L., Mayeux, R., Ringman, J. M., Rossor, M. N., Salloway, S., Schofield, P. R., Sperling, R. A., Marcus, D., Cairns, N. J., Buckles, V. D., Ladenson, J. H., Morris, J. C., Holtzman, D. M., & Dominantly Inherited Alzheimer, N. (2014). Longitudinal change in CSF biomarkers in autosomal-dominant Alzheimer's disease. *Science translational medicine, 6*(226), 226ra230-226ra230. doi:10.1126/scitranslmed.3007901

Fahy, E., Subramaniam, S., Brown, H. A., Glass, C. K., Merrill, A. H., Jr., Murphy, R. C., Raetz, C. R., Russell, D. W., Seyama, Y., Shaw, W., Shimizu, T., Spener, F., van Meer, G., VanNieuwenhze, M. S., White, S. H., Witztum, J. L., & Dennis, E. A. (2005). A comprehensive classification system for lipids. *J Lipid Res, 46*(5), 839-861. doi:10.1194/jlr.E400004-JLR200

Fanani, M. L., & Maggio, B. (2017). The many faces (and phases) of ceramide and sphingomyelin I - single lipids. *Biophys Rev, 9*(5), 589-600. doi:10.1007/s12551-017-0297-z

Fanò, G., Mariggiò, M. A., Angelella, P., Nicoletti, I., Antonica, A., Fulle, S., & Calissano, P. (1993). The S-100 protein causes an increase of intracellular calcium and death of PC12 cells. *Neuroscience, 53*(4), 919-925. doi:10.1016/0306-4522(93)90477-w

Ferreira, D., Perestelo-Pérez, L., Westman, E., Wahlund, L. O., Sarría, A., & Serrano-Aguilar, P. (2014). Meta-Review of CSF Core Biomarkers in Alzheimer's Disease: The State-of-the-Art after the New Revised Diagnostic Criteria. *Frontiers in aging neuroscience, 6*, 47. doi:10.3389/fnagi.2014.00047

Ferri, G. L., Probert, L., Cocchia, D., Michetti, F., Marangos, P. J., & Polak, J. M. (1982). Evidence for the presence of S-100 protein in the glial component of the human enteric nervous system. *Nature, 297*(5865), 409-410. doi:10.1038/297409a0

Filippov, V., Song, M. A., Zhang, K., Vinters, H. V., Tung, S., Kirsch, W. M., Yang, J., & Duerksen-Hughes, P. J. (2012). Increased ceramide in brains with Alzheimer's and other neurodegenerative diseases. *J Alzheimers Dis, 29*(3), 537-547. doi:10.3233/jad-2011-111202

Fisher, S. K., & Wonnacott, S. (2012). Chapter 13 - Acetylcholine. In S. T. Brady, G. J. Siegel, R. W. Albers, & D. L. Price (Eds.), *Basic Neurochemistry (Eighth Edition)* (pp. 258-282). New York: Academic Press.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res, 12*(3), 189-198. doi:10.1016/0022-3956(75)90026-6

Fonteh, A. N., Ormseth, C., Chiang, J., Cipolla, M., Arakaki, X., & Harrington, M. G. (2015). Sphingolipid metabolism correlates with cerebrospinal fluid Beta amyloid levels in Alzheimer's disease. *PLoS One, 10*(5), e0125597-e0125597. doi:10.1371/journal.pone.0125597

Fukuyama, R., Izumoto, T., & Fushiki, S. (2001). The cerebrospinal fluid level of glial fibrillary acidic protein is increased in cerebrospinal fluid from Alzheimer's disease patients and correlates with severity of dementia. *Eur Neurol, 46*(1), 35-38. doi:10.1159/000050753

Gabriel, A., Almeida, M., Ribeiro, M., Durães, J., Tábuas-Pereira, M., Pinheiro, A., Pascoal, R., Santana, I., & Baldeiras, I. (2017). Association between butyrylcholinesterase and cerebrospinal fluid biomarkers in Alzheimer’s disease patients. *Neurosci Lett, 641*. doi:10.1016/j.neulet.2017.01.036

Gallagher, M., & Koh, M. T. (2011). Episodic memory on the path to Alzheimer's disease. *Current opinion in neurobiology, 21*(6), 929-934. doi:10.1016/j.conb.2011.10.021

Gangishetti, U., Christina Howell, J., Perrin, R. J., Louneva, N., Watts, K. D., Kollhoff, A., Grossman, M., Wolk, D. A., Shaw, L. M., Morris, J. C., Trojanowski, J. Q., Fagan, A. M., Arnold, S. E., & Hu, W. T. (2018). Non-beta-amyloid/tau cerebrospinal fluid markers inform staging and progression in Alzheimer's disease. *Alzheimer's research & therapy, 10*(1), 98. doi:10.1186/s13195-018-0426-3

Ganguli, M., Snitz, B. E., Lee, C.-W., Vanderbilt, J., Saxton, J. A., & Chang, C.-C. H. (2010). Age and education effects and norms on a cognitive test battery from a population-based cohort: the Monongahela-Youghiogheny Healthy Aging Team. *Aging & mental health, 14*(1), 100-107. doi:10.1080/13607860903071014

García-Ayllón, M. S., Silveyra, M. X., & Sáez-Valero, J. (2008). Association between acetylcholinesterase and beta-amyloid peptide in Alzheimer's cerebrospinal fluid. *Chem Biol Interact, 175*(1-3), 209-215. doi:10.1016/j.cbi.2008.04.047

Gatz, M., Reynolds, C. A., Fratiglioni, L., Johansson, B., Mortimer, J. A., Berg, S., Fiske, A., & Pedersen, N. L. (2006). Role of genes and environments for explaining Alzheimer disease. *Arch Gen Psychiatry, 63*(2), 168-174. doi:10.1001/archpsyc.63.2.168

Geula, C., & Mesulam, M. M. (1995). Cholinesterases and the pathology of Alzheimer disease. *Alzheimer Dis Assoc Disord, 9 Suppl 2*, 23-28. doi:10.1097/00002093-199501002-00005

Giacobini, E. (2003). Cholinesterases: new roles in brain function and in Alzheimer's disease. *Neurochem Res, 28*(3-4), 515-522. doi:10.1023/a:1022869222652

Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., & et al. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature, 349*(6311), 704-706. doi:10.1038/349704a0

Goedert, M., Jakes, R., Qi, Z., Wang, J. H., & Cohen, P. (1995). Protein phosphatase 2A is the major enzyme in brain that dephosphorylates tau protein phosphorylated by proline-directed protein kinases or cyclic AMP-dependent protein kinase. *J Neurochem, 65*(6), 2804-2807. doi:10.1046/j.1471-4159.1995.65062804.x

Gomez-Munoz, A., Presa, N., Gomez-Larrauri, A., Rivera, I. G., Trueba, M., & Ordonez, M. (2016). Control of inflammatory responses by ceramide, sphingosine 1-phosphate and ceramide 1-phosphate. *Prog Lipid Res, 61*, 51-62. doi:10.1016/j.plipres.2015.09.002

Gong, C. X., Grundke-Iqbal, I., & Iqbal, K. (1994). Dephosphorylation of Alzheimer's disease abnormally phosphorylated tau by protein phosphatase-2A. *Neuroscience, 61*(4), 765-772. doi:10.1016/0306-4522(94)90400-6

González-Reyes, R. E., Nava-Mesa, M. O., Vargas-Sánchez, K., Ariza-Salamanca, D., & Mora-Muñoz, L. (2017). Involvement of Astrocytes in Alzheimer's Disease from a Neuroinflammatory and Oxidative Stress Perspective. *Frontiers in Molecular Neuroscience, 10*(427). doi:10.3389/fnmol.2017.00427

Gouras, G. K., Tampellini, D., Takahashi, R. H., & Capetillo-Zarate, E. (2010). Intraneuronal beta-amyloid accumulation and synapse pathology in Alzheimer's disease. *Acta neuropathologica, 119*(5), 523-541. doi:10.1007/s00401-010-0679-9

Graff-Radford, J., Yong, K. X. X., Apostolova, L. G., Bouwman, F. H., Carrillo, M., Dickerson, B. C., Rabinovici, G. D., Schott, J. M., Jones, D. T., & Murray, M. E. (2021). New insights into atypical Alzheimer's disease in the era of biomarkers. *The Lancet. Neurology, 20*(3), 222-234. doi:10.1016/s1474-4422(20)30440-3

Grafman, J., & Litvan, I. (1999). Importance of deficits in executive functions. *The Lancet, 354*(9194), 1921-1923. doi:https://doi.org/10.1016/S0140-6736(99)90438-5

Greenberg, S. M., Bacskai, B. J., Hernandez-Guillamon, M., Pruzin, J., Sperling, R., & van Veluw, S. J. (2020). Cerebral amyloid angiopathy and Alzheimer disease — one peptide, two pathways. *Nature Reviews Neurology, 16*(1), 30-42. doi:10.1038/s41582-019-0281-2

Greig, N. H., Utsuki, T., Yu, Q., Zhu, X., Holloway, H. W., Perry, T., Lee, B., Ingram, D. K., & Lahiri, D. K. (2001). A new therapeutic target in Alzheimer's disease treatment: attention to butyrylcholinesterase. *Curr Med Res Opin, 17*(3), 159-165. doi:10.1185/0300799039117057

Grimm, M. O., Grimm, H. S., Patzold, A. J., Zinser, E. G., Halonen, R., Duering, M., Tschape, J. A., De Strooper, B., Muller, U., Shen, J., & Hartmann, T. (2005). Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. *Nat Cell Biol, 7*(11), 1118-1123. doi:10.1038/ncb1313

Grimmer, T., Riemenschneider, M., Förstl, H., Henriksen, G., Klunk, W. E., Mathis, C. A., Shiga, T., Wester, H. J., Kurz, A., & Drzezga, A. (2009). Beta amyloid in Alzheimer's disease: increased deposition in brain is reflected in reduced concentration in cerebrospinal fluid. *Biol Psychiatry, 65*(11), 927-934. doi:10.1016/j.biopsych.2009.01.027

Guarino, A., Favieri, F., Boncompagni, I., Agostini, F., Cantone, M., & Casagrande, M. (2019). Executive Functions in Alzheimer Disease: A Systematic Review. *Frontiers in aging neuroscience, 10*(437). doi:10.3389/fnagi.2018.00437

Hakala, B. E., White, C., & Recklies, A. D. (1993). Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem, 268*(34), 25803-25810.

Han, X., M. Holtzman, D., W. McKeel Jr, D., Kelley, J., & Morris, J. C. (2002). Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. *J Neurochem, 82*(4), 809-818. doi:10.1046/j.1471-4159.2002.00997.x

Hansen, D. V., Hanson, J. E., & Sheng, M. (2017). Microglia in Alzheimer's disease. *Journal of Cell Biology, 217*(2), 459-472. doi:10.1083/jcb.201709069

Hansson, O., Zetterberg, H., Buchhave, P., Andreasson, U., Londos, E., Minthon, L., & Blennow, K. (2007). Prediction of Alzheimer's disease using the CSF Abeta42/Abeta40 ratio in patients with mild cognitive impairment. *Dement Geriatr Cogn Disord, 23*(5), 316-320. doi:10.1159/000100926

Hanzel, D. K., Trojanowski, J. Q., Johnston, R. F., & Loring, J. F. (1999). High-throughput quantitative histological analysis of Alzheimer's disease pathology using a confocal digital microscanner. *Nat Biotechnol, 17*(1), 53-57. doi:10.1038/5225

Hartmann, T., Kuchenbecker, J., & Grimm, M. O. (2007). Alzheimer's disease: the lipid connection. *J Neurochem, 103 Suppl 1*, 159-170. doi:10.1111/j.1471-4159.2007.04715.x

Haughey, N. J., Bandaru, V. V., Bae, M., & Mattson, M. P. (2010). Roles for dysfunctional sphingolipid metabolism in Alzheimer's disease neuropathogenesis. *Biochim Biophys Acta, 1801*(8), 878-886. doi:10.1016/j.bbalip.2010.05.003

He, X., Huang, Y., Li, B., Gong, C. X., & Schuchman, E. H. (2010). Deregulation of sphingolipid metabolism in Alzheimer's disease. *Neurobiol Aging, 31*(3), 398-408. doi:10.1016/j.neurobiolaging.2008.05.010

Heizmann, C. W., Fritz, G., & Schäfer, B. W. (2002). S100 proteins: structure, functions and pathology. *Front Biosci, 7*, d1356-1368.

Heller, C., Foiani, M. S., Moore, K., Convery, R., Bocchetta, M., Neason, M., Cash, D. M., Thomas, D., Greaves, C. V., Woollacott, I. O., Shafei, R., Van Swieten, J. C., Moreno, F., Sanchez-Valle, R., Borroni, B., Laforce, R., Jr., Masellis, M., Tartaglia, M. C., Graff, C., Galimberti, D., Rowe, J. B., Finger, E., Synofzik, M., Vandenberghe, R., de Mendonca, A., Tagliavini, F., Santana, I., Ducharme, S., Butler, C. R., Gerhard, A., Levin, J., Danek, A., Frisoni, G., Sorbi, S., Otto, M., Heslegrave, A. J., Zetterberg, H., & Rohrer, J. D. (2020). Plasma glial fibrillary acidic protein is raised in progranulin-associated frontotemporal dementia. *J Neurol Neurosurg Psychiatry, 91*(3), 263-270. doi:10.1136/jnnp-2019-321954

Henry, J. D., Crawford, J. R., & Phillips, L. H. (2004). Verbal fluency performance in dementia of the Alzheimer's type: a meta-analysis. *Neuropsychologia, 42*(9), 1212-1222. doi:10.1016/j.neuropsychologia.2004.02.001

Henry, M. L., & Gorno-Tempini, M. L. (2010). The logopenic variant of primary progressive aphasia. *Current opinion in neurology, 23*(6), 633-637. doi:10.1097/WCO.0b013e32833fb93e

Heppner, F. L., Ransohoff, R. M., & Becher, B. (2015). Immune attack: the role of inflammation in Alzheimer disease. *Nat Rev Neurosci, 16*(6), 358-372. doi:10.1038/nrn3880

Hesse, C., Rosengren, L., Andreasen, N., Davidsson, P., Vanderstichele, H., Vanmechelen, E., & Blennow, K. (2001). Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neurosci Lett, 297*(3), 187-190. doi:10.1016/s0304-3940(00)01697-9

Hinsinger, G., Galéotti, N., Nabholz, N., Urbach, S., Rigau, V., Demattei, C., Lehmann, S., Camu, W., Labauge, P., Castelnovo, G., Brassat, D., Loussouarn, D., Salou, M., Laplaud, D., Casez, O., Bockaert, J., Marin, P., & Thouvenot, E. (2015). Chitinase 3-like proteins as diagnostic and prognostic biomarkers of multiple sclerosis. *Mult Scler, 21*(10), 1251-1261. doi:10.1177/1352458514561906

Hippius, H., & Neundörfer, G. (2003). The discovery of Alzheimer's disease. *Dialogues in clinical neuroscience, 5*(1), 101-108. doi:10.31887/DCNS.2003.5.1/hhippius

Hodges, J. R. (2000). Memory in the dementias. In *The Oxford handbook of memory.* (pp. 441-459). New York, NY, US: Oxford University Press.

Hodges, J. R., & Patterson, K. (1995). Is semantic memory consistently impaired early in the course of Alzheimer's disease? Neuroanatomical and diagnostic implications. *Neuropsychologia, 33*(4), 441-459. doi:10.1016/0028-3932(94)00127-b

Hofner, B., Boccuto, L., & Göker, M. (2015). Controlling false discoveries in high-dimensional situations: boosting with stability selection. *BMC Bioinformatics, 16*, 144. doi:10.1186/s12859-015-0575-3

Hoshi, M., Takashima, A., Murayama, M., Yasutake, K., Yoshida, N., Ishiguro, K., Hoshino, T., & Imahori, K. (1997). Nontoxic amyloid beta peptide 1-42 suppresses acetylcholine synthesis. Possible role in cholinergic dysfunction in Alzheimer's disease. *J Biol Chem, 272*(4), 2038-2041. doi:10.1074/jbc.272.4.2038

Hov, K. R., Bolstad, N., Idland, A. V., Zetterberg, H., Blennow, K., Chaudhry, F. A., Frihagen, F., Ræder, J., Wyller, T. B., & Watne, L. O. (2017). Cerebrospinal Fluid S100B and Alzheimer's Disease Biomarkers in Hip Fracture Patients with Delirium. *Dement Geriatr Cogn Dis Extra, 7*(3), 374-385. doi:10.1159/000481853

Hu, J., Ferreira, A., & Van Eldik, L. J. (1997). S100beta induces neuronal cell death through nitric oxide release from astrocytes. *J Neurochem, 69*(6), 2294-2301. doi:10.1046/j.1471-4159.1997.69062294.x

Huttunen, H. J., Kuja-Panula, J., Sorci, G., Agneletti, A. L., Donato, R., & Rauvala, H. (2000). Coregulation of neurite outgrowth and cell survival by amphoterin and S100 proteins through receptor for advanced glycation end products (RAGE) activation. *J Biol Chem, 275*(51), 40096-40105. doi:10.1074/jbc.M006993200

Ikonomovic, M. D., Klunk, W. E., Abrahamson, E. E., Mathis, C. A., Price, J. C., Tsopelas, N. D., Lopresti, B. J., Ziolko, S., Bi, W., Paljug, W. R., Debnath, M. L., Hope, C. E., Isanski, B. A., Hamilton, R. L., & DeKosky, S. T. (2008). Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. *Brain, 131*(Pt 6), 1630-1645. doi:10.1093/brain/awn016

Ishiki, A., Kamada, M., Kawamura, Y., Terao, C., Shimoda, F., Tomita, N., Arai, H., & Furukawa, K. (2016). Glial fibrillar acidic protein in the cerebrospinal fluid of Alzheimer's disease, dementia with Lewy bodies, and frontotemporal lobar degeneration. *J Neurochem, 136*(2), 258-261. doi:10.1111/jnc.13399

Ittner, L. M., & Götz, J. (2011). Amyloid-β and tau--a toxic pas de deux in Alzheimer's disease. *Nat Rev Neurosci, 12*(2), 65-72. doi:10.1038/nrn2967

Jack, C. R., Jr., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., Holtzman, D. M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J. L., Montine, T., Phelps, C., Rankin, K. P., Rowe, C. C., Scheltens, P., Siemers, E., Snyder, H. M., Sperling, R., & Contributors. (2018). NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement, 14*(4), 535-562. doi:10.1016/j.jalz.2018.02.018

Jack, C. R., Jr., Dickson, D. W., Parisi, J. E., Xu, Y. C., Cha, R. H., O'Brien, P. C., Edland, S. D., Smith, G. E., Boeve, B. F., Tangalos, E. G., Kokmen, E., & Petersen, R. C. (2002). Antemortem MRI findings correlate with hippocampal neuropathology in typical aging and dementia. *Neurology, 58*(5), 750-757. doi:10.1212/wnl.58.5.750

Jack, C. R., Jr., Knopman, D. S., Jagust, W. J., Shaw, L. M., Aisen, P. S., Weiner, M. W., Petersen, R. C., & Trojanowski, J. Q. (2010). Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *The Lancet. Neurology, 9*(1), 119-128. doi:10.1016/s1474-4422(09)70299-6

Jagust, W. J., Landau, S. M., Shaw, L. M., Trojanowski, J. Q., Koeppe, R. A., Reiman, E. M., Foster, N. L., Petersen, R. C., Weiner, M. W., Price, J. C., & Mathis, C. A. (2009). Relationships between biomarkers in aging and dementia. *Neurology, 73*(15), 1193-1199. doi:10.1212/WNL.0b013e3181bc010c

Jazvinšćak Jembrek, M., Hof, P. R., & Šimić, G. (2015). Ceramides in Alzheimer's Disease: Key Mediators of Neuronal Apoptosis Induced by Oxidative Stress and Aβ Accumulation. *Oxidative medicine and cellular longevity, 2015*, 346783-346783. doi:10.1155/2015/346783

Jesse, S., Steinacker, P., Cepek, L., von Arnim, C. A., Tumani, H., Lehnert, S., Kretzschmar, H. A., Baier, M., & Otto, M. (2009). Glial fibrillary acidic protein and protein S-100B: different concentration pattern of glial proteins in cerebrospinal fluid of patients with Alzheimer's disease and Creutzfeldt-Jakob disease. *J Alzheimers Dis, 17*(3), 541-551. doi:10.3233/jad-2009-1075

Jessen, F., Amariglio, R. E., van Boxtel, M., Breteler, M., Ceccaldi, M., Chételat, G., Dubois, B., Dufouil, C., Ellis, K. A., van der Flier, W. M., Glodzik, L., van Harten, A. C., de Leon, M. J., McHugh, P., Mielke, M. M., Molinuevo, J. L., Mosconi, L., Osorio, R. S., Perrotin, A., Petersen, R. C., Rabin, L. A., Rami, L., Reisberg, B., Rentz, D. M., Sachdev, P. S., de la Sayette, V., Saykin, A. J., Scheltens, P., Shulman, M. B., Slavin, M. J., Sperling, R. A., Stewart, R., Uspenskaya, O., Vellas, B., Visser, P. J., Wagner, M., & Subjective Cognitive Decline Initiative Working, G. (2014). A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. *Alzheimers Dement, 10*(6), 844-852. doi:10.1016/j.jalz.2014.01.001

Johannsson, M., Snaedal, J., Johannesson, G. H., Gudmundsson, T. E., & Johnsen, K. (2015). The acetylcholine index: an electroencephalographic marker of cholinergic activity in the living human brain applied to Alzheimer's disease and other dementias. *Dement Geriatr Cogn Disord, 39*(3-4), 132-142. doi:10.1159/000367889

Johansen, J. S. (2006). Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibroses and cancer. *Dan Med Bull, 53*(2), 172-209.

Johansson, P., Almqvist, E. G., Johansson, J. O., Mattsson, N., Andreasson, U., Hansson, O., Wallin, A., Blennow, K., Zetterberg, H., & Svensson, J. (2013). Cerebrospinal fluid (CSF) 25-hydroxyvitamin D concentration and CSF acetylcholinesterase activity are reduced in patients with Alzheimer's disease. *PLoS One, 8*(11), e81989. doi:10.1371/journal.pone.0081989

Jonsson, T., Atwal, J. K., Steinberg, S., Snaedal, J., Jonsson, P. V., Bjornsson, S., Stefansson, H., Sulem, P., Gudbjartsson, D., Maloney, J., Hoyte, K., Gustafson, A., Liu, Y., Lu, Y., Bhangale, T., Graham, R. R., Huttenlocher, J., Bjornsdottir, G., Andreassen, O. A., Jönsson, E. G., Palotie, A., Behrens, T. W., Magnusson, O. T., Kong, A., Thorsteinsdottir, U., Watts, R. J., & Stefansson, K. (2012). A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature, 488*(7409), 96-99. doi:10.1038/nature11283

Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P. V., Snaedal, J., Bjornsson, S., Huttenlocher, J., Levey, A. I., Lah, J. J., Rujescu, D., Hampel, H., Giegling, I., Andreassen, O. A., Engedal, K., Ulstein, I., Djurovic, S., Ibrahim-Verbaas, C., Hofman, A., Ikram, M. A., van Duijn, C. M., Thorsteinsdottir, U., Kong, A., & Stefansson, K. (2013). Variant of TREM2 associated with the risk of Alzheimer's disease. *The New England journal of medicine, 368*(2), 107-116. doi:10.1056/NEJMoa1211103

Jorm, A. F. (2004). The Informant Questionnaire on cognitive decline in the elderly (IQCODE): a review. *Int Psychogeriatr, 16*(3), 275-293. doi:10.1017/s1041610204000390

Kadekaro, M., Crane, A. M., & Sokoloff, L. (1985). Differential effects of electrical stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat. *Proc Natl Acad Sci U S A, 82*(17), 6010-6013. doi:10.1073/pnas.82.17.6010

Kalvodova, L., Kahya, N., Schwille, P., Ehehalt, R., Verkade, P., Drechsel, D., & Simons, K. (2005). Lipids as modulators of proteolytic activity of BACE: involvement of cholesterol, glycosphingolipids, and anionic phospholipids in vitro. *J Biol Chem, 280*(44), 36815-36823. doi:10.1074/jbc.M504484200

Kar, S., Issa, A. M., Seto, D., Auld, D. S., Collier, B., & Quirion, R. (1998). Amyloid beta-peptide inhibits high-affinity choline uptake and acetylcholine release in rat hippocampal slices. *J Neurochem, 70*(5), 2179-2187. doi:10.1046/j.1471-4159.1998.70052179.x

Kar, S., Seto, D., Gaudreau, P., & Quirion, R. (1996). Beta-amyloid-related peptides inhibit potassium-evoked acetylcholine release from rat hippocampal slices. *J Neurosci, 16*(3), 1034-1040. doi:10.1523/jneurosci.16-03-01034.1996

Karch, C. M., & Goate, A. M. (2015). Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Biol Psychiatry, 77*(1), 43-51. doi:10.1016/j.biopsych.2014.05.006

Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W., & Glabe, C. G. (2003). Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science, 300*(5618), 486-489. doi:10.1126/science.1079469

Kind, T., Liu, K.-H., Lee, D. Y., DeFelice, B., Meissen, J. K., & Fiehn, O. (2013). LipidBlast in silico tandem mass spectrometry database for lipid identification. *Nature Methods, 10*(8), 755-758. doi:10.1038/nmeth.2551

Kirkpatrick, R. B., Emery, J. G., Connor, J. R., Dodds, R., Lysko, P. G., & Rosenberg, M. (1997). Induction and Expression of Human Cartilage Glycoprotein 39 in Rheumatoid Inflammatory and Peripheral Blood Monocyte-Derived Macrophages. *Experimental Cell Research, 237*(1), 46-54. doi:https://doi.org/10.1006/excr.1997.3764

Kishi, T., Matsunaga, S., Oya, K., Nomura, I., Ikuta, T., & Iwata, N. (2017). Memantine for Alzheimer's Disease: An Updated Systematic Review and Meta-analysis. *J Alzheimers Dis, 60*(2), 401-425. doi:10.3233/jad-170424

Koffie, R. M., Meyer-Luehmann, M., Hashimoto, T., Adams, K. W., Mielke, M. L., Garcia-Alloza, M., Micheva, K. D., Smith, S. J., Kim, M. L., Lee, V. M., Hyman, B. T., & Spires-Jones, T. L. (2009). Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. *Proc Natl Acad Sci U S A, 106*(10), 4012-4017. doi:10.1073/pnas.0811698106

Kotronoulas, A., Jónasdóttir, H. S., Sigurðardóttir, R. S., Halldórsson, S., Haraldsson, G. G., & Rolfsson, Ó. (2020). Wound healing grafts: Omega-3 fatty acid lipid content differentiates the lipid profiles of acellular Atlantic cod skin from traditional dermal substitutes. *J Tissue Eng Regen Med, 14*(3), 441-451. doi:10.1002/term.3005

Kuhle, J., Barro, C., Disanto, G., Mathias, A., Soneson, C., Bonnier, G., Yaldizli, Ö., Regeniter, A., Derfuss, T., Canales, M., Schluep, M., Du Pasquier, R., Krueger, G., & Granziera, C. (2016). Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. *Mult Scler, 22*(12), 1550-1559. doi:10.1177/1352458515623365

Kumar, R., Nordberg, A., & Darreh-Shori, T. (2016). Amyloid-β peptides act as allosteric modulators of cholinergic signalling through formation of soluble BAβACs. *Brain, 139*(Pt 1), 174-192. doi:10.1093/brain/awv318

Landqvist Waldö, M., Frizell Santillo, A., Passant, U., Zetterberg, H., Rosengren, L., Nilsson, C., & Englund, E. (2013). Cerebrospinal fluid neurofilament light chain protein levels in subtypes of frontotemporal dementia. *BMC Neurol, 13*, 54. doi:10.1186/1471-2377-13-54

Lane, R. M., & Darreh-Shori, T. (2015). Understanding the beneficial and detrimental effects of donepezil and rivastigmine to improve their therapeutic value. *J Alzheimers Dis, 44*(4), 1039-1062. doi:10.3233/jad-142268

Lane, R. M., & He, Y. (2013). Butyrylcholinesterase genotype and gender influence Alzheimer's disease phenotype. *Alzheimers Dement, 9*(2), e1-73. doi:10.1016/j.jalz.2010.12.005

Lane, R. M., Potkin, S. G., & Enz, A. (2006). Targeting acetylcholinesterase and butyrylcholinesterase in dementia. *Int J Neuropsychopharmacol, 9*(1), 101-124. doi:10.1017/s1461145705005833

Lautner, R., Insel, P. S., Skillbäck, T., Olsson, B., Landén, M., Frisoni, G. B., Herukka, S.-K., Hampel, H., Wallin, A., Minthon, L., Hansson, O., Blennow, K., Mattsson, N., & Zetterberg, H. (2017). Preclinical effects of APOE ε4 on cerebrospinal fluid Aβ42 concentrations. *Alzheimer's research & therapy, 9*(1), 87-87. doi:10.1186/s13195-017-0313-3

Le Prince, G., Delaere, P., Fages, C., Duyckaerts, C., Hauw, J. J., & Tardy, M. (1993). Alterations of glial fibrillary acidic protein mRNA level in the aging brain and in senile dementia of the Alzheimer type. *Neurosci Lett, 151*(1), 71-73. doi:10.1016/0304-3940(93)90048-p

Lee, J. T., Xu, J., Lee, J. M., Ku, G., Han, X., Yang, D. I., Chen, S., & Hsu, C. Y. (2004). Amyloid-beta peptide induces oligodendrocyte death by activating the neutral sphingomyelinase-ceramide pathway. *J Cell Biol, 164*(1), 123-131. doi:10.1083/jcb.200307017

Leng, F., & Edison, P. (2021). Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? *Nature Reviews Neurology, 17*(3), 157-172. doi:10.1038/s41582-020-00435-y

Lépinoux-Chambaud, C., & Eyer, J. (2013). Review on intermediate filaments of the nervous system and their pathological alterations. *Histochemistry and Cell Biology, 140*(1), 13-22. doi:10.1007/s00418-013-1101-1

Levenson, R. W., Sturm, V. E., & Haase, C. M. (2014). Emotional and behavioral symptoms in neurodegenerative disease: a model for studying the neural bases of psychopathology. *Annual review of clinical psychology, 10*, 581-606. doi:10.1146/annurev-clinpsy-032813-153653

Lewczuk, P., Esselmann, H., Otto, M., Maler, J. M., Henkel, A. W., Henkel, M. K., Eikenberg, O., Antz, C., Krause, W. R., Reulbach, U., Kornhuber, J., & Wiltfang, J. (2004). Neurochemical diagnosis of Alzheimer's dementia by CSF Abeta42, Abeta42/Abeta40 ratio and total tau. *Neurobiol Aging, 25*(3), 273-281. doi:10.1016/s0197-4580(03)00086-1

Lewczuk, P., Lelental, N., Spitzer, P., Maler, J. M., & Kornhuber, J. (2015). Amyloid-β 42/40 cerebrospinal fluid concentration ratio in the diagnostics of Alzheimer's disease: validation of two novel assays. *J Alzheimers Dis, 43*(1), 183-191. doi:10.3233/jad-140771

Lewczuk, P., Riederer, P., O'Bryant, S. E., Verbeek, M. M., Dubois, B., Visser, P. J., Jellinger, K. A., Engelborghs, S., Ramirez, A., Parnetti, L., Jack, C. R., Jr., Teunissen, C. E., Hampel, H., Lleó, A., Jessen, F., Glodzik, L., de Leon, M. J., Fagan, A. M., Molinuevo, J. L., Jansen, W. J., Winblad, B., Shaw, L. M., Andreasson, U., Otto, M., Mollenhauer, B., Wiltfang, J., Turner, M. R., Zerr, I., Handels, R., Thompson, A. G., Johansson, G., Ermann, N., Trojanowski, J. Q., Karaca, I., Wagner, H., Oeckl, P., van Waalwijk van Doorn, L., Bjerke, M., Kapogiannis, D., Kuiperij, H. B., Farotti, L., Li, Y., Gordon, B. A., Epelbaum, S., Vos, S. J. B., Klijn, C. J. M., Van Nostrand, W. E., Minguillon, C., Schmitz, M., Gallo, C., Lopez Mato, A., Thibaut, F., Lista, S., Alcolea, D., Zetterberg, H., Blennow, K., & Kornhuber, J. (2018). Cerebrospinal fluid and blood biomarkers for neurodegenerative dementias: An update of the Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry. *World J Biol Psychiatry, 19*(4), 244-328. doi:10.1080/15622975.2017.1375556

Lezak, M. D., Howieson, D. B., Loring, D. W., & Fischer, J. S. (2004). *Neuropsychological assessment*: Oxford University Press, USA.

Lian, H., Yang, L., Cole, A., Sun, L., Chiang, A. C., Fowler, S. W., Shim, D. J., Rodriguez-Rivera, J., Taglialatela, G., Jankowsky, J. L., Lu, H. C., & Zheng, H. (2015). NFκB-activated astroglial release of complement C3 compromises neuronal morphology and function associated with Alzheimer's disease. *Neuron, 85*(1), 101-115. doi:10.1016/j.neuron.2014.11.018

Liddelow, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L., Bennett, M. L., Münch, A. E., Chung, W. S., Peterson, T. C., Wilton, D. K., Frouin, A., Napier, B. A., Panicker, N., Kumar, M., Buckwalter, M. S., Rowitch, D. H., Dawson, V. L., Dawson, T. M., Stevens, B., & Barres, B. A. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature, 541*(7638), 481-487. doi:10.1038/nature21029

Liebisch, G., Vizcaíno, J. A., Köfeler, H., Trötzmüller, M., Griffiths, W. J., Schmitz, G., Spener, F., & Wakelam, M. J. O. (2013). Shorthand notation for lipid structures derived from mass spectrometry. *J Lipid Res, 54*(6), 1523-1530. doi:10.1194/jlr.M033506

Ludwin, S. K., Kosek, J. C., & Eng, L. F. (1976). The topographical distribution of S-100 and GFA proteins in the adult rat brain: an immunohistochemical study using horseradish peroxidase-labelled antibodies. *J Comp Neurol, 165*(2), 197-207. doi:10.1002/cne.901650206

Maia, L. F., Kaeser, S. A., Reichwald, J., Hruscha, M., Martus, P., Staufenbiel, M., & Jucker, M. (2013). Changes in amyloid-β and Tau in the cerebrospinal fluid of transgenic mice overexpressing amyloid precursor protein. *Science translational medicine, 5*(194), 194re192. doi:10.1126/scitranslmed.3006446

Masson, P., Schopfer, L. M., Bartels, C. F., Froment, M. T., Ribes, F., Nachon, F., & Lockridge, O. (2002). Substrate activation in acetylcholinesterase induced by low pH or mutation in the pi-cation subsite. *Biochim Biophys Acta, 1594*(2), 313-324. doi:10.1016/s0167-4838(01)00323-5

Masson, P., Xie, W., Froment, M. T., Levitsky, V., Fortier, P. L., Albaret, C., & Lockridge, O. (1999). Interaction between the peripheral site residues of human butyrylcholinesterase, D70 and Y332, in binding and hydrolysis of substrates. *Biochim Biophys Acta, 1433*(1-2), 281-293. doi:10.1016/s0167-4838(99)00115-6

Mattsson, N., Insel, P. S., Palmqvist, S., Portelius, E., Zetterberg, H., Weiner, M., Blennow, K., & Hansson, O. (2016). Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. *EMBO Mol Med, 8*(10), 1184-1196. doi:10.15252/emmm.201606540

Mattsson, N., Tabatabaei, S., Johansson, P., Hansson, O., Andreasson, U., Månsson, J. E., Johansson, J. O., Olsson, B., Wallin, A., Svensson, J., Blennow, K., & Zetterberg, H. (2011). Cerebrospinal fluid microglial markers in Alzheimer's disease: elevated chitotriosidase activity but lack of diagnostic utility. *Neuromolecular Med, 13*(2), 151-159. doi:10.1007/s12017-011-8147-9

McKeith, I. G., Boeve, B. F., Dickson, D. W., Halliday, G., Taylor, J.-P., Weintraub, D., Aarsland, D., Galvin, J., Attems, J., Ballard, C. G., Bayston, A., Beach, T. G., Blanc, F., Bohnen, N., Bonanni, L., Bras, J., Brundin, P., Burn, D., Chen-Plotkin, A., Duda, J. E., El-Agnaf, O., Feldman, H., Ferman, T. J., ffytche, D., Fujishiro, H., Galasko, D., Goldman, J. G., Gomperts, S. N., Graff-Radford, N. R., Honig, L. S., Iranzo, A., Kantarci, K., Kaufer, D., Kukull, W., Lee, V. M. Y., Leverenz, J. B., Lewis, S., Lippa, C., Lunde, A., Masellis, M., Masliah, E., McLean, P., Mollenhauer, B., Montine, T. J., Moreno, E., Mori, E., Murray, M., O'Brien, J. T., Orimo, S., Postuma, R. B., Ramaswamy, S., Ross, O. A., Salmon, D. P., Singleton, A., Taylor, A., Thomas, A., Tiraboschi, P., Toledo, J. B., Trojanowski, J. Q., Tsuang, D., Walker, Z., Yamada, M., & Kosaka, K. (2017). Diagnosis and management of dementia with Lewy bodies. *Fourth consensus report of the DLB Consortium, 89*(1), 88-100. doi:10.1212/wnl.0000000000004058

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology, 34*(7), 939-944. doi:10.1212/wnl.34.7.939

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R., Jr., Kawas, C. H., Klunk, W. E., Koroshetz, W. J., Manly, J. J., Mayeux, R., Mohs, R. C., Morris, J. C., Rossor, M. N., Scheltens, P., Carrillo, M. C., Thies, B., Weintraub, S., & Phelps, C. H. (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement, 7*(3), 263-269. doi:10.1016/j.jalz.2011.03.005

Medeiros, R., & LaFerla, F. M. (2013). Astrocytes: conductors of the Alzheimer disease neuroinflammatory symphony. *Exp Neurol, 239*, 133-138. doi:10.1016/j.expneurol.2012.10.007

Mehrabian, S., Alexopoulos, P., Ortner, M., Traykov, L., Grimmer, T., Kurz, A., Förstl, H., Bickel, H., & Diehl-Schmid, J. (2015). Cerebrospinal fluid biomarkers for Alzheimer's disease: the role of apolipoprotein E genotype, age, and sex. *Neuropsychiatric disease and treatment, 11*, 3105-3110. doi:10.2147/NDT.S95018

Meinshausen, N., & Bühlmann, P. (2010). Stability selection. *Journal of the Royal Statistical Society: Series B (Statistical Methodology), 72*(4), 417-473. doi:https://doi.org/10.1111/j.1467-9868.2010.00740.x

Melah, K. E., Lu, S. Y., Hoscheidt, S. M., Alexander, A. L., Adluru, N., Destiche, D. J., Carlsson, C. M., Zetterberg, H., Blennow, K., Okonkwo, O. C., Gleason, C. E., Dowling, N. M., Bratzke, L. C., Rowley, H. A., Sager, M. A., Asthana, S., Johnson, S. C., & Bendlin, B. B. (2016). Cerebrospinal Fluid Markers of Alzheimer's Disease Pathology and Microglial Activation are Associated with Altered White Matter Microstructure in Asymptomatic Adults at Risk for Alzheimer's Disease. *J Alzheimers Dis, 50*(3), 873-886. doi:10.3233/jad-150897

Mesulam, M., Guillozet, A., Shaw, P., & Quinn, B. (2002). Widely spread butyrylcholinesterase can hydrolyze acetylcholine in the normal and Alzheimer brain. *Neurobiol Dis, 9*(1), 88-93. doi:10.1006/nbdi.2001.0462

Mesulam, M. M. (2013). Cholinergic circuitry of the human nucleus basalis and its fate in Alzheimer's disease. *J Comp Neurol, 521*(18), 4124-4144. doi:10.1002/cne.23415

Mesulam, M. M., & Asuncion Morán, M. (1987). Cholinesterases within neurofibrillary tangles related to age and Alzheimer's disease. *Ann Neurol, 22*(2), 223-228. doi:10.1002/ana.410220206

Middeldorp, J., & Hol, E. M. (2011). GFAP in health and disease. *Progress in Neurobiology, 93*(3), 421-443. doi:https://doi.org/10.1016/j.pneurobio.2011.01.005

Mielke, M. M., Bandaru, V. V., Haughey, N. J., Rabins, P. V., Lyketsos, C. G., & Carlson, M. C. (2010a). Serum sphingomyelins and ceramides are early predictors of memory impairment. *Neurobiol Aging, 31*(1), 17-24. doi:10.1016/j.neurobiolaging.2008.03.011

Mielke, M. M., Haughey, N. J., Bandaru, V. V., Schech, S., Carrick, R., Carlson, M. C., Mori, S., Miller, M. I., Ceritoglu, C., Brown, T., Albert, M., & Lyketsos, C. G. (2010b). Plasma ceramides are altered in mild cognitive impairment and predict cognitive decline and hippocampal volume loss. *Alzheimers Dement, 6*(5), 378-385. doi:10.1016/j.jalz.2010.03.014

Mielke, M. M., Haughey, N. J., Bandaru, V. V. R., Zetterberg, H., Blennow, K., Andreasson, U., Johnson, S. C., Gleason, C. E., Blazel, H. M., Puglielli, L., Sager, M. A., Asthana, S., & Carlsson, C. M. (2014). Cerebrospinal fluid sphingolipids, β-amyloid, and tau in adults at risk for Alzheimer's disease. *Neurobiol Aging, 35*(11), 2486-2494. doi:10.1016/j.neurobiolaging.2014.05.019

Mitchell, A. J., & Shiri-Feshki, M. (2009). Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. *Acta Psychiatr Scand, 119*(4), 252-265. doi:10.1111/j.1600-0447.2008.01326.x

Molinuevo, J. L., Ayton, S., Batrla, R., Bednar, M. M., Bittner, T., Cummings, J., Fagan, A. M., Hampel, H., Mielke, M. M., Mikulskis, A., O'Bryant, S., Scheltens, P., Sevigny, J., Shaw, L. M., Soares, H. D., Tong, G., Trojanowski, J. Q., Zetterberg, H., & Blennow, K. (2018). Current state of Alzheimer's fluid biomarkers. *Acta neuropathologica, 136*(6), 821-853. doi:10.1007/s00401-018-1932-x

Motter, R., Vigo-Pelfrey, C., Kholodenko, D., Barbour, R., Johnson-Wood, K., Galasko, D., Chang, L., Miller, B., Clark, C., Green, R., & et al. (1995). Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol, 38*(4), 643-648. doi:10.1002/ana.410380413

Mukhopadhyay, A., Saddoughi, S. A., Song, P., Sultan, I., Ponnusamy, S., Senkal, C. E., Snook, C. F., Arnold, H. K., Sears, R. C., Hannun, Y. A., & Ogretmen, B. (2009). Direct interaction between the inhibitor 2 and ceramide via sphingolipid-protein binding is involved in the regulation of protein phosphatase 2A activity and signaling. *Faseb j, 23*(3), 751-763. doi:10.1096/fj.08-120550

Muramori, F., Kobayashi, K., & Nakamura, I. (1998). A quantitative study of neurofibrillary tangles, senile plaques and astrocytes in the hippocampal subdivisions and entorhinal cortex in Alzheimer's disease, normal controls and non-Alzheimer neuropsychiatric diseases. *Psychiatry Clin Neurosci, 52*(6), 593-599. doi:10.1111/j.1440-1819.1998.tb02706.x

Musiek, E. S., & Bennett, D. A. (2021). Aducanumab and the “post-amyloid” era of Alzheimer research? *Neuron*. doi:https://doi.org/10.1016/j.neuron.2021.09.007

Nebes, R. D. (1989). Semantic memory in Alzheimer's disease. *Psychological Bulletin, 106*(3), 377-394. doi:10.1037/0033-2909.106.3.377

Nizri, E., Hamra-Amitay, Y., Sicsic, C., Lavon, I., & Brenner, T. (2006). Anti-inflammatory properties of cholinergic up-regulation: A new role for acetylcholinesterase inhibitors. *Neuropharmacology, 50*(5), 540-547. doi:10.1016/j.neuropharm.2005.10.013

Nooijen, P. T., Schoonderwaldt, H. C., Wevers, R. A., Hommes, O. R., & Lamers, K. J. (1997). Neuron-specific enolase, S-100 protein, myelin basic protein and lactate in CSF in dementia. *Dement Geriatr Cogn Disord, 8*(3), 169-173. doi:10.1159/000106627

Nunes-Tavares, N., Santos, L. E., Stutz, B., Brito-Moreira, J., Klein, W. L., Ferreira, S. T., & de Mello, F. G. (2012). Inhibition of choline acetyltransferase as a mechanism for cholinergic dysfunction induced by amyloid-β peptide oligomers. *J Biol Chem, 287*(23), 19377-19385. doi:10.1074/jbc.M111.321448

Olsson, B., Lautner, R., Andreasson, U., Öhrfelt, A., Portelius, E., Bjerke, M., Hölttä, M., Rosén, C., Olsson, C., Strobel, G., Wu, E., Dakin, K., Petzold, M., Blennow, K., & Zetterberg, H. (2016). CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *The Lancet. Neurology, 15*(7), 673-684. doi:10.1016/s1474-4422(16)00070-3

Olsson, B., Portelius, E., Cullen, N. C., Sandelius, Å., Zetterberg, H., Andreasson, U., Höglund, K., Irwin, D. J., Grossman, M., Weintraub, D., Chen-Plotkin, A., Wolk, D., McCluskey, L., Elman, L., Shaw, L. M., Toledo, J. B., McBride, J., Hernandez-Con, P., Lee, V. M., Trojanowski, J. Q., & Blennow, K. (2019). Association of Cerebrospinal Fluid Neurofilament Light Protein Levels With Cognition in Patients With Dementia, Motor Neuron Disease, and Movement Disorders. *JAMA Neurol, 76*(3), 318-325. doi:10.1001/jamaneurol.2018.3746

Olubiyi, O. O., & Strodel, B. (2012). Structures of the amyloid β-peptides Aβ1-40 and Aβ1-42 as influenced by pH and a D-peptide. *J Phys Chem B, 116*(10), 3280-3291. doi:10.1021/jp2076337

Ousman, S. S., & Kubes, P. (2012). Immune surveillance in the central nervous system. *Nature Neuroscience, 15*(8), 1096-1101. doi:10.1038/nn.3161

Ovod, V., Ramsey, K. N., Mawuenyega, K. G., Bollinger, J. G., Hicks, T., Schneider, T., Sullivan, M., Paumier, K., Holtzman, D. M., Morris, J. C., Benzinger, T., Fagan, A. M., Patterson, B. W., & Bateman, R. J. (2017). Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimers Dement, 13*(8), 841-849. doi:10.1016/j.jalz.2017.06.2266

Parnetti, L., Tiraboschi, P., Lanari, A., Peducci, M., Padiglioni, C., D'Amore, C., Pierguidi, L., Tambasco, N., Rossi, A., & Calabresi, P. (2008). Cerebrospinal fluid biomarkers in Parkinson's disease with dementia and dementia with Lewy bodies. *Biol Psychiatry, 64*(10), 850-855. doi:10.1016/j.biopsych.2008.02.016

Pavlov, V. A., Parrish, W. R., Rosas-Ballina, M., Ochani, M., Puerta, M., Ochani, K., Chavan, S., Al-Abed, Y., & Tracey, K. J. (2009). Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. *Brain Behav Immun, 23*(1), 41-45. doi:10.1016/j.bbi.2008.06.011

Pedersen, W. A., & Blusztajn, J. K. (1997). Characterization of the acetylcholine-reducing effect of the amyloid-beta peptide in mouse SN56 cells. *Neurosci Lett, 239*(2-3), 77-80. doi:10.1016/s0304-3940(97)00913-0

Pedersen, W. A., Kloczewiak, M. A., & Blusztajn, J. K. (1996). Amyloid beta-protein reduces acetylcholine synthesis in a cell line derived from cholinergic neurons of the basal forebrain. *Proc Natl Acad Sci U S A, 93*(15), 8068-8071. doi:10.1073/pnas.93.15.8068

Pereira, J. B., Janelidze, S., Ossenkoppele, R., Kvartsberg, H., Brinkmalm, A., Mattsson-Carlgren, N., Stomrud, E., Smith, R., Zetterberg, H., Blennow, K., & Hansson, O. (2020). Untangling the association of amyloid-β and tau with synaptic and axonal loss in Alzheimer's disease. *Brain, 144*(1), 310-324. doi:10.1093/brain/awaa395

Perrin, R. J., Fagan, A. M., & Holtzman, D. M. (2009). Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. *Nature, 461*(7266), 916-922. doi:10.1038/nature08538

Perry, E. K., Tomlinson, B. E., Blessed, G., Bergmann, K., Gibson, P. H., & Perry, R. H. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br Med J, 2*(6150), 1457-1459. doi:10.1136/bmj.2.6150.1457

Perry, R. H., Blessed, G., Perry, E. K., & Tomlinson, B. E. (1980). Histochemical observations on cholinesterase activities in the brains of elderly normal and demented (Alzheimer-type) patients. *Age Ageing, 9*(1), 9-16. doi:10.1093/ageing/9.1.9

Perry, R. J., & Hodges, J. R. (1999). Attention and executive deficits in Alzheimer's disease: A critical review. *Brain, 122*(3), 383-404. doi:10.1093/brain/122.3.383

Peskind, E. R., Griffin, W. S. T., Akama, K. T., Raskind, M. A., & Van Eldik, L. J. (2001). Cerebrospinal fluid S100B is elevated in the earlier stages of Alzheimer's disease. *Neurochemistry International, 39*(5), 409-413. doi:https://doi.org/10.1016/S0197-0186(01)00048-1

Peter, J., Lahr, J., Minkova, L., Lauer, E., Grothe, M. J., Teipel, S., Köstering, L., Kaller, C. P., Heimbach, B., Hüll, M., Normann, C., Nissen, C., Reis, J., & Klöppel, S. (2016). Contribution of the Cholinergic System to Verbal Memory Performance in Mild Cognitive Impairment. *J Alzheimers Dis, 53*(3), 991-1001. doi:10.3233/JAD-160273

Petersen, R. C. (2004). Mild cognitive impairment as a diagnostic entity. *J Intern Med, 256*(3), 183-194. doi:10.1111/j.1365-2796.2004.01388.x

Petzold, A. (2005). Neurofilament phosphoforms: Surrogate markers for axonal injury, degeneration and loss. *Journal of the Neurological Sciences, 233*(1), 183-198. doi:https://doi.org/10.1016/j.jns.2005.03.015

Pijnenburg, Y. A., Janssen, J. C., Schoonenboom, N. S., Petzold, A., Mulder, C., Stigbrand, T., Norgren, N., Heijst, H., Hack, C. E., Scheltens, P., & Teunissen, C. E. (2007). CSF neurofilaments in frontotemporal dementia compared with early onset Alzheimer's disease and controls. *Dement Geriatr Cogn Disord, 23*(4), 225-230. doi:10.1159/000099473

Pike, C. J., Cummings, B. J., & Cotman, C. W. (1995). Early association of reactive astrocytes with senile plaques in Alzheimer's disease. *Exp Neurol, 132*(2), 172-179. doi:10.1016/0014-4886(95)90022-5

Porchet, R., Probst, A., Bouras, C., Dráberová, E., Dráber, P., & Riederer, B. M. (2003). Analysis of glial acidic fibrillary protein in the human entorhinal cortex during aging and in Alzheimer's disease. *Proteomics, 3*(8), 1476-1485. doi:10.1002/pmic.200300456

Portelius, E., Tran, A. J., Andreasson, U., Persson, R., Brinkmalm, G., Zetterberg, H., Blennow, K., & Westman-Brinkmalm, A. (2007). Characterization of amyloid beta peptides in cerebrospinal fluid by an automated immunoprecipitation procedure followed by mass spectrometry. *J Proteome Res, 6*(11), 4433-4439. doi:10.1021/pr0703627

Portelius, E., Westman-Brinkmalm, A., Zetterberg, H., & Blennow, K. (2006). Determination of beta-amyloid peptide signatures in cerebrospinal fluid using immunoprecipitation-mass spectrometry. *J Proteome Res, 5*(4), 1010-1016. doi:10.1021/pr050475v

Prince, M., Albanese, E., Guerchet, M., & Prina, M. (2014). *World Alzheimer Report 2014: Dementia and Risk Reduction. An Analysis of Protective and Modifiable Factors*.

Prince, M., Wimo, A., Guerchet, M., Ali, G.-C., Wu, Y.-T., & Prina, M. (2015). *World Alzheimer Report 2015. The Global Impact of Dementia. An Analysis of Prevalence, Incidence, Cost and Trends*.

Puglielli, L., Ellis, B. C., Saunders, A. J., & Kovacs, D. M. (2003). Ceramide stabilizes beta-site amyloid precursor protein-cleaving enzyme 1 and promotes amyloid beta-peptide biogenesis. *J Biol Chem, 278*(22), 19777-19783. doi:10.1074/jbc.M300466200

Qaseem, A., Snow, V., Cross, J. T., Jr., Forciea, M. A., Hopkins, R., Jr., Shekelle, P., Adelman, A., Mehr, D., Schellhase, K., Campos-Outcalt, D., Santaguida, P., & Owens, D. K. (2008). Current pharmacologic treatment of dementia: a clinical practice guideline from the American College of Physicians and the American Academy of Family Physicians. *Ann Intern Med, 148*(5), 370-378. doi:10.7326/0003-4819-148-5-200803040-00008

Querol-Vilaseca, M., Colom-Cadena, M., Pegueroles, J., San Martín-Paniello, C., Clarimon, J., Belbin, O., Fortea, J., & Lleó, A. (2017). YKL-40 (Chitinase 3-like I) is expressed in a subset of astrocytes in Alzheimer's disease and other tauopathies. *J Neuroinflammation, 14*(1), 118. doi:10.1186/s12974-017-0893-7

Rajan, K. B., Aggarwal, N. T., McAninch, E. A., Weuve, J., Barnes, L. L., Wilson, R. S., DeCarli, C., & Evans, D. A. (2020). Remote Blood Biomarkers of Longitudinal Cognitive Outcomes in a Population Study. *Ann Neurol, 88*(6), 1065-1076. doi:https://doi.org/10.1002/ana.25874

Ramani, S., Berard, J. A., & Walker, L. A. S. (2021). The relationship between neurofilament light chain and cognition in neurological disorders: A scoping review. *J Neurol Sci, 420*, 117229. doi:10.1016/j.jns.2020.117229

Rao, M. V., Campbell, J., Yuan, A., Kumar, A., Gotow, T., Uchiyama, Y., & Nixon, R. A. (2003). The neurofilament middle molecular mass subunit carboxyl-terminal tail domains is essential for the radial growth and cytoskeletal architecture of axons but not for regulating neurofilament transport rate. *J Cell Biol, 163*(5), 1021-1031. doi:10.1083/jcb.200308076

Rathcke, C. N., & Vestergaard, H. (2009). YKL-40 - an emerging biomarker in cardiovascular disease and diabetes. *Cardiovascular Diabetology, 8*(1), 61. doi:10.1186/1475-2840-8-61

Rehli, M., Krause, S. W., & Andreesen, R. (1997). Molecular characterization of the gene for human cartilage gp-39 (CHI3L1), a member of the chitinase protein family and marker for late stages of macrophage differentiation. *Genomics, 43*(2), 221-225. doi:10.1006/geno.1997.4778

Rehli, M., Niller, H. H., Ammon, C., Langmann, S., Schwarzfischer, L., Andreesen, R., & Krause, S. W. (2003). Transcriptional regulation of CHI3L1, a marker gene for late stages of macrophage differentiation. *J Biol Chem, 278*(45), 44058-44067. doi:10.1074/jbc.M306792200

Reijs, B. L. R., Teunissen, C. E., Goncharenko, N., Betsou, F., Blennow, K., Baldeiras, I., Brosseron, F., Cavedo, E., Fladby, T., Froelich, L., Gabryelewicz, T., Gurvit, H., Kapaki, E., Koson, P., Kulic, L., Lehmann, S., Lewczuk, P., Lleó, A., Maetzler, W., de Mendonça, A., Miller, A.-M., Molinuevo, J. L., Mollenhauer, B., Parnetti, L., Rot, U., Schneider, A., Simonsen, A. H., Tagliavini, F., Tsolaki, M., Verbeek, M. M., Verhey, F. R. J., Zboch, M., Winblad, B., Scheltens, P., Zetterberg, H., & Visser, P. J. (2015). The Central Biobank and Virtual Biobank of BIOMARKAPD: A Resource for Studies on Neurodegenerative Diseases. *Frontiers in neurology, 6*, 216-216. doi:10.3389/fneur.2015.00216

Resnick, S. M., Sojkova, J., Zhou, Y., An, Y., Ye, W., Holt, D. P., Dannals, R. F., Mathis, C. A., Klunk, W. E., Ferrucci, L., Kraut, M. A., & Wong, D. F. (2010). Longitudinal cognitive decline is associated with fibrillar amyloid-beta measured by [11C]PiB. *Neurology, 74*(10), 807-815. doi:10.1212/WNL.0b013e3181d3e3e9

Rickmann, M., & Wolff, J. R. (1995). S100 protein expression in subpopulations of neurons of rat brain. *Neuroscience, 67*(4), 977-991. doi:https://doi.org/10.1016/0306-4522(94)00615-C

Riemenschneider, M., Wagenpfeil, S., Vanderstichele, H., Otto, M., Wiltfang, J., Kretzschmar, H., Vanmechelen, E., Förstl, H., & Kurz, A. (2003). Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. *Mol Psychiatry, 8*(3), 343-347. doi:10.1038/sj.mp.4001220

Rocher, A. B., Chapon, F., Blaizot, X., Baron, J.-C., & Chavoix, C. (2003). Resting-state brain glucose utilization as measured by PET is directly related to regional synaptophysin levels: a study in baboons. *NeuroImage, 20*(3), 1894-1898. doi:https://doi.org/10.1016/j.neuroimage.2003.07.002

Rosén, C., Mattsson, N., Johansson, P. M., Andreasson, U., Wallin, A., Hansson, O., Johansson, J.-O., Lamont, J., Svensson, J., Blennow, K., & Zetterberg, H. (2011). Discriminatory Analysis of Biochip-Derived Protein Patterns in CSF and Plasma in Neurodegenerative Diseases. *Frontiers in aging neuroscience, 3*, 1-1. doi:10.3389/fnagi.2011.00001

Rothermundt, M., Peters, M., Prehn, J. H., & Arolt, V. (2003). S100B in brain damage and neurodegeneration. *Microsc Res Tech, 60*(6), 614-632. doi:10.1002/jemt.10303

Saleem, M., Ratnam Bandaru, V. V., Herrmann, N., Swardfager, W., Mielke, M. M., Oh, P. I., Shammi, P., Kiss, A., Haughey, N. J., Rovinski, R., & Lanctôt, K. L. (2013). Ceramides predict verbal memory performance in coronary artery disease patients undertaking exercise: a prospective cohort pilot study. *BMC Geriatrics, 13*(1), 135. doi:10.1186/1471-2318-13-135

Salmon, D. P., & Bondi, M. W. (2009). Neuropsychological assessment of dementia. *Annual review of psychology, 60*, 257-282. doi:10.1146/annurev.psych.57.102904.190024

Salter, M. W., & Stevens, B. (2017). Microglia emerge as central players in brain disease. *Nature Medicine, 23*(9), 1018-1027. doi:10.1038/nm.4397

Salthouse, T. A. (2000). Aging and measures of processing speed. *Biol Psychol, 54*(1-3), 35-54. doi:10.1016/s0301-0511(00)00052-1

Sato, C., Barthélemy, N. R., Mawuenyega, K. G., Patterson, B. W., Gordon, B. A., Jockel-Balsarotti, J., Sullivan, M., Crisp, M. J., Kasten, T., Kirmess, K. M., Kanaan, N. M., Yarasheski, K. E., Baker-Nigh, A., Benzinger, T. L. S., Miller, T. M., Karch, C. M., & Bateman, R. J. (2018). Tau Kinetics in Neurons and the Human Central Nervous System. *Neuron, 98*(4), 861-864. doi:10.1016/j.neuron.2018.04.035

Satoh, Y., Hirakura, Y., Shibayama, S., Hirashima, N., Suzuki, T., & Kirino, Y. (2001). Beta-amyloid peptides inhibit acetylcholine release from cholinergic presynaptic nerve endings isolated from an electric ray. *Neurosci Lett, 302*(2-3), 97-100. doi:10.1016/s0304-3940(01)01665-2

Satoi, H., Tomimoto, H., Ohtani, R., Kitano, T., Kondo, T., Watanabe, M., Oka, N., Akiguchi, I., Furuya, S., Hirabayashi, Y., & Okazaki, T. (2005). Astroglial expression of ceramide in Alzheimer's disease brains: a role during neuronal apoptosis. *Neuroscience, 130*(3), 657-666. doi:10.1016/j.neuroscience.2004.08.056

Schliebs, R., & Arendt, T. (2006). The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. *J Neural Transm (Vienna), 113*(11), 1625-1644. doi:10.1007/s00702-006-0579-2

Schliebs, R., & Arendt, T. (2011). The cholinergic system in aging and neuronal degeneration. *Behav Brain Res, 221*(2), 555-563. doi:10.1016/j.bbr.2010.11.058

Schöll, M., Lockhart, S. N., Schonhaut, D. R., O'Neil, J. P., Janabi, M., Ossenkoppele, R., Baker, S. L., Vogel, J. W., Faria, J., Schwimmer, H. D., Rabinovici, G. D., & Jagust, W. J. (2016). PET Imaging of Tau Deposition in the Aging Human Brain. *Neuron, 89*(5), 971-982. doi:10.1016/j.neuron.2016.01.028

Selinfreund, R. H., Barger, S. W., Pledger, W. J., & Van Eldik, L. J. (1991). Neurotrophic protein S100 beta stimulates glial cell proliferation. *Proc Natl Acad Sci U S A, 88*(9), 3554-3558. doi:10.1073/pnas.88.9.3554

Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med, 8*(6), 595-608. doi:10.15252/emmm.201606210

Serrano-Pozo, A., Frosch, M. P., Masliah, E., & Hyman, B. T. (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med, 1*(1), a006189. doi:10.1101/cshperspect.a006189

Shao, Z., Janse, E., Visser, K., & Meyer, A. S. (2014). What do verbal fluency tasks measure? Predictors of verbal fluency performance in older adults. *Front Psychol, 5*, 772. doi:10.3389/fpsyg.2014.00772

Sherrington, R., Froelich, S., Sorbi, S., Campion, D., Chi, H., Rogaeva, E. A., Levesque, G., Rogaev, E. I., Lin, C., Liang, Y., Ikeda, M., Mar, L., Brice, A., Agid, Y., Percy, M. E., Clerget-Darpoux, F., Piacentini, S., Marcon, G., Nacmias, B., Amaducci, L., Frebourg, T., Lannfelt, L., Rommens, J. M., & St George-Hyslop, P. H. (1996). Alzheimer's disease associated with mutations in presenilin 2 is rare and variably penetrant. *Hum Mol Genet, 5*(7), 985-988. doi:10.1093/hmg/5.7.985

Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Tsuda, T., Mar, L., Foncin, J. F., Bruni, A. C., Montesi, M. P., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., Chumakov, I., Pollen, D., Brookes, A., Sanseau, P., Polinsky, R. J., Wasco, W., Da Silva, H. A., Haines, J. L., Perkicak-Vance, M. A., Tanzi, R. E., Roses, A. D., Fraser, P. E., Rommens, J. M., & St George-Hyslop, P. H. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature, 375*(6534), 754-760. doi:10.1038/375754a0

Silveyra, M. X., García-Ayllón, M. S., de Barreda, E. G., Small, D. H., Martínez, S., Avila, J., & Sáez-Valero, J. (2012). Altered expression of brain acetylcholinesterase in FTDP-17 human tau transgenic mice. *Neurobiol Aging, 33*(3), 624.e623-634. doi:10.1016/j.neurobiolaging.2011.03.006

Sims, R., van der Lee, S. J., Naj, A. C., Bellenguez, C., Badarinarayan, N., Jakobsdottir, J., Kunkle, B. W., Boland, A., Raybould, R., Bis, J. C., Martin, E. R., Grenier-Boley, B., Heilmann-Heimbach, S., Chouraki, V., Kuzma, A. B., Sleegers, K., Vronskaya, M., Ruiz, A., Graham, R. R., Olaso, R., Hoffmann, P., Grove, M. L., Vardarajan, B. N., Hiltunen, M., Nöthen, M. M., White, C. C., Hamilton-Nelson, K. L., Epelbaum, J., Maier, W., Choi, S. H., Beecham, G. W., Dulary, C., Herms, S., Smith, A. V., Funk, C. C., Derbois, C., Forstner, A. J., Ahmad, S., Li, H., Bacq, D., Harold, D., Satizabal, C. L., Valladares, O., Squassina, A., Thomas, R., Brody, J. A., Qu, L., Sánchez-Juan, P., Morgan, T., Wolters, F. J., Zhao, Y., Garcia, F. S., Denning, N., Fornage, M., Malamon, J., Naranjo, M. C. D., Majounie, E., Mosley, T. H., Dombroski, B., Wallon, D., Lupton, M. K., Dupuis, J., Whitehead, P., Fratiglioni, L., Medway, C., Jian, X., Mukherjee, S., Keller, L., Brown, K., Lin, H., Cantwell, L. B., Panza, F., McGuinness, B., Moreno-Grau, S., Burgess, J. D., Solfrizzi, V., Proitsi, P., Adams, H. H., Allen, M., Seripa, D., Pastor, P., Cupples, L. A., Price, N. D., Hannequin, D., Frank-García, A., Levy, D., Chakrabarty, P., Caffarra, P., Giegling, I., Beiser, A. S., Giedraitis, V., Hampel, H., Garcia, M. E., Wang, X., Lannfelt, L., Mecocci, P., Eiriksdottir, G., Crane, P. K., Pasquier, F., Boccardi, V., Henández, I., Barber, R. C., Scherer, M., Tarraga, L., Adams, P. M., Leber, M., Chen, Y., Albert, M. S., Riedel-Heller, S., Emilsson, V., Beekly, D., Braae, A., Schmidt, R., Blacker, D., Masullo, C., Schmidt, H., Doody, R. S., Spalletta, G., Longstreth, W. T., Jr., Fairchild, T. J., Bossù, P., Lopez, O. L., Frosch, M. P., Sacchinelli, E., Ghetti, B., Yang, Q., Huebinger, R. M., Jessen, F., Li, S., Kamboh, M. I., Morris, J., Sotolongo-Grau, O., Katz, M. J., Corcoran, C., Dunstan, M., Braddel, A., Thomas, C., Meggy, A., Marshall, R., Gerrish, A., Chapman, J., Aguilar, M., Taylor, S., Hill, M., Fairén, M. D., Hodges, A., Vellas, B., Soininen, H., Kloszewska, I., Daniilidou, M., Uphill, J., Patel, Y., Hughes, J. T., Lord, J., Turton, J., Hartmann, A. M., Cecchetti, R., Fenoglio, C., Serpente, M., Arcaro, M., Caltagirone, C., Orfei, M. D., Ciaramella, A., Pichler, S., Mayhaus, M., Gu, W., Lleó, A., Fortea, J., Blesa, R., Barber, I. S., Brookes, K., Cupidi, C., Maletta, R. G., Carrell, D., Sorbi, S., Moebus, S., Urbano, M., Pilotto, A., Kornhuber, J., Bosco, P., Todd, S., Craig, D., Johnston, J., Gill, M., Lawlor, B., Lynch, A., Fox, N. C., Hardy, J., Albin, R. L., Apostolova, L. G., Arnold, S. E., Asthana, S., Atwood, C. S., Baldwin, C. T., Barnes, L. L., Barral, S., Beach, T. G., Becker, J. T., Bigio, E. H., Bird, T. D., Boeve, B. F., Bowen, J. D., Boxer, A., Burke, J. R., Burns, J. M., Buxbaum, J. D., Cairns, N. J., Cao, C., Carlson, C. S., Carlsson, C. M., Carney, R. M., Carrasquillo, M. M., Carroll, S. L., Diaz, C. C., Chui, H. C., Clark, D. G., Cribbs, D. H., Crocco, E. A., DeCarli, C., Dick, M., Duara, R., Evans, D. A., Faber, K. M., Fallon, K. B., Fardo, D. W., Farlow, M. R., Ferris, S., Foroud, T. M., Galasko, D. R., Gearing, M., Geschwind, D. H., Gilbert, J. R.,

Graff-Radford, N. R., Green, R. C., Growdon, J. H., Hamilton, R. L., Harrell, L. E., Honig, L. S., Huentelman, M. J., Hulette, C. M., Hyman, B. T., Jarvik, G. P., Abner, E., Jin, L. W., Jun, G., Karydas, A., Kaye, J. A., Kim, R., Kowall, N. W., Kramer, J. H., LaFerla, F. M., Lah, J. J., Leverenz, J. B., Levey, A. I., Li, G., Lieberman, A. P., Lunetta, K. L., Lyketsos, C. G., Marson, D. C., Martiniuk, F., Mash, D. C., Masliah, E., McCormick, W. C., McCurry, S. M., McDavid, A. N., McKee, A. C., Mesulam, M., Miller, B. L., Miller, C. A., Miller, J. W., Morris, J. C., Murrell, J. R., Myers, A. J., O'Bryant, S., Olichney, J. M., Pankratz, V. S., Parisi, J. E., Paulson, H. L., Perry, W., Peskind, E., Pierce, A., Poon, W. W., Potter, H., Quinn, J. F., Raj, A., Raskind, M., Reisberg, B., Reitz, C., Ringman, J. M., Roberson, E. D., Rogaeva, E., Rosen, H. J., Rosenberg, R. N., Sager, M. A., Saykin, A. J., Schneider, J. A., Schneider, L. S., Seeley, W. W., Smith, A. G., Sonnen, J. A., Spina, S., Stern, R. A., Swerdlow, R. H., Tanzi, R. E., Thornton-Wells, T. A., Trojanowski, J. Q., Troncoso, J. C., Van Deerlin, V. M., Van Eldik, L. J., Vinters, H. V., Vonsattel, J. P., Weintraub, S., Welsh-Bohmer, K. A., Wilhelmsen, K. C., Williamson, J., Wingo, T. S., Woltjer, R. L., Wright, C. B., Yu, C. E., Yu, L., Garzia, F., Golamaully, F., Septier, G., Engelborghs, S., Vandenberghe, R., De Deyn, P. P., Fernadez, C. M., Benito, Y. A., Thonberg, H., Forsell, C., Lilius, L., Kinhult-Stählbom, A., Kilander, L., Brundin, R., Concari, L., Helisalmi, S., Koivisto, A. M., Haapasalo, A., Dermecourt, V., Fievet, N., Hanon, O., Dufouil, C., Brice, A., Ritchie, K., Dubois, B., Himali, J. J., Keene, C. D., Tschanz, J., Fitzpatrick, A. L., Kukull, W. A., Norton, M., Aspelund, T., Larson, E. B., Munger, R., Rotter, J. I., Lipton, R. B., Bullido, M. J., Hofman, A., Montine, T. J., Coto, E., Boerwinkle, E., Petersen, R. C., Alvarez, V., Rivadeneira, F., Reiman, E. M., Gallo, M., O'Donnell, C. J., Reisch, J. S., Bruni, A. C., Royall, D. R., Dichgans, M., Sano, M., Galimberti, D., St George-Hyslop, P., Scarpini, E., Tsuang, D. W., Mancuso, M., Bonuccelli, U., Winslow, A. R., Daniele, A., Wu, C. K., Peters, O., Nacmias, B., Riemenschneider, M., Heun, R., Brayne, C., Rubinsztein, D. C., Bras, J., Guerreiro, R., Al-Chalabi, A., Shaw, C. E., Collinge, J., Mann, D., Tsolaki, M., Clarimón, J., Sussams, R., Lovestone, S., O'Donovan, M. C., Owen, M. J., Behrens, T. W., Mead, S., Goate, A. M., Uitterlinden, A. G., Holmes, C., Cruchaga, C., Ingelsson, M., Bennett, D. A., Powell, J., Golde, T. E., Graff, C., De Jager, P. L., Morgan, K., Ertekin-Taner, N., Combarros, O., Psaty, B. M., Passmore, P., Younkin, S. G., Berr, C., Gudnason, V., Rujescu, D., Dickson, D. W., Dartigues, J. F., DeStefano, A. L., Ortega-Cubero, S., Hakonarson, H., Campion, D., Boada, M., Kauwe, J. K., Farrer, L. A., Van Broeckhoven, C., Ikram, M. A., Jones, L., Haines, J. L., Tzourio, C., Launer, L. J., Escott-Price, V., Mayeux, R., Deleuze, J. F., Amin, N., Holmans, P. A., Pericak-Vance, M. A., Amouyel, P., van Duijn, C. M., Ramirez, A., Wang, L. S., Lambert, J. C., Seshadri, S.,

Williams, J., & Schellenberg, G. D. (2017). Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease. *Nat Genet, 49*(9), 1373-1384. doi:10.1038/ng.3916

Sjögren, M., Rosengren, L., Minthon, L., Davidsson, P., Blennow, K., & Wallin, A. (2000). Cytoskeleton proteins in CSF distinguish frontotemporal dementia from AD. *Neurology, 54*(10), 1960-1964. doi:10.1212/wnl.54.10.1960

Skillbäck, T., Farahmand, B., Bartlett, J. W., Rosén, C., Mattsson, N., Nägga, K., Kilander, L., Religa, D., Wimo, A., Winblad, B., Rosengren, L., Schott, J. M., Blennow, K., Eriksdotter, M., & Zetterberg, H. (2014a). CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. *Neurology, 83*(21), 1945-1953. doi:10.1212/wnl.0000000000001015

Skillbäck, T., Farahmand, B. Y., Rosén, C., Mattsson, N., Nägga, K., Kilander, L., Religa, D., Wimo, A., Winblad, B., Schott, J. M., Blennow, K., Eriksdotter, M., & Zetterberg, H. (2015). Cerebrospinal fluid tau and amyloid-β1-42 in patients with dementia. *Brain, 138*(Pt 9), 2716-2731. doi:10.1093/brain/awv181

Skillbäck, T., Rosén, C., Asztely, F., Mattsson, N., Blennow, K., & Zetterberg, H. (2014b). Diagnostic performance of cerebrospinal fluid total tau and phosphorylated tau in Creutzfeldt-Jakob disease: results from the Swedish Mortality Registry. *JAMA Neurol, 71*(4), 476-483. doi:10.1001/jamaneurol.2013.6455

Smith, G. E., & Bondi, M. W. (2013). *Mild cognitive impairment and dementia: Definitions, diagnosis, and treatment*. New York, NY, US: Oxford University Press.

Snaedal, J., Johannesson, G. H., Gudmundsson, T. E., Blin, N. P., Emilsdottir, A. L., Einarsson, B., & Johnsen, K. (2012). Diagnostic accuracy of statistical pattern recognition of electroencephalogram registration in evaluation of cognitive impairment and dementia. *Dement Geriatr Cogn Disord, 34*(1), 51-60. doi:10.1159/000339996

Sofroniew, M. V., & Vinters, H. V. (2010). Astrocytes: biology and pathology. *Acta neuropathologica, 119*(1), 7-35. doi:10.1007/s00401-009-0619-8

Spies, P. E., Verbeek, M. M., Groen, T. v., & Claassen, J. A. H. R. (2012). Reviewing reasons for the decreased CSF Abeta42 concentration in Alzheimer disease. *Frontiers in Bioscience-Landmark, 17*(6), 2024-2034. doi:10.2741/4035

Spires-Jones, T. L., & Hyman, B. T. (2014). The intersection of amyloid beta and tau at synapses in Alzheimer's disease. *Neuron, 82*(4), 756-771. doi:10.1016/j.neuron.2014.05.004

Squire, L. R., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science, 253*(5026), 1380-1386. doi:10.1126/science.1896849

St George-Hyslop, P., Haines, J., Rogaev, E., Mortilla, M., Vaula, G., Pericak-Vance, M., Foncin, J. F., Montesi, M., Bruni, A., Sorbi, S., Rainero, I., Pinessi, L., Pollen, D., Polinsky, R., Nee, L., Kennedy, J., Macciardi, F., Rogaeva, E., Liang, Y., Alexandrova, N., Lukiw, W., Schlumpf, K., Tanzi, R., Tsuda, T., Farrer, L., Cantu, J. M., Duara, R., Amaducci, L., Bergamini, L., Gusella, J., Roses, A., Crapper McLachlan, D., & et al. (1992). Genetic evidence for a novel familial Alzheimer's disease locus on chromosome 14. *Nat Genet, 2*(4), 330-334. doi:10.1038/ng1292-330

St George-Hyslop, P. H., Tanzi, R. E., Polinsky, R. J., Haines, J. L., Nee, L., Watkins, P. C., Myers, R. H., Feldman, R. G., Pollen, D., Drachman, D., & et al. (1987). The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science, 235*(4791), 885-890. doi:10.1126/science.2880399

Steardo, L., Jr., Bronzuoli, M. R., Iacomino, A., Esposito, G., Steardo, L., & Scuderi, C. (2015). Does neuroinflammation turn on the flame in Alzheimer's disease? Focus on astrocytes. *Front Neurosci, 9*, 259. doi:10.3389/fnins.2015.00259

Stranahan, A. M., Cutler, R. G., Button, C., Telljohann, R., & Mattson, M. P. (2011). Diet-induced elevations in serum cholesterol are associated with alterations in hippocampal lipid metabolism and increased oxidative stress. *J Neurochem, 118*(4), 611-615. doi:10.1111/j.1471-4159.2011.07351.x

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of experimental psychology, 18*(6), 643.

Strozyk, D., Blennow, K., White, L. R., & Launer, L. J. (2003). CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology, 60*(4), 652-656. doi:10.1212/01.wnl.0000046581.81650.d0

Tapiola, T., Alafuzoff, I., Herukka, S. K., Parkkinen, L., Hartikainen, P., Soininen, H., & Pirttilä, T. (2009). Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain. *Arch Neurol, 66*(3), 382-389. doi:10.1001/archneurol.2008.596

Teitsdottir, U. D., Halldorsson, S., Rolfsson, O., Lund, S. H., Jonsdottir, M. K., Snaedal, J., & Petersen, P. H. (2021). Cerebrospinal Fluid C18 Ceramide Associates with Markers of Alzheimer's Disease and Inflammation at the Pre- and Early Stages of Dementia. *J Alzheimers Dis, 81*(1), 231-244. doi:10.3233/jad-200964

Teitsdottir, U. D., Jonsdottir, M. K., Lund, S. H., Darreh-Shori, T., Snaedal, J., & Petersen, P. H. (2020). Association of glial and neuronal degeneration markers with Alzheimer's disease cerebrospinal fluid profile and cognitive functions. *Alzheimer's research & therapy, 12*(1), 92. doi:10.1186/s13195-020-00657-8

Theuns, J., Del-Favero, J., Dermaut, B., van Duijn, C. M., Backhovens, H., Van den Broeck, M. V., Serneels, S., Corsmit, E., Van Broeckhoven, C. V., & Cruts, M. (2000). Genetic variability in the regulatory region of presenilin 1 associated with risk for Alzheimer's disease and variable expression. *Hum Mol Genet, 9*(3), 325-331. doi:10.1093/hmg/9.3.325

Tibshirani, R. (1996). Regression Shrinkage and Selection Via the Lasso. *Journal of the Royal Statistical Society: Series B (Methodological), 58*(1), 267-288. doi:https://doi.org/10.1111/j.2517-6161.1996.tb02080.x

Tolboom, N., van der Flier, W. M., Yaqub, M., Boellaard, R., Verwey, N. A., Blankenstein, M. A., Windhorst, A. D., Scheltens, P., Lammertsma, A. A., & van Berckel, B. N. (2009). Relationship of cerebrospinal fluid markers to 11C-PiB and 18F-FDDNP binding. *J Nucl Med, 50*(9), 1464-1470. doi:10.2967/jnumed.109.064360

Tombaugh, T. N. (2004). Trail Making Test A and B: normative data stratified by age and education. *Arch Clin Neuropsychol, 19*(2), 203-214. doi:10.1016/s0887-6177(03)00039-8

Tramontina, F., Conte, S., Gonçalves, D., Gottfried, C., Portela, L. V., Vinade, L., Salbego, C., & Gonçalves, C. A. (2002). Developmental changes in S100B content in brain tissue, cerebrospinal fluid, and astrocyte cultures of rats. *Cell Mol Neurobiol, 22*(3), 373-378. doi:10.1023/a:1020732304591

Tromp, D., Dufour, A., Lithfous, S., Pebayle, T., & Després, O. (2015). Episodic memory in normal aging and Alzheimer disease: Insights from imaging and behavioral studies. *Ageing Research Reviews, 24*, 232-262. doi:https://doi.org/10.1016/j.arr.2015.08.006

Tsugawa, H., Cajka, T., Kind, T., Ma, Y., Higgins, B., Ikeda, K., Kanazawa, M., VanderGheynst, J., Fiehn, O., & Arita, M. (2015). MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nature Methods, 12*(6), 523-526. doi:10.1038/nmeth.3393

Tulving, E. (1983). *Elements of Episodic Memory*: Oxford University Press.

Ulrich, J., Meier-Ruge, W., Probst, A., Meier, E., & Ipsen, S. (1990). Senile plaques: staining for acetylcholinesterase and A4 protein: a comparative study in the hippocampus and entorhinal cortex. *Acta neuropathologica, 80*(6), 624-628. doi:10.1007/bf00307630

Vågberg, M., Norgren, N., Dring, A., Lindqvist, T., Birgander, R., Zetterberg, H., & Svenningsson, A. (2015). Levels and Age Dependency of Neurofilament Light and Glial Fibrillary Acidic Protein in Healthy Individuals and Their Relation to the Brain Parenchymal Fraction. *PLoS One, 10*(8), e0135886. doi:10.1371/journal.pone.0135886

Van Broeckhoven, C., Backhovens, H., Cruts, M., De Winter, G., Bruyland, M., Cras, P., & Martin, J. J. (1992). Mapping of a gene predisposing to early-onset Alzheimer's disease to chromosome 14q24.3. *Nat Genet, 2*(4), 335-339. doi:10.1038/ng1292-335

Van Cauwenberghe, C., Van Broeckhoven, C., & Sleegers, K. (2016). The genetic landscape of Alzheimer disease: clinical implications and perspectives. *Genetics in medicine : official journal of the American College of Medical Genetics, 18*(5), 421-430. doi:10.1038/gim.2015.117

van der Kant, R., Goldstein, L. S. B., & Ossenkoppele, R. (2020). Amyloid-β-independent regulators of tau pathology in Alzheimer disease. *Nature Reviews Neuroscience, 21*(1), 21-35. doi:10.1038/s41583-019-0240-3

van Deursen, J. A., Vuurman, E. F. P. M., Smits, L. L., Verhey, F. R. J., & Riedel, W. J. (2009). Response speed, contingent negative variation and P300 in Alzheimer's disease and MCI. *Brain and Cognition, 69*(3), 592-599. doi:https://doi.org/10.1016/j.bandc.2008.12.007

van Meer, G., Voelker, D. R., & Feigenson, G. W. (2008). Membrane lipids: where they are and how they behave. *Nature reviews. Molecular cell biology, 9*(2), 112-124. doi:10.1038/nrm2330

van Westerloo, D. J., Giebelen, I. A. J., Florquin, S., Daalhuisen, J., Bruno, M. J., de Vos, A. F., Tracey, K. J., & van der Poll, T. (2005). The Cholinergic Anti-Inflammatory Pathway Regulates the Host Response during Septic Peritonitis. *The Journal of Infectious Diseases, 191*(12), 2138-2148. doi:10.1086/430323

Verberk, I., Laarhuis, M., Bosch, K., Ebenau, J., Leeuwenstijn, M., Prins, N., Scheltens, P., Teunissen, C., & Flier, W. (2021). Serum markers glial fibrillary acidic protein and neurofilament light for prognosis and monitoring in cognitively normal older people: a prospective memory clinic-based cohort study. *The Lancet Healthy Longevity, 2*. doi:10.1016/S2666-7568(20)30061-1

Verghese, P. B., Castellano, J. M., & Holtzman, D. M. (2011). Apolipoprotein E in Alzheimer's disease and other neurological disorders. *The Lancet. Neurology, 10*(3), 241-252. doi:10.1016/S1474-4422(10)70325-2

Verma, M., & Howard, R. J. (2012). Semantic memory and language dysfunction in early Alzheimer's disease: a review. *Int J Geriatr Psychiatry, 27*(12), 1209-1217. doi:https://doi.org/10.1002/gps.3766

Vetrivel, K. S., Zhang, Y.-w., Xu, H., & Thinakaran, G. (2006). Pathological and physiological functions of presenilins. *Molecular neurodegeneration, 1*, 4-4. doi:10.1186/1750-1326-1-4

Vijayaraghavan, S., Karami, A., Aeinehband, S., Behbahani, H., Grandien, A., Nilsson, B., Ekdahl, K. N., Lindblom, R. P., Piehl, F., & Darreh-Shori, T. (2013). Regulated Extracellular Choline Acetyltransferase Activity- The Plausible Missing Link of the Distant Action of Acetylcholine in the Cholinergic Anti-Inflammatory Pathway. *PLoS One, 8*(6), e65936. doi:10.1371/journal.pone.0065936

Villain, N., & Dubois, B. (2019). Alzheimer's Disease Including Focal Presentations. *Semin Neurol, 39*(2), 213-226. doi:10.1055/s-0039-1681041

Villemagne, V. L., Burnham, S., Bourgeat, P., Brown, B., Ellis, K. A., Salvado, O., Szoeke, C., Macaulay, S. L., Martins, R., Maruff, P., Ames, D., Rowe, C. C., & Masters, C. L. (2013). Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *The Lancet. Neurology, 12*(4), 357-367. doi:10.1016/s1474-4422(13)70044-9

Walter, J., & van Echten-Deckert, G. (2013). Cross-talk of membrane lipids and Alzheimer-related proteins. *Molecular neurodegeneration, 8*, 34. doi:10.1186/1750-1326-8-34

Wang, F., Li, S., Xiang, J., & Li, F. (2019). Transcriptome analysis reveals the activation of neuroendocrine-immune system in shrimp hemocytes at the early stage of WSSV infection. *BMC genomics, 20*(1), 247-247. doi:10.1186/s12864-019-5614-4

Wang, Y., & Mandelkow, E. (2016). Tau in physiology and pathology. *Nat Rev Neurosci, 17*(1), 5-21. doi:10.1038/nrn.2015.1

Warkentin, S., Erikson, C., & Janciauskiene, S. (2008). rCBF pathology in Alzheimer's disease is associated with slow processing speed. *Neuropsychologia, 46*(5), 1193-1200. doi:10.1016/j.neuropsychologia.2007.08.029

Wechsler, D. (1955). Wechsler adult intelligence scale.

Wechsler, D. (1987). *WMS-R: Wechsler memory scale-revised*: Psychological Corporation.

Weissberger, G. H., Strong, J. V., Stefanidis, K. B., Summers, M. J., Bondi, M. W., & Stricker, N. H. (2017). Diagnostic Accuracy of Memory Measures in Alzheimer's Dementia and Mild Cognitive Impairment: a Systematic Review and Meta-Analysis. *Neuropsychol Rev, 27*(4), 354-388. doi:10.1007/s11065-017-9360-6

Wennström, M., Surova, Y., Hall, S., Nilsson, C., Minthon, L., Hansson, O., & Nielsen, H. M. (2015). The Inflammatory Marker YKL-40 Is Elevated in Cerebrospinal Fluid from Patients with Alzheimer's but Not Parkinson's Disease or Dementia with Lewy Bodies. *PLoS One, 10*(8), e0135458. doi:10.1371/journal.pone.0135458

Wilke, C., Preische, O., Deuschle, C., Roeben, B., Apel, A., Barro, C., Maia, L., Maetzler, W., Kuhle, J., & Synofzik, M. (2016). Neurofilament light chain in FTD is elevated not only in cerebrospinal fluid, but also in serum. *J Neurol Neurosurg Psychiatry, 87*(11), 1270-1272. doi:10.1136/jnnp-2015-312972

Wiltfang, J., Esselmann, H., Bibl, M., Hüll, M., Hampel, H., Kessler, H., Frölich, L., Schröder, J., Peters, O., Jessen, F., Luckhaus, C., Perneczky, R., Jahn, H., Fiszer, M., Maler, J. M., Zimmermann, R., Bruckmoser, R., Kornhuber, J., & Lewczuk, P. (2007). Amyloid beta peptide ratio 42/40 but not A beta 42 correlates with phospho-Tau in patients with low- and high-CSF A beta 40 load. *J Neurochem, 101*(4), 1053-1059. doi:10.1111/j.1471-4159.2006.04404.x

Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L. O., Nordberg, A., Bäckman, L., Albert, M., Almkvist, O., Arai, H., Basun, H., Blennow, K., de Leon, M., DeCarli, C., Erkinjuntti, T., Giacobini, E., Graff, C., Hardy, J., Jack, C., Jorm, A., Ritchie, K., van Duijn, C., Visser, P., & Petersen, R. C. (2004). Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *J Intern Med, 256*(3), 240-246. doi:10.1111/j.1365-2796.2004.01380.x

Woolf, N. J., & Butcher, L. L. (2011). Cholinergic systems mediate action from movement to higher consciousness. *Behav Brain Res, 221*(2), 488-498. doi:10.1016/j.bbr.2009.12.046

Wright, C. I., Geula, C., & Mesulam, M. M. (1993). Neurological cholinesterases in the normal brain and in Alzheimer's disease: relationship to plaques, tangles, and patterns of selective vulnerability. *Ann Neurol, 34*(3), 373-384. doi:10.1002/ana.410340312

Xing, Y., Tang, Y., Zhao, L., Wang, Q., Qin, W., Ji, X., Zhang, J., & Jia, J. (2016). Associations between plasma ceramides and cognitive and neuropsychiatric manifestations in Parkinson's disease dementia. *J Neurol Sci, 370*, 82-87. doi:10.1016/j.jns.2016.09.028

Yang, Q., Hamberger, A., Hyden, H., Wang, S., Stigbrand, T., & Haglid, K. G. (1995). S-100 beta has a neuronal localisation in the rat hindbrain revealed by an antigen retrieval method. *Brain Res, 696*(1-2), 49-61. doi:10.1016/0006-8993(95)00755-f

Yang, T., Li, S., Xu, H., Walsh, D. M., & Selkoe, D. J. (2017). Large Soluble Oligomers of Amyloid β-Protein from Alzheimer Brain Are Far Less Neuroactive Than the Smaller Oligomers to Which They Dissociate. *J Neurosci, 37*(1), 152-163. doi:10.1523/jneurosci.1698-16.2016

Yang, Z., & Wang, K. K. (2015). Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. *Trends Neurosci, 38*(6), 364-374. doi:10.1016/j.tins.2015.04.003

Yoshikai, S., Sasaki, H., Doh-ura, K., Furuya, H., & Sakaki, Y. (1990). Genomic organization of the human amyloid beta-protein precursor gene. *Gene, 87*(2), 257-263. doi:10.1016/0378-1119(90)90310-n

Zannis, V. I., Breslow, J. L., Utermann, G., Mahley, R. W., Weisgraber, K. H., Havel, R. J., Goldstein, J. L., Brown, M. S., Schonfeld, G., Hazzard, W. R., & Blum, C. (1982). Proposed nomenclature of apoE isoproteins, apoE genotypes, and phenotypes. *J Lipid Res, 23*(6), 911-914.

Zempel, H., Thies, E., Mandelkow, E., & Mandelkow, E. M. (2010). Abeta oligomers cause localized Ca(2+) elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. *J Neurosci, 30*(36), 11938-11950. doi:10.1523/jneurosci.2357-10.2010

Zetterberg, H. (2016). Neurofilament Light: A Dynamic Cross-Disease Fluid Biomarker for Neurodegeneration. *Neuron, 91*(1), 1-3. doi:https://doi.org/10.1016/j.neuron.2016.06.030

Zetterberg, H., Hietala, M. A., Jonsson, M., Andreasen, N., Styrud, E., Karlsson, I., Edman, A., Popa, C., Rasulzada, A., Wahlund, L. O., Mehta, P. D., Rosengren, L., Blennow, K., & Wallin, A. (2006). Neurochemical aftermath of amateur boxing. *Arch Neurol, 63*(9), 1277-1280. doi:10.1001/archneur.63.9.1277

Zetterberg, H., Jacobsson, J., Rosengren, L., Blennow, K., & Andersen, P. M. (2007). Cerebrospinal fluid neurofilament light levels in amyotrophic lateral sclerosis: impact of SOD1 genotype. *Eur J Neurol, 14*(12), 1329-1333. doi:10.1111/j.1468-1331.2007.01972.x

Zetterberg, H., Skillbäck, T., Mattsson, N., Trojanowski, J. Q., Portelius, E., Shaw, L. M., Weiner, M. W., & Blennow, K. (2016). Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression. *JAMA Neurol, 73*(1), 60-67. doi:10.1001/jamaneurol.2015.3037

Zhou, B., Teramukai, S., Yoshimura, K., & Fukushima, M. (2009). Validity of cerebrospinal fluid biomarkers as endpoints in early-phase clinical trials for Alzheimer's disease. *J Alzheimers Dis, 18*(1), 89-102. doi:10.3233/jad-2009-1124

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Paper I

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Association of glial and neuronal degeneration markers with Alzheimer's disease cerebrospinal fluid profile and cognitive functions

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Abstract

Background: Neuroinflammation has gained increasing attention as a potential contributing factor in the onset and progression of Alzheimer's disease (AD). The objective of this study was to examine the association of selected cerebrospinal fluid (CSF) inflammatory and neuronal degeneration markers with signature CSF AD profile and cognitive functions among subjects at the symptomatic pre- and early dementia stages.

Methods: In this cross-sectional study, 52 subjects were selected from an Icelandic memory clinic cohort. Subjects were classified as having AD ($n = 28$, age = 70, 39% female, Mini-Mental State Examination [MMSE] = 27) or non-AD ($n = 24$, age = 67, 33% female, MMSE = 28) profile based on the ratio between CSF total-tau (T-tau) and amyloid-β_{1-42} ($A\beta_{42}$) values (cut-off point chosen as 0.52). Novel CSF biomarkers included neurofilament light (NFL), YKL-40, S100 calcium-binding protein B (S100B) and glial fibrillary acidic protein (GFAP), measured with enzyme-linked immunosorbent assays (ELISAs). Subjects underwent neuropsychological assessment for evaluation of different cognitive domains, including verbal episodic memory, non-verbal episodic memory, language, processing speed, and executive functions.

Results: Accuracy coefficient for distinguishing between the two CSF profiles was calculated for each CSF marker and test. Novel CSF markers performed poorly (area under curve [AUC] coefficients ranging from 0.61 to 0.64) compared to tests reflecting verbal episodic memory, which all performed fair (AUC > 70). LASSO regression with a stability approach was applied for the selection of CSF markers and demographic variables predicting performance on each cognitive domain, both among all subjects and only those with a CSF AD profile. Relationships between CSF markers and cognitive domains, where the CSF marker reached stability selection criteria of > 75%, were visualized with scatter plots. Before calculations of corresponding Pearson's correlations coefficients, composite scores for cognitive domains were adjusted for age and education. GFAP correlated with executive functions ($r = -0.37$, $p = 0.01$) overall, while GFAP correlated with processing speed ($r = -0.68$, $p < 0.001$) and NFL with verbal episodic memory ($r = -0.43$, $p = 0.02$) among subjects with a CSF AD profile.

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Conclusions: The novel CSF markers NFL and GFAP show potential as markers for cognitive decline among individuals with core AD pathology at the symptomatic pre- and early stages of dementia.

Keywords: Alzheimer's disease, Cerebrospinal fluid, Neurofilament light, YKL-40, S100 calcium-binding protein B, Glial fibrillary acidic protein, AD biomarker profile, Cognitive domains

Introduction

In recent years, a paradigm shift in the research criteria of Alzheimer's disease (AD) has occurred as the primary focus has shifted from clinical to biological criteria. The emphasis is now on the pathology [1], which is believed to start decades before the appearance of clinical symptoms [2]. The core cerebrospinal fluid (CSF) biomarkers reflecting the hallmarks of AD pathology, extracellular amyloid plaques (Aβ), and neurodegeneration (total tau [T-tau] and phosphorylated tau [P-tau]) have been at the center of this shift and have been extensively studied [3]. Although the diagnostic accuracies of these markers are generally satisfactory [4], their levels are relatively constant in the symptomatic stages of the disease and do not correlate well with the progression of cognitive decline [5–7]. This necessitates the need for exploration of novel biomarkers that help in better understanding the different aspects of AD pathology, its progression, and clinical manifestation.

Increasing evidence shows that inflammation is a contributing factor in the pathogenesis and development of AD and other neurodegenerative diseases [8, 9]. A number of studies show that Aβ toxicity and plaques induce an immune response, including activation of astrocytes and microglia, the immune cells of the brain [10–12]. Furthermore, activation of these cells is also thought to play a role in the formation and progression of neurofibrillary tangles (NFTs), contributing to neuronal dysfunction and loss [13]. Glial activation markers are, therefore, of high interest when it comes to exploring new biomarkers for the diagnosis of dementia.

The glial proteins YKL-40 (also known as chitinase-3-like-1 protein), S100 calcium-binding protein B (S100B), and glial fibrillary acidic protein (GFAP) have previously been associated with AD pathology [14]. All are expressed in astrocytes within the central nervous system (CNS), primarily (YKL-40 and S100B) [15, 16] or exclusively (GFAP) [17]. YKL-40, a chitin-binding glycoprotein and a glial activation marker [18], has been identified inside reactive astrocytes in close proximity to amyloid plaques [19]. YKL-40 expression also correlates with tau pathology in AD brain tissues, demonstrating an association between glial activation and neurodegeneration [20]. S100B is a calcium-binding protein, exerting both intracellular and extracellular functions and has been found to be upregulated in AD tissues [21, 22]. GFAP is a key intermediate filament protein and marker of reactive astrocytes, whose expression has been associated with amyloid plaque load and, to a lesser extent, the number of NFTs [23–25].

Inflammation in the brain and its role in AD can be studied indirectly through the analysis of CSF proteins. Increased levels of CSF YKL-40, S100B, and GFAP have been observed in AD patients compared to healthy controls, although results have not been consistent [26]. The relationship between inflammatory and core AD markers (Aβ, tau) in CSF has also been explored. Previous studies have found a strong positive association between CSF YKL-40 and tau proteins but not between YKL-40 and $A\beta_{42}$ [19, 27–29]. YKL-40 has also been shown to strongly correlate with neuronal degeneration marker neurofilament light (NFL) in CSF [30], further supporting the association between glial activation and neurodegeneration. NFL is mainly located in myelinated axons. Therefore, its levels also reflect white matter changes, with recent studies indicating a potential for this protein as both a diagnostic and progression marker in AD and other neurodegenerative diseases [26, 31]. Few studies have examined the relationship between S100B and GFAP with core AD markers in CSF. Hov et al. [32] found an association between S100B and P-tau but not $A\beta_{42}$ among elective surgery patients free from dementia and delirium. Ishiki et al. [33] did not find an association between CSF GFAP and core markers within a dementia cohort.

Loss of memory is typically among the first clinical symptoms of AD, marking the beginning of cognitive decline. The medial temporal lobe is an early site of tau accumulation, and its dysfunction may underlie episodic memory decline [34]. Other cognitive domains are also involved in AD, such as language, non-verbal episodic memory, and executive functions [35].

In the most recent research criteria from the International Working Group for the diagnosis of AD published in 2014 [36], the diagnosis of prodromal AD requires both the presence of cognitive symptoms and AD signature biomarker profile (increased amyloid positron emission tomography [PET] deposition or the combination of lowered CSF amyloid-β_{1-42} and elevated CSF tau). It is essential for the evaluation of novel biomarkers to examine their relationship with both entities separately, independent of diagnosis. That type of approach could enhance both understanding of the underlying

pathology of AD and the sequence of events leading to cognitive impairment. The first aim of this study was to assess the ability of glial (YKL-40, S100B, GFAP) and neurodegeneration (NFL) markers in CSF to discriminate between different CSF profiles (AD and non-AD) among subjects at the symptomatic pre- and early stages of dementia. In addition, the results were compared to the discrimination ability of neuropsychological tests, which are commonly used to aid AD diagnosis. The second aim was to investigate the relationship between the CSF markers with neuropsychological tests reflecting different cognitive domains.

Methods

Subjects

Individuals, referred to The National University Hospital of Iceland Memory Clinic during a 4-year period which had (1) a score between 24 and 30 on the Mini-Mental State Examination (MMSE) and (2) a score of 4.0 or less on the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) [37], were invited to join a prospective study on mild cognitive impairment (MCI, n = 218). The exclusion criteria were (1) cognitive impairment that, without a doubt, could be explained by a condition other than dementia; (2) difficulties participating due to health or social issues; and (3) residency outside the Reykjavík Capital Area. In entering the study, each subject underwent various assessments, including a standard clinical and neuropsychological assessment and brain magnetic resonance imaging (MRI) for evaluation of medial temporal lobe atrophy (MTA). Lumbar puncture for collection of CSF, which was optional by the requirement of the National Bioethics Committee, was also carried out. For this particular study (Fig. 1), only subjects with CSF samples and complete neuropsychological assessment were selected from the cohort (n = 56). The final sample included 52 subjects as four were removed due to excessively high value on CSF GFAP (n = 1) or blood-contamination in the CSF sample (n = 3). Clinical diagnosis of AD was based on the criteria for probable AD dementia defined by the National Institute on Aging-Alzheimer's Association (NIA-AA) [38], with evidence of AD pathophysiological processes (based on MTA score or/and analysis of core CSF markers). Patients with Lewy body dementia (LBD) were diagnosed based on the consensus criteria of McKeith et al. [39]. MCI diagnosis required the fulfillment of the Winblad criteria [40], with those not fulfilling the criteria diagnosed as having subjective mild cognitive impairment (SCI).

CSF collection and analysis

CSF was collected via lumbar puncture with a 22-gauge spinal needle at the L3/4 or L4/5 interspace. Uncentrifuged samples were frozen in 2-ml polypropylene tubes and stored at − 80 °C. Commercially available sandwich enzyme-linked immunosorbent assays (ELISAs) were used for measurements of all proteins. Analyses of core AD markers T-tau (IBL International, Hamburg, Germany) and $A\beta_{42}$ (IBL International, Hamburg, Germany) were carried out in the ISO 15189 accredited medical laboratory MVZ Labor P.D. Dr. Volkmann und Kollegen GbR (Karlsruhe, Germany). Assays for novel markers NFL (Uman Diagnostics, Umeå, Sweden), YKL-40 (Quantikine ELISA Human Chitinase-3–like 1; R&D systems, M.N., USA), S100B (BioVendor GmbH, Heidelberg, Germany), and GFAP (BioVendor GmbH, Heidelberg, Germany) were performed in technical duplicates

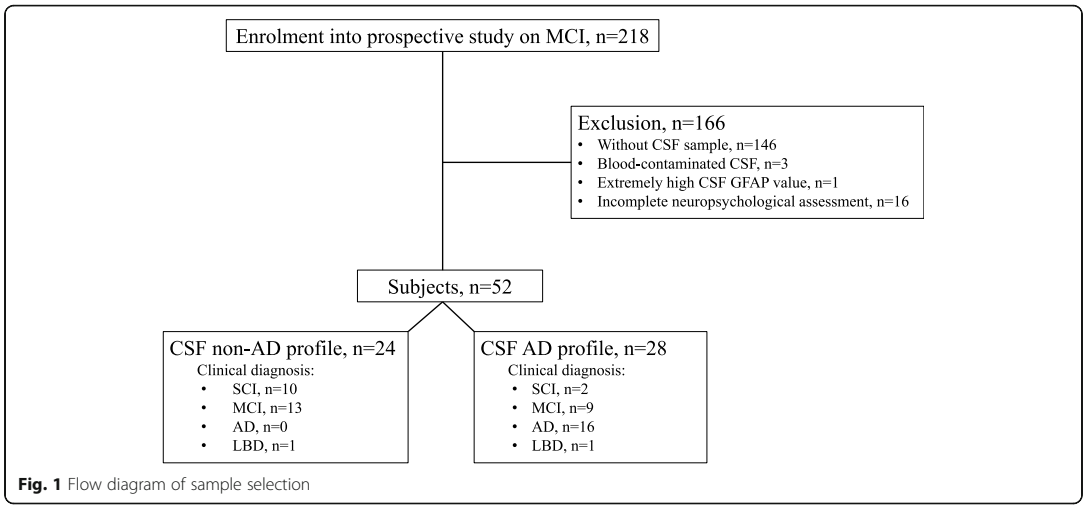

Fig. 1 Flow diagram of sample selection

and according to the manufacturer's instructions in a laboratory at the University of Iceland. The mean Intra-assay CV was < 10% and mean Inter-assay CV < 15% for all assays.

Subject grouping based on CSF measures

Each subject was classified independently of clinical diagnosis on the basis of CSF T-tau and $A\beta_{42}$ values. T-tau/$A\beta_{42}$ ratio cut-off of 0.52 was chosen based on results from a large memory clinic cohort study [41], giving a sensitivity of 93% for AD and specificity of 83% for controls. A positive CSF AD profile was defined as T-tau/$A\beta_{42}$ ratio > 0.52. The same ratio was also used as a part of the clinical diagnosis of AD, explaining full concordance with CSF AD profile.

Neuropsychological tests

All subjects underwent a detailed neuropsychological assessment performed by licensed psychologists. Five cognitive domains, commonly affected by aging and AD, were assessed using seven tests (Table 1). For the evaluation of verbal episodic memory, two tests were used. The first, Rey Auditory Verbal Learning Test (RAVLT), consisted of 15 nouns read aloud by the examiner for five consecutive trials. Each trial was followed by a free-recall test. After a 30-min delay, subjects were required to recall the words without being reread the list [42]. The second test was composed of a story [43], which included 25 ideas verbally presented by the examiner. Right after the story was presented (immediate recall), the subject was asked to repeat what they remembered without being given any clues (free recall). Thirty minutes later, subjects were asked to recall the story again (delayed recall). The Rey–Osterrieth complex figure test (ROCF) was used to assess non-verbal episodic memory [42]. The subject was asked to reproduce a complicated line drawing, first by copying it free-hand, second by drawing from memory (immediate recall), and third by drawing it after a 30-min delay (delay recall). Verbal fluency [44] was evaluated with subjects having to produce as many animal names and words starting with the letters H and S as possible in 60 s. Two subtests were used to evaluate processing speed. Part A of The Trail Making Test (TMT-A) [45] required subjects to connect 25 numbered circles positioned randomly on a piece of paper. The first and the most simple part of the Stroop test—Word reading—was also used for the evaluation of the same cognitive domain [46]. Subjects were shown a list of color names (red, green, yellow, or blue), each printed in black ink, and told to read out loud as rapidly as possible. For evaluation of executive functions, The Digit Symbol Substitution Test (DSST), Trail making Test B (TMT-B), and Stroop 4th/3rd parts were used. DSST [47] is a paper-and-pencil test that requires the participant to match symbols to numbers according to a key located at the top of the page. The subject copied the symbol into spaces below a row of numbers. The number of correct symbols within 120 s, constituted the score. TMT-B includes both numbers (1–13) and letters (A-L), with the subject drawing lines between circles, alternating between numbers and letters (1-A-2-B-3-C, etc.). In Stroop—part 4, subjects had to name the color of words when color and meaning were incongruent. Part 3—naming of squares of given colors—was used to control for speed by calculating the ratio between the two parts.

Table 1 List of neuropsychological tests administrated

Cognitive domain	Neuropsychological test	Scores (range)
Verbal episodic memory	RAVLT immediate recall	Free recall—the sum of the number of words recalled from trials 1 through 5 (0 to 75)
	RAVLT delayed recall	Delayed free recall—number of words recalled after 30-min delay (0 to 15)
	RAVLT recognition—false positives	Recognition—number of words recognized from a list of 45 words. Number of false positives subtracted from the score (– 30 to 15)
	Story immediate recall	Recall of a story containing 25 ideas (0 to 25)
	Story delayed recall	Recall of a story containing 25 ideas again after 30-min delay (0 to 25)
Non-verbal episodic memory	ROCF immediate recall	Complicated drawing reproduced (0 to 36)
	ROCF delayed recall	Complicated drawing reproduced again after 30-min delay (0 to 36)
Language	Verbal fluency animals	Number of animal names produced in 60 s
	Verbal fluency H+S	Number of words that begin with H/S in 60 s
Processing speed	TMT-A	Time in seconds to connect a set of 25 numbered dots in sequential order
	Stroop test, part I	Time in seconds to read a set of color words written in black
Executive functions	DSST	Number of symbols correctly produced in 120 s
	TMT-B	Time in seconds to connect 25 targets, alternating between numbers and letters
	Stroop 4th/3rd part	Part 3—time in seconds it takes to name squares of given colors Part 4—time in seconds it takes to name the color of a word

Abbreviations: *RAVLT* Rey Auditory Verbal Learning Test, *ROCF* Rey–Osterrieth complex figure, *DSST* Digit symbol substitution test, *TMT* Trail Making Test

Statistical analysis

Descriptive group comparisons were performed using Mann-Whitney *U* tests and chi-square tests for continuous and categorical variables, respectively. Raw values of CSF measures and selected neuropsychological tests (TMT, Stroop test, DSST) were naturally log-transformed to account for a non-normal distribution. Composite scores for each cognitive domain were calculated by averaging neuropsychological test *z*-scores and subsequently converting those scores into *z*-scores. Before the computation of composite scores, *z*-scores for tests measuring reaction time were reversed (TMT, Stroop test, DSST) for the purpose of test consistency (higher scores always indicating better performance). Receiver operating characteristic (ROC) curves were constructed for the differentiation between CSF AD and non-AD profiles. The discrimination abilities of each CSF marker and cognitive domain were compared using the area under the curve (AUC) method, according to DeLong et al. [48]. The AUC is the probability that a randomly selected pair of subjects from each CSF profile group is correctly classified. Stability selection was employed in combination with least absolute shrinkage and selection operator (LASSO) regression for the purpose of identifying stable predictors in multivariable models [49]. LASSO is a penalized approach to multiple regression and especially useful when dealing with multicollinearity (highly correlated predictors). A penalty is introduced, reducing large variance due to multicollinearity in exchange for a tolerable amount of bias. It also performs variable selection as it imposes coefficients of some variables to shrink towards zero. Stable selection is based on resampling the data for avoidance of overfitting, which can be advantageous when dealing with smaller data sets. Instead of fitting one model on a whole sample, many models are fitted on subsamples drawn from it. Stability selection was performed by the use of the function stabsel in the package stabs, implementing the package glmnet for LASSO model fitting [50, 51]. Cut-off value for stable selection was set to 75% (the percentage of times a variable was selected into a model) and per-family error rate (PFER) to 1 for all analyses. Each subsample was half the size of the original one, with 100 subsamples being drawn. LASSO logistic regression was applied for the selection of novel CSF markers and composite tests, most accurately distinguishing between the two CSF profiles. LASSO linear regression was used to select variables, out of CSF markers and demographic variables, predicting with most accuracy the composite *z*-score for each cognitive domain. Two LASSO regressions with a stability selection were performed for each cognitive domain, one which included all subjects and the other, which only included those with a CSF AD profile. Scatter plots were used for visualization of the selected relationships between CSF markers and cognitive domains. Cognitive domain measures were adjusted for age and education before the calculations of corresponding Pearson's correlations coefficients. For the adjustment, linear regression models were created with each composite test *z*-score as the dependent variable and age and education as independent variables. The residual for each subject was subsequently calculated (observed minus predicted score). Significance values were not adjusted for multiple comparisons, as this study was viewed as explorative with emphasis on discovering relationships. All statistical analyses were performed using R (version 3.6.1, The R Foundation for Statistical Computing).

Results

Sample characteristics

Table 2 shows the demographic, pathophysiological, and clinical characteristics of the cohort by CSF profile. There were no significant differences between the groups in age, length of education, novel CSF protein levels, or gender frequencies. Boxplots comparing distributions in CSF protein levels (NFL, YKL-40, S100B, GFAP) between profile groups are presented in Additional file 1, S1a-d. The CSF AD profile group showed significantly worse performance on the MMSE, RAVLT, Story, ROCF immediate recall, and Verbal fluency animal tests compared to the non-AD group ($p < 0.05$).

Pearson's correlations between CSF markers

Pearson's correlations between the CSF markers, age, and length of education are presented in Fig. 2, respectively. Inflammatory markers YKL-40 and S100B and neurodegeneration markers NFL and T-tau all correlated positively and significantly with each other. The highest correlation was found between NFL and YKL-40 (NFL: $r = 0.62$, $p < 0.001$). GFAP did only significantly correlate with the CSF marker S100B ($r = 0.53$, $p < 0.001$). No CSF markers correlated significantly with $A\beta_{42}$. All the CSF markers, except for $A\beta_{42}$, correlated positively with age. Length of education correlated weakly and negatively with T-tau ($r = -0.29$, $p = 0.03$).

Accuracy of CSF markers and cognitive domains in distinguishing between CSF profiles

Accuracies for distinguishing between CSF AD and non-AD profiles were based on univariable ROC analyses (Table 3). AUCs for novel CSF markers ranged from 0.61 to 0.64, with a lower limit of each confidence interval below the value of 0.5. In comparison, neuropsychological tests reflecting verbal episodic memory had the highest accuracy compared to other measurements, with all AUCs over 0.70, which is considered fair [52]. The scores for the verbal episodic memory composite test

Table 2 Subject demographics, CSF marker levels, and neuropsychological test scores by CSF profile

	CSF profile		
	Non-AD T-tau/Aβ_{42} ≤ 0.52 (***n*** = 24)	AD T-tau/Aβ_{42} > 0.52 (***n*** = 28)	***p*** value[a]
Demographics			
Gender (M/F)	16/8	17/11	0.66
Age, years	67 (46–80)	70 (51–84)	0.17
Education, years	14.0 (9–20)	12.5 (6–17)	0.11
Clinical diagnosis			
SCI/MCI/AD/LBD	10/13/0/1	2/9/16/1	N/A[b]
CSF measures			
Aβ_{42} (pg/ml)	703 (374–2332)	454 (160–822)	N/A[c]
T-tau (pg/ml)	173 (100–722)	416 (132–838)	N/A[c]
NFL (ng/ml)	1.9 (0.9–6.5)	2.5 (1.2–4.5)	0.15
YKL-40 (ng/ml)	165 (83–399)	203 (124–367)	0.12
S100B (pg/ml)	215 (132–335)	230 (129–458)	0.17
GFAP (ng/ml)	1.0 (0.1–7.1)	1.3 (0.5–21.3)	0.09
Cognitive domains			
Global cognition			
MMSE, score	28 (24–30)	27 (24–30)	0.01
Verbal episodic memory			
RAVLT immediate recall, score	36 (23–66)	26.5 (13–51)	0.003
RAVLT delayed recall, score	6.5 (0–15)	1.5 (0–12)	< 0.001
RAVLT recognition-fp, score	9.0 (3–15)	5.5 (−3–15)	0.003
Story immediate recall, score	13.5 (5–17)	8 (1–18)	0.005
Story delayed recall, score	12.0 (1–19)	5.5 (0–16)	0.002
Non-verbal episodic memory			
ROCF immediate recall, score	13.3 (0–27)	7.3 (0–26)	0.04
ROCF delayed recall, score	12.8 (0–25)	8.5 (0–26)	0.07
Language			
Verbal fluency animal, score	20 (8–33)	14 (4–27)	0.02
Verbal fluency H+S, score	24.0 (14–48)	25.5 (6–63)	1.00
Processing speed			
TMT-A, seconds	43.5 (21–133)	48.0 (27–116)	0.22
Stroop—part I, seconds	23.5 (20–42)	24.5 (17–34)	0.64
Executive functions			
TMT-B, seconds	109 (44–340)	153 (60–343)	0.06
DSST, score	8.5 (3–51)	7.0 (2–61)	0.24
Stroop 4th/3rd part, seconds	2.1 (1.4–4.0)	2.1 (1.6–5.8)	0.25

Abbreviations: *AD* Alzheimer's disease, *CSF* cerebrospinal fluid, *DDST* Digit Symbol Substitution Test, *fp* false positives, *LBD* Lewy body dementia, *MCI* mild cognitive impairment, *MMSE* Mini-Mental State Examination, *N/A* not applicable, *RAVLT* Rey Auditory-Verbal Learning Test, *ROCF* Rey–Osterrieth complex figure, *SCI* subjective cognitive impairment, *TMT* Trail Making Test

Values are shown as median (range) or as numbers per group, [a]Mann-Whitney U non-parametric tests used for continuous variables and chi-square tests for categorical variables, *p* values not applicable for [b]clinical diagnosis due to CSF profiles being part of the diagnostic criteria for AD and [c]Aβ_{42} and T-tau due to their values used for defining CSF profiles

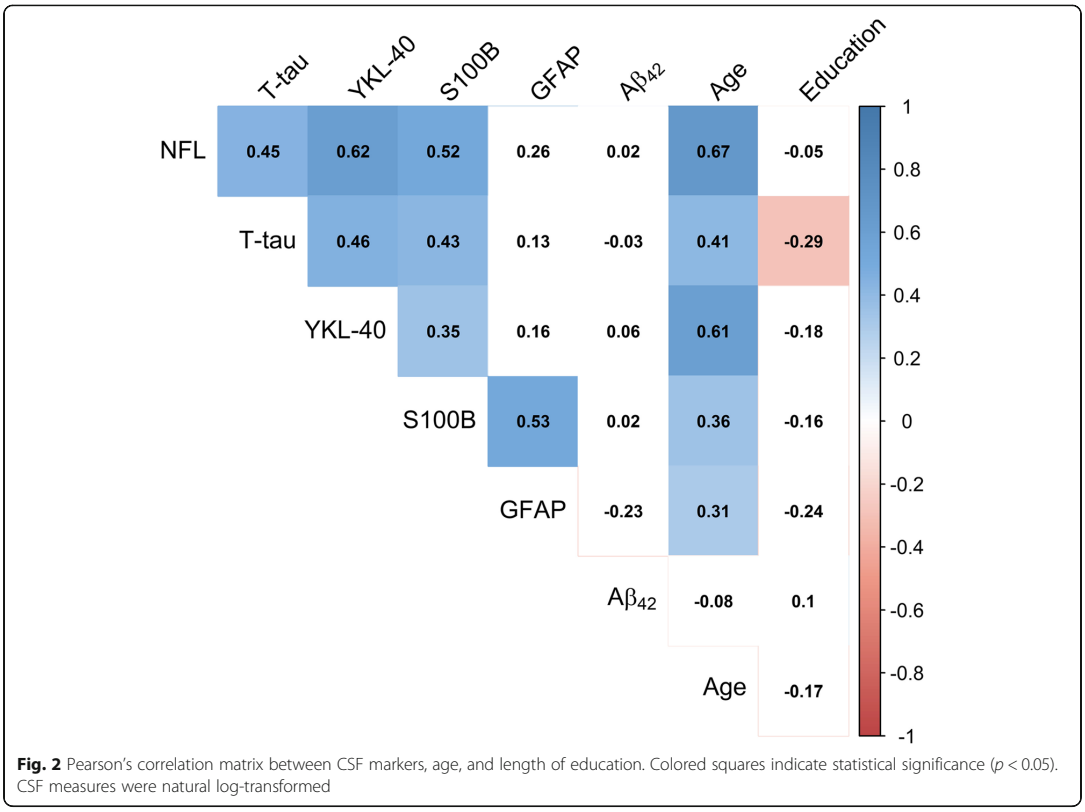

Fig. 2 Pearson's correlation matrix between CSF markers, age, and length of education. Colored squares indicate statistical significance ($p < 0.05$). CSF measures were natural log-transformed

(AUC = 0.80, CI 0.69–0.92) and RAVLT delayed recall (AUC = 0.80, CI 0.68–0.93) distinguished the best between the CSF profile groups. A similar trend in results was found when ROC analyses were stratified by gender (Table S1, Additional file 1), although AUC coefficients were overall higher for women ($n = 19$) compared to men ($n = 33$). LASSO logistic regression with stability selection was performed for the selection of variables distinguishing between the CSF profile groups with the highest consistency. Nine possible predictors could be selected, the four novel CSF markers and the five composite tests presenting each cognitive domain. Only the test reflecting verbal episodic memory was selected as a predictor, with selection frequency (96%) above the cut-off value. All other possible predictors had a much lower selection frequency (≤ 20%).

Figure 3 illustrates the ROC curves for the two cognitive domains and the CSF measure with the highest AUC from Table 3. Verbal episodic memory (AUC = 0.80) was superior in distinguishing between CSF AD vs. non-AD profiles compared to non-verbal episodic memory (AUC = 0.65) and CSF GFAP (0.64).

Selection of predictors for scores on each cognitive domain

LASSO linear regression with a stability selection was applied for identifying a set of variables (CSF markers and demographic variables) predicting cognitive scores with the highest consistency (Fig. 4). Two analyses were performed for each of the five domains, one including all subjects ($n = 52$) and the other only among those with a CSF AD profile ($n = 28$). Variables with stability selection above 75% were considered reliable predictors. GFAP (78%) was selected as a predictor for executive functions (Fig. 4a) and age (95%) as a predictor for non-verbal memory (Fig. 4b) within the whole cohort. Among subjects with a CSF AD profile, GFAP (87%) and age (81%) were selected as predictors for processing speed (Fig. 4c) and NFL (80%) for verbal episodic memory (Fig. 4d). No variables reached the stability selection criteria as predictors of score reflecting language (Fig. 4e).

Pearson's correlations between selected CSF markers and cognitive domains

Relationships between CSF measures and cognitive domains, as selected with LASSO regression—stability

Table 3 Accuracy in distinguishing between CSF AD and non-AD profiles

	Univariable ROC analyses		Multivariable LASSO logistic regression[b]
	AUC	95% CI (AUC)*	Stability selection (%)
CSF measures[a]			
GFAP (ng/ml)	0.64	0.48–0.79	10
YKL-40 (ng/ml)	0.63	0.47–0.78	18
NFL (ng/ml)	0.62	0.45–0.78	2
S100B (pg/ml)	0.61	0.46–0.77	20
Cognitive domains			
Verbal episodic memory			
Composite *z*-score	0.80	0.69–0.92	96[c]
RAVLT delayed recall, score	0.80	0.68–0.93	–
Story delayed recall, score	0.75	0.62–0.89	–
RAVLT immediate recall, score	0.74	0.61–0.88	–
RAVLT recognition-fp, score	0.74	0.61–0.87	–
Story immediate recall, score	0.73	0.59–0.86	–
Non-verbal episodic memory			
Composite *z*-score	0.65	0.50–0.81	14
ROCF immediate recall, score	0.66	0.51–0.81	–
ROCF delayed recall, score	0.65	0.49–0.80	–
Executive functions			
Composite *z*-score	0.64	0.49–0.80	16
TMT-B, seconds[a]	0.66	0.50–0.81	–
DSST, score[a]	0.60	0.44–0.75	–
Stroop 4th/3rd part, seconds[a]	0.59	0.43–0.75	–
Language			
Composite *z*-score	0.60	0.44–0.76	4
Verbal fluency animals, score	0.68	0.54–0.83	–
Verbal fluency H+S, score	0.50	0.34–0.66	–
Processing speed			
Composite *z*-score	0.56	0.39–0.72	9
TMT-A, seconds[a]	0.60	0.44–0.76	–
Stroop test—part I, seconds[a]	0.54	0.38–0.70	–

AUC is the probability that a randomly selected pair of subjects from each CSF profile group is correctly classified
Abbreviations: AD Alzheimer's disease, *AUC* area under curve, *CI* confidence intervals, *CSF* cerebrospinal fluid, *DDST* Digit Symbol Substitution Test, *fp* false positives, *LASSO* Least absolute shrinkage and selection operator, *RAVLT* Rey Auditory-Verbal Learning Test, *ROCF* Rey–Osterrieth complex figure, *TMT* Trail Making Test
*Confidence intervals calculated with DeLong method
[a]Values are natural log-transformed
[b]LASSO logistic regression model was fitted on 100 subsamples, with different predictors (CSF measures and composite test scores) possibly selected into each model. Numbers present the frequency (%) of each possible predictor selected. The per-family error rate (PFER) was set at 1, and the cut-off value at 75% for stability selection
[c]The composite test for verbal episodic memory was the only measure to have selection frequency above the cut-off value

selection analyses (Fig. 4), were visualized using scatter plots. It is well established that normal aging and level and quality of education can influence cognitive test performance [53]. Composite *z*-scores were therefore adjusted for age and education prior to Pearson's correlations calculations.

CSF NFL levels did not significantly correlate with verbal episodic memory among all subjects ($r = -0.26$, $p = 0.06$, Fig. 5a). Analysis by CSF profile (Fig. 5b) revealed moderate, significant correlation among subjects with a CSF AD profile ($r = -0.43$, $p = 0.02$) compared to none among those without ($r = -0.05$, $p = 0.82$). Correlations

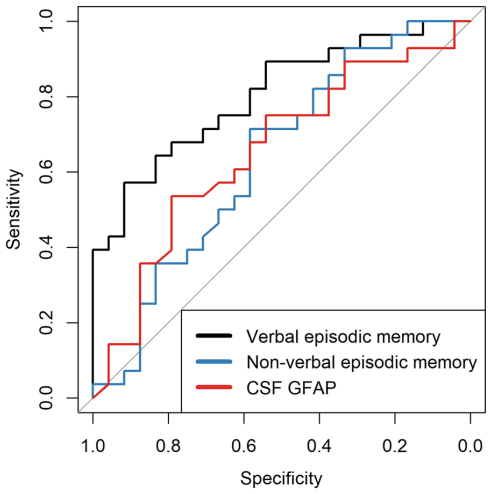

Fig. 3 Comparison between ROC curves of the two cognitive domains and the CSF marker with the highest area under the curve (AUC) coefficients

between the NFL levels and individual neuropsychological tests reflecting verbal episodic memory are presented in Additional file 1, S2a-e. T-tau did not reach the selection criteria for any cognitive domain. It is, nonetheless, of interest to compare the results of T-tau to NFL as both proteins are markers of neurodegeneration. The association between T-tau and verbal episodic memory was similar to NFL within the whole cohort ($r = -0.28$, $p < 0.04$, Fig. 5c) but did not reach significance within the CSF AD group ($r = -0.15$, $p = 0.45$) when analyzed by CSF profile (Fig. 5d).

Correlation between CSF GFAP levels and processing speed did not reach significance within the whole cohort ($r = -0.27$, $p = 0.06$, Fig. 5e) or among those with a CSF non-AD profile ($r = 0.02$, $p = 0.94$, Fig. 5f). A moderately strong correlation was, on the other hand, detected among those with a CSF AD profile ($r = -0.68$, $p < 0.001$, Fig. 5f). A weak, negative correlation was found between CSF GFAP levels and executive functions, both within the whole cohort ($r = -0.37$, $p = 0.01$, Fig. 5g) and among subjects with a CSF AD profile ($r = -0.39$, $p = 0.04$, Fig. 5h). The corresponding correlations between CSF GFAP levels with individual neuropsychological tests reflecting processing speed and executive functions are presented in Additional file 1, Fig. S3a-e. Additional file 1 also includes scatter plots identical to those shown in Fig. 5 without adjustment for age and education (Fig. S4a-h) and Pearson's correlations between CSF markers, age, and education and composite scores of each cognitive domain, both unadjusted and adjusted for age and education (Table S2).

Discussion

We compared different CSF biomarkers reflecting neurodegeneration (NFL) and inflammation (YKL-40, S100B and GFAP) in relation to core CSF AD markers and cognitive functions in a cohort of subjects at the pre- and early symptomatic dementia stages. While our results indicated that these CSF markers did not accurately distinguish between AD and non-AD CSF profiles, they exhibited different patterns of association with certain cognitive domains, as evaluated by various neuropsychological tests. This pattern was mainly observed among subjects with a CSF AD profile. Within that group, levels of the neurodegeneration marker NFL associated with verbal episodic memory while inflammatory marker GFAP associated with processing speed. In addition, GFAP associated weakly with executive functions within the whole cohort. Overall, these results indicate that CSF NFL and GFAP levels do relate to cognitive functions, specifically among those with a CSF AD profile.

Both CSF NFL and YKL-40 levels correlated with T-tau but not with $A\beta_{42}$, in accordance with previous studies [54–56]; thereby, NFL and YKL-40 levels most likely reflect processes that are independent of Aβ pathology [55, 57, 58]. The putative inflammatory marker, S100B, did show a similar trend as YKL-40 within the whole cohort, correlating strongly with CSF neurodegeneration markers (NFL and T-tau) but not with $A\beta_{42}$ levels. In contrast, GFAP did not correlate with the CSF neurodegeneration markers nor with CSF $A\beta_{42}$ levels. Neither CSF S100B nor GFAP have been much studied in terms of correlation with CSF core AD markers. Hov et al. [32] found similar results among elective surgery patients free from dementia and delirium, with S100B positively correlating with P-tau but not with $A\beta_{42}$ in CSF. Ishiki et al. [33] did not find an association between GFAP and the core AD markers within a sample of healthy subjects and dementia patients. Here we found that CSF NFL, YKL-40, S100B, and GFAP all performed poorly in differentiating between the CSF AD and non-AD profiles. In summary, these results are in accordance with previous findings that have suggested markers NFL, YKL-40, S100B, and GFAP to be not AD specific.

The neuropsychological tests reflecting verbal episodic memory did show the best accuracy in differentiating between the CSF profiles out of all the evaluated cognitive measures and the novel CSF markers. The accuracy was good for the composite score of verbal episodic memory and RAVLT delayed recall test (80%), but fair for all the other verbal episodic memory tests (between 70 and 80%). A recent meta-analysis [59] based on 47 studies has shown that immediate and delayed memory tests consistently show good accuracy (above 80%) for differentiating between AD and healthy controls, especially those involving list recall. Importantly, these studies are based on the

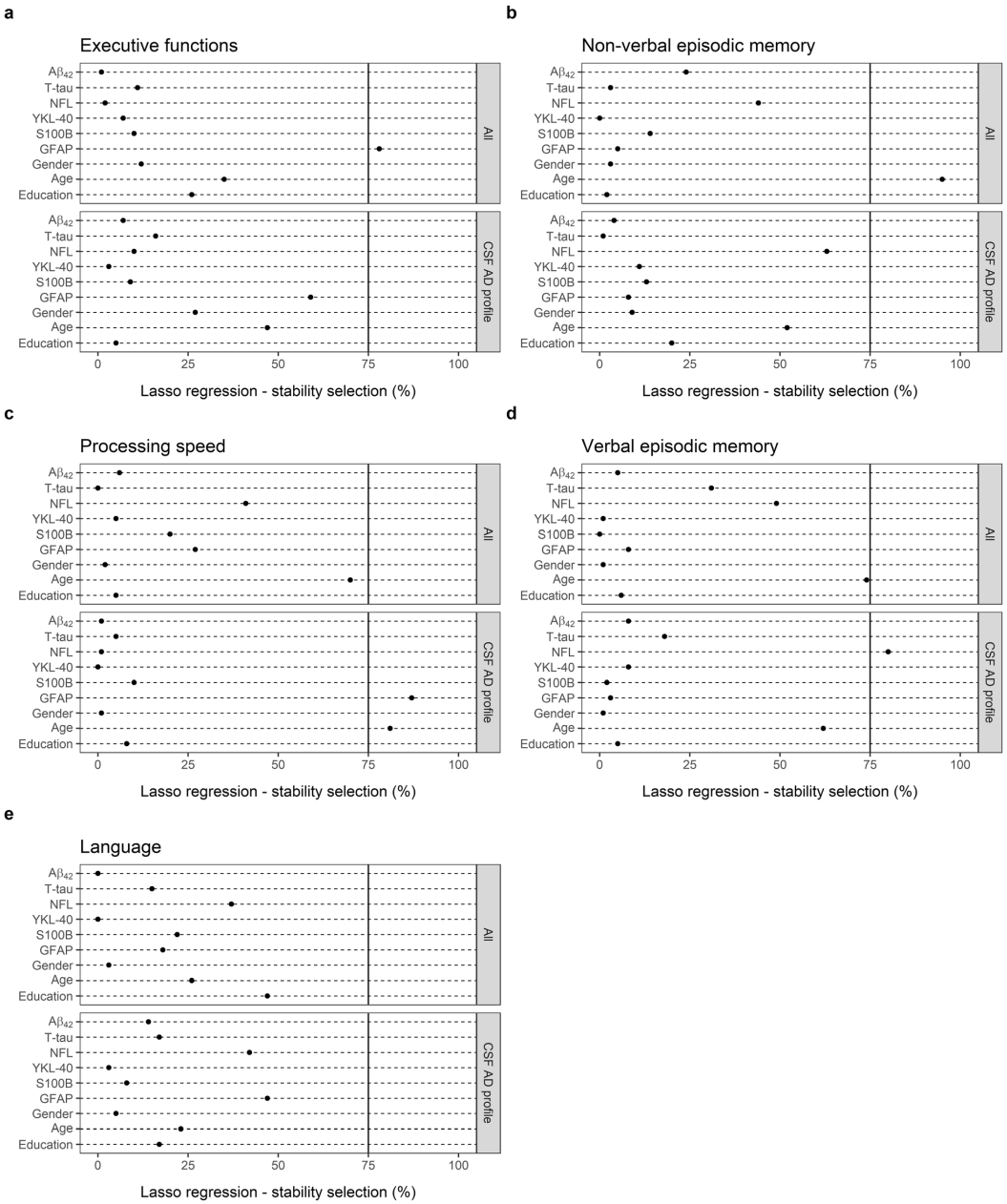

Fig. 4 LASSO linear regression—stability selection analyses for prediction of composite *z*-scores reflecting **a** executive functions, **b** non-verbal episodic memory, **c** processing speed, **d** verbal episodic memory, and **e** language. Two analyses were created for each domain, one including all participants ($n = 52$) and the other only the CSF AD profile group ($n = 28$). The cut-off selection value was set at 75% and the per-family error rate (PFER) at 1 for all analyses

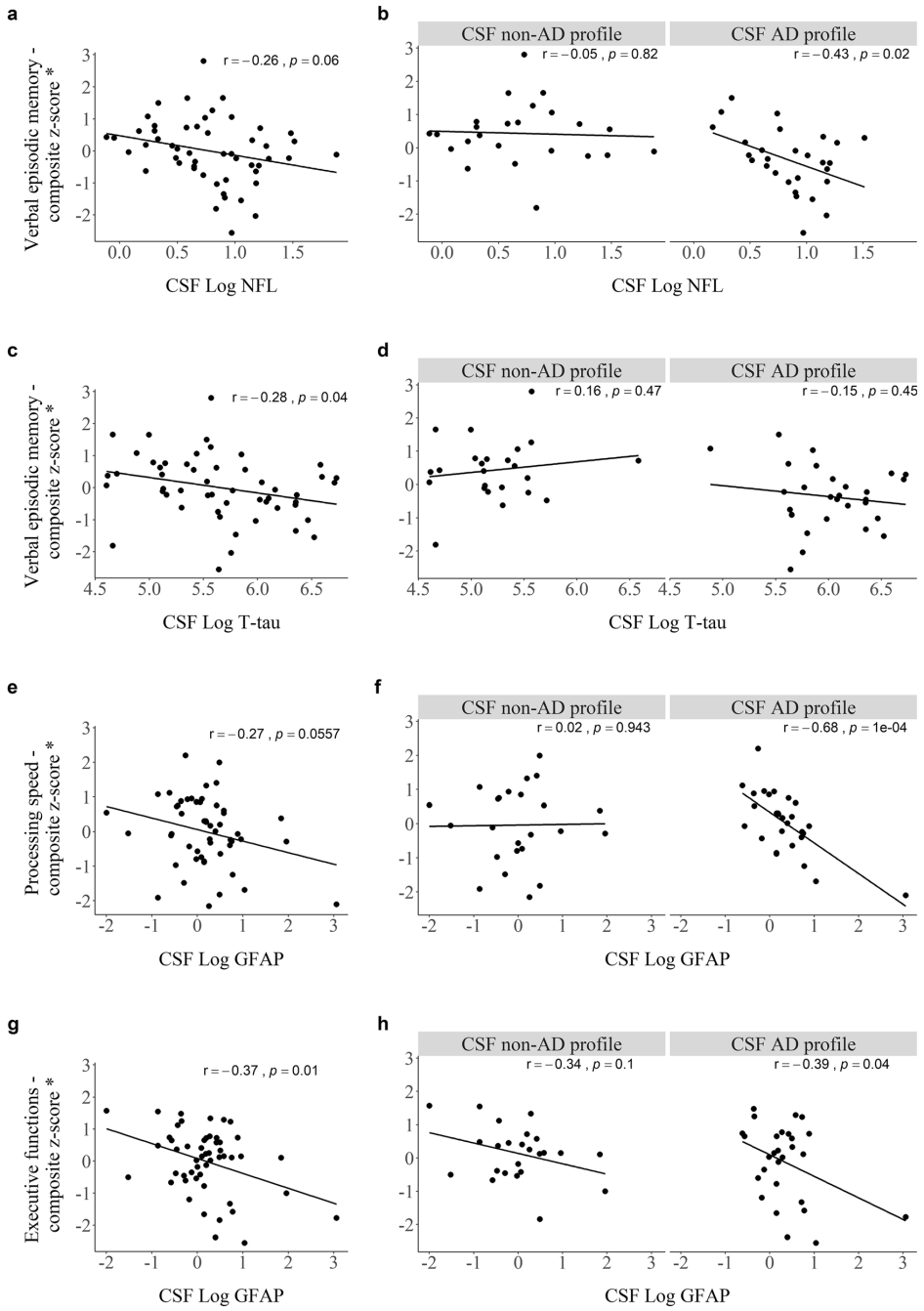

Fig. 5 (See legend on next page.)

(See figure on previous page.)
Fig. 5 Scatter plots presenting Pearson's correlations between CSF levels of NFL and verbal episodic memory (**a**, **b**), T-tau and verbal episodic memory (**c**, **d**), GFAP and processing speed (**e**, **f**), and GFAP and executive functions (**g**, **h**) within the whole cohort and by CSF profile. *Cognitive domains were adjusted for covariates (age and education). Without the bottom corner GFAP outlier in the CSF AD profile group, Pearson's correlations were slightly lower for **f** processing speed ($r = -0.58$, $p = 0.001$) and **h** executive functions ($r = -0.28$, $p = 0.15$)

clinical diagnosis of AD, while our focus was on the signature of the CSF AD biomarker profile.

CSF markers related in different ways to cognitive measures. Both CSF NFL [56, 60] and YKL-40 [58] have been previously reported to associate with cognitive decline, with correlation found between CSF levels and global cognition assessed by MMSE test scores among AD patients. In the same studies, the correlation did not hold for patients with MCI. Thus, NFL and YKL-40 might not be sensitive to very early changes in cognition in the earliest symptomatic stages of dementia (SCI, MCI) as in more advanced stages. In this study, the relationship between NFL and YKL-40 with different cognitive domains within the whole cohort could not be confirmed. A possible explanation could be that a majority of subjects ($n = 34$) were at the SCI or MCI stages, with 23 of those without a CSF AD profile.

Knowledge regarding the relationship between core CSF biomarkers and cognition remains incomplete. Overall, $A\beta_{42}$ and T-tau appear to associate with memory and executive functions in some studies [61, 62], although results have not been consistent in terms of which cognitive domains they are associated with, which particular tests are most suitable and the strength of relationships in different clinical stages [61, 63, 64]. However, the levels of core CSF marker have shown evidence of reaching a plateau early in the clinical course of the disease and are therefore not considered ideal for tracking the progression of disease at later stages [65].

Increased CSF levels of inflammatory marker GFAP was found weakly associated with worse performance on tests reflecting executive functions, both within the whole cohort and among subjects with CSF AD profile. Few studies have examined the relationship between CSF GFAP levels and cognitive functions. Ishiki et al. [33] did not find an association between CSF GFAP levels and MMSE scores in a sample of healthy subjects and dementia patients. Darreh-Shori et al. [66] also reported no correlation between CSF GFAP levels and MMSE scores among AD patients. As with CSF GFAP, little research has been conducted on the association between CSF S100B levels and cognition. In the same study [66], a weak, positive relationship was found between levels of CSF S100B and MMSE scores within the same patient group.

Associations between selected CSF markers and cognitive domains were also examined within each CSF profile. CSF NFL levels moderately related to verbal episodic memory among those with CSF AD profile but not among those without. Higher levels of CSF GFAP also moderately associated with worse performance on processing speed only within the CSF AD profile group. This is of interest because the CSF markers did not directly relate to the CSF AD profile (ability in discriminating between CSF profiles was poor). This outcome could possibly be explained by the additive effects of distinctive processes on cognitive functions. A previous study [67] showed a similar trend where CSF YKL-40 levels associated with less preservation of global cognition only in individuals with low Aβ levels (Aβ positive). CSF Aβ levels did though not correlate with YKL-40 or cognitive decline, but to brain atrophy in Aβ positive subjects.

This study has several limitations. First, the sample was relatively small, and hence, present findings need to be validated in a larger study. The sample did not include healthy controls, which could underestimate associations between the studied variables. Another limitation of the study is the lack of information about the ApoE genotype. However, it is unlikely that the ApoE genotype affects the outcome as previous studies have suggested that ApoE ε4 status does not influence CSF NFL or YKL-40 levels [19, 68, 69].

Conclusions

Our findings suggest that levels of CSF markers NFL and GFAP relate to different cognitive profiles at the symptomatic pre- and early dementia stages. The relationships between the levels of NFL with verbal episodic memory and GFAP with processing speed were only observed among those with CSF AD profile, although the CSF markers did not directly relate to the CSF AD profile. These CSF markers could be of potential use as progression markers, monitoring subtle cognitive changes at the earliest symptomatic stages of dementia among those with AD pathology. Further studies with bigger group sizes are needed to validate these results and to evaluate their potential in tracking changes in the more advanced stages of AD and other types of dementia.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13195-020-00657-8.

Additional file 1: Figure 1. Levels of CSF NFL, YKL-40, S100B and GFAP by CSF profile. **Table 1.** Univariable ROC analysis for distinguishing between CSF profile groups stratified by gender. **Figure 2.** Pearson's correlations between levels of CSF NFL with neuropsychological tests reflecting verbal episodic memory by CSF profile. **Figure 3.** Pearson's correlations between levels of CSF GFAP with neuropsychological tests reflecting processing speed and executive functions by CSF profile. **Figure 4**. Pearson's correlations between CSF levels of NFL and T-tau with verbal episodic memory and GFAP with processing speed and executive functions, within the whole cohort and by CSF profile. **Table 2.** Pearson's correlations between CSF markers, age, education and composite z-scores reflecting cognitive domains.

Abbreviations

AD: Alzheimer's disease; AUC: Area under curve; $A\beta_{42}$: Amyloid-β_{1-42}; CSF: Cerebrospinal fluid; DSST: Digit Symbol Substitution Test; FP: False positives; GFAP: Glial fibrillary acidic protein; IQCODE: Informant Questionnaire on Cognitive Decline in the Elderly; LASSO: Least absolute shrinkage and selection operator; LBD: Lewy body dementia; MCI: Mild cognitive impairment; MMSE: Mini-Mental State Examination; MTA: Medial temporal lobe atrophy; NFL: Neurofilament light; NFTs: Neurofibrillary tangles; PET: Positron emission tomography; PFER: Per-family error rate; P-tau: Phosphorylated tau; RAVLT: Rey Auditory Verbal Learning Test; ROC: Receiver operating characteristic; ROCF: Rey–Osterrieth Complex Figure; S100B: S100 calcium-binding protein B; SCI: Subjective cognitive impairment; TMT: Trail Making Test; T-tau: Total-tau

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Authors' contributions

UDT, J.S., and PHP contributed to the conception and design of the study. UDT and MKJ contributed to the collection of data. UDT performed the statistical analysis and drafted the manuscript. SHL provided guidance on statistical analysis and verified the results. PHP, J.S., T.D., SHL, and MJK revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

The data which support this study are not publicly available, but may be provided upon reasonable request.

Ethics approval and consent to participate

The study has been approved by the National Research Ethics Committee of Iceland (VSN-14-028), and all subjects signed an informed consent. The study was conducted in accordance with the Helsinki Declaration latest revision of 2013.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Jack CR Jr, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14(4):535–62.
2. Bateman RJ, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012;367(9):795–804.
3. Ittner LM, Gotz J. Amyloid-beta and tau–a toxic pas de deux in Alzheimer's disease. Nat Rev Neurosci. 2011;12(2):65–72.
4. Ferreira D, et al. Meta-review of CSF core biomarkers in Alzheimer's disease: the state-of-the-art after the new revised diagnostic criteria. Front Aging Neurosci. 2014;6:47.
5. Perrin RJ, Fagan AM, Holtzman DM. Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. Nature. 2009;461(7266):916–22.
6. Zhou B, et al. Validity of cerebrospinal fluid biomarkers as endpoints in early-phase clinical trials for Alzheimer's disease. J Alzheimers Dis. 2009;18(1):89–102.
7. Jack CR Jr, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 2010;9(1):119–28.
8. Ardura-Fabregat A, et al. Targeting neuroinflammation to treat Alzheimer's disease. CNS Drugs. 2017;31(12):1057–82.
9. Calsolaro V, Edison P. Neuroinflammation in Alzheimer's disease: current evidence and future directions. Alzheimers Dement. 2016;12(6):719–32.
10. Medeiros R, LaFerla FM. Astrocytes: conductors of the Alzheimer disease neuroinflammatory symphony. Exp Neurol. 2013;239:133–8.
11. El Khoury JB, et al. CD36 mediates the innate host response to beta-amyloid. J Exp Med. 2003;197(12):1657–66.
12. Steardo L Jr, et al. Does neuroinflammation turn on the flame in Alzheimer's disease? *Focus on astrocytes*. Front Neurosci. 2015;9:259.
13. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. Nat Rev Neurosci. 2015;16(6):358–72.
14. Carter SF, et al. Astrocyte biomarkers in Alzheimer's disease. Trends Mol Med. 2019;25(2):77–95.
15. Bonneh-Barkay D, et al. Astrocyte and macrophage regulation of YKL-40 expression and cellular response in neuroinflammation. Brain pathology (Zurich). 2012;22(4):530–46.
16. Donato R, et al. S100B's double life: intracellular regulator and extracellular signal. Biochim Biophys Acta. 2009;1793(6):1008–22.
17. Yang Z, Wang KK. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci. 2015;38(6):364–74.
18. Rehli M, et al. Transcriptional regulation of CHI3L1, a marker gene for late stages of macrophage differentiation. J Biol Chem. 2003;278(45):44058–67.
19. Craig-Schapiro R, et al. YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's disease. Biol Psychiatry. 2010;68(10):903–12.
20. Querol-Vilaseca M, et al. YKL-40 (Chitinase 3-like I) is expressed in a subset of astrocytes in Alzheimer's disease and other tauopathies. J Neuroinflammation. 2017;14(1):118.
21. Griffin WS, et al. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci U S A. 1989;86(19):7611–5.
22. Mrak RE, Sheng JG, Griffin WS. Correlation of astrocytic S100 beta expression with dystrophic neurites in amyloid plaques of Alzheimer's disease. J Neuropathol Exp Neurol. 1996;55(3):273–9.
23. Hanzel DK, et al. High-throughput quantitative histological analysis of Alzheimer's disease pathology using a confocal digital microscanner. Nat Biotechnol. 1999;17(1):53–7.
24. Muramori F, Kobayashi K, Nakamura I. A quantitative study of neurofibrillary tangles, senile plaques and astrocytes in the hippocampal subdivisions and entorhinal cortex in Alzheimer's disease, normal controls and non-Alzheimer neuropsychiatric diseases. Psychiatry Clin Neurosci. 1998;52(6):593–9.
25. Vehmas AK, et al. Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. Neurobiol Aging. 2003;24(2):321–31.

26. Olsson B, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol. 2016;15(7):673–84.
27. Alcolea D, et al. Relationship between cortical thickness and cerebrospinal fluid YKL-40 in predementia stages of Alzheimer's disease. Neurobiol Aging. 2015;36(6):2018–23.
28. Alcolea D, et al. Relationship between beta-Secretase, inflammation and core cerebrospinal fluid biomarkers for Alzheimer's disease. J Alzheimers Dis. 2014;42(1):157–67.
29. Antonell A, et al. Cerebrospinal fluid level of YKL-40 protein in preclinical and prodromal Alzheimer's disease. J Alzheimers Dis. 2014;42(3):901–8.
30. Melah KE, et al. Cerebrospinal fluid markers of Alzheimer's disease pathology and microglial activation are associated with altered white matter microstructure in asymptomatic adults at risk for Alzheimer's disease. J Alzheimers Dis. 2016;50(3):873–86.
31. Olsson B, et al. Association of cerebrospinal fluid neurofilament light protein levels with cognition in patients with dementia, motor neuron disease, and movement disorders. JAMA Neurol. 2019;76(3):318–25.
32. Hov KR, et al. Cerebrospinal fluid S100B and Alzheimer's disease biomarkers in hip fracture patients with delirium. Dement Geriatr Cogn Dis Extra. 2017;7(3):374–85.
33. Ishiki A, et al. Glial fibrillar acidic protein in the cerebrospinal fluid of Alzheimer's disease, dementia with Lewy bodies, and frontotemporal lobar degeneration. J Neurochem. 2016;136(2):258–61.
34. Small SA, et al. A pathophysiological framework of hippocampal dysfunction in ageing and disease. Nat Rev Neurosci. 2011;12(10):585–601.
35. Snowden JS, et al. Cognitive phenotypes in Alzheimer's disease and genetic risk. Cortex. 2007;43(7):835–45.
36. Dubois B, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol. 2014;13(6):614–29.
37. Jorm AF. A short form of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): development and cross-validation. Psychol Med. 1994;24(1):145–53.
38. McKhann GM, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's Dement. 2011;7(3):263–9.
39. McKeith IG, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB consortium. Neurology. 2017;89(1):88–100.
40. Winblad B, et al. Mild cognitive impairment – beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med. 2004;256(3):240–6.
41. Duits FH, et al. The cerebrospinal fluid "Alzheimer profile": easily said, but what does it mean? Alzheimers Dement. 2014;10(6):713–723.e2.
42. Lezak MD. Neuropsychological assessment. Oxford: Oxford University Press; 2012.
43. Wechsler D. WMS-R : Wechsler Memory Scale-Revised : manual. San Antonio: Harcourt Brace Jovanovich; 1987.
44. Shao Z, et al. What do verbal fluency tasks measure? Predictors of verbal fluency performance in older adults. Front Psychol. 2014;5:772.
45. Tombaugh TN. Trail Making Test A and B: normative data stratified by age and education. Arch Clin Neuropsychol. 2004;19(2):203–14.
46. Stroop JR. Studies of interference in serial verbal reactions. J Exp Psychol. 1935;18(6):643–62.
47. Wechsler D. Wechsler adult intelligence scale–Fourth Edition (WAIS–IV), vol. 22. San Antonio: NCS Pearson; 2008. p. 498.
48. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44(3):837–45.
49. Tibshirani R. Regression shrinkage and selection via the lasso. J Royal Stat Soc Series B (Methodological). 1996;58(1):267–88.
50. Hofner B, Boccuto L, Göker M. Controlling false discoveries in high-dimensional situations: boosting with stability selection. BMC Bioinformatics. 2015;16(1):144.
51. Meinshausen N, Bühlmann P. Stability selection. J Royal Stat Soc: Series B (Statistical Methodology). 2010;72(4):417–73.
52. Consensus report of the Working Group on: Molecular and biochemical markers of Alzheimer's disease. The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. Neurobiol Aging, 1998. 19(2): p. 109–116.
53. Ganguli M, et al. Age and education effects and norms on a cognitive test battery from a population-based cohort: the Monongahela-Youghiogheny Healthy Aging Team. Aging Ment Health. 2010;14(1):100–7.
54. Alcolea D, et al. Amyloid precursor protein metabolism and inflammation markers in preclinical Alzheimer disease. Neurology. 2015;85(7):626–33.
55. Mattsson N, et al. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. EMBO Mol Med. 2016;8(10):1184–96.
56. Olsson, B., et al., Association of cerebrospinal fluid neurofilament light protein levels with cognition in patients with dementia, motor neuron disease, and movement disorders. JAMA Neurol. 2019;76(3):318–25.
57. Gangishetti U, et al. Non-beta-amyloid/tau cerebrospinal fluid markers inform staging and progression in Alzheimer's disease. Alzheimers Res Ther. 2018;10(1):98.
58. Kester MI, et al. Cerebrospinal fluid VILIP-1 and YKL-40, candidate biomarkers to diagnose, predict and monitor Alzheimer's disease in a memory clinic cohort. Alzheimers Res Ther. 2015;7(1):59.
59. Weissberger GH, et al. Diagnostic accuracy of memory measures in Alzheimer's dementia and mild cognitive impairment: a systematic review and meta-analysis. Neuropsychol Rev. 2017;27(4):354–88.
60. Zetterberg H, et al. Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression. JAMA neurology. 2016;73(1):60–7.
61. Guhra M, et al. Linking CSF and cognition in Alzheimer's disease: reanalysis of clinical data. Exp Gerontol. 2016;73:107–13.
62. Nordlund A, et al. Episodic memory and speed/attention deficits are associated with Alzheimer-typical CSF abnormalities in MCI. J Int Neuropsychol Soc. 2008;14(4):582–90.
63. Rolstad S, et al. Amyloid-beta(4)(2) is associated with cognitive impairment in healthy elderly and subjective cognitive impairment. J Alzheimers Dis. 2011;26(1):135–42.
64. Bendlin BB, et al. CSF T-Tau/Abeta42 predicts white matter microstructure in healthy adults at risk for Alzheimer's disease. PLoS One. 2012;7(6):e37720.
65. Bertens D, et al. Temporal evolution of biomarkers and cognitive markers in the asymptomatic, MCI, and dementia stage of Alzheimer's disease. Alzheimers Dement. 2015;11(5):511–22.
66. Darreh-Shori T, et al. Functional variability in butyrylcholinesterase activity regulates intrathecal cytokine and astroglial biomarker profiles in patients with Alzheimer's disease. Neurobiol Aging. 2013;34(11):2465–81.
67. Sala-Llonch R, et al. Inflammation, amyloid, and atrophy in the aging brain: relationships with longitudinal changes in cognition. J Alzheimers Dis. 2017;58(3):829–40.
68. Bos I, et al. Cerebrospinal fluid biomarkers of neurodegeneration, synaptic integrity, and astroglial activation across the clinical Alzheimer's disease spectrum. Alzheimers Dement. 2019;15(5):644–54.
69. Sutphen CL, et al. Longitudinal cerebrospinal fluid biomarker changes in preclinical Alzheimer disease during middle age. JAMA Neurol. 2015;72(9):1029–42.

Publisher's Note

Paper II

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Cerebrospinal Fluid C18 Ceramide Associates with Markers of Alzheimer's Disease and Inflammation at the Pre- and Early Stages of Dementia

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Abstract.
Background: Understanding how dysregulation in lipid metabolism relates to the severity of Alzheimer's disease (AD) pathology might be critical in developing effective treatments.
Objective: To identify lipid species in cerebrospinal fluid (CSF) associated with signature AD pathology and to explore their relationships with measures reflecting AD-related processes (neurodegeneration, inflammation, deficits in verbal episodic memory) among subjects at the pre- and early symptomatic stages of dementia.
Methods: A total of 60 subjects that had been referred to an Icelandic memory clinic cohort were classified as having CSF AD ($n = 34$) or non-AD ($n = 26$) pathology profiles. Untargeted CSF lipidomic analysis was performed using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS) for the detection of mass-to-charge ratio (m/z) features. CSF proteins reflecting neurodegeneration (neurofilament light [NFL]) and inflammation (chitinase-3-like protein 1 [YKL-40], S100 calcium-binding protein B [S100B], glial fibrillary acidic protein [GFAP]) were also measured. Rey Auditory Verbal Learning (RAVLT) and Story tests were used for the assessment of verbal episodic memory.
Results: Eight out of 1008 features were identified as best distinguishing between the CSF profile groups. Of those, only the annotation of the m/z feature assigned to lipid species C18 ceramide was confirmed with a high confidence. Multiple regression analyses, adjusted for age, gender, and education, demonstrated significant associations of CSF core AD markers ($A\beta_{42}$: st.$\beta = -0.36$, $p = 0.007$; T-tau: st.$\beta = 0.41$, $p = 0.005$) and inflammatory marker S100B (st.$\beta = 0.51$, $p = 0.001$) with C18 ceramide levels.
Conclusion: Higher levels of C18 ceramide associated with increased AD pathology and inflammation, suggesting its potential value as a therapeutic target.

Keywords: Alzheimer's disease, biomarkers, cerebrospinal fluid, inflammation, lipidomics

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease that leads to progressive cognitive impairment and dementia. The pathology of AD, which starts decades before the appearance of clinical symptoms [1], is characterized by extracellular amyloid plaques containing amyloid-β (Aβ) and intracellular neurofibrillary tangles (NFTs) comprised of phosphorylated tau (P-tau) [2, 3]. The most widely accepted hypothesis regarding AD pathogenesis proposes that the deposition of Aβ leads to formation of NFTs, neuronal dysfunction, and dementia [4]. Although abundant evidence places Aβ and tau pathology at the center of AD pathogenesis, the mechanisms linking the two together and to eventual neuronal dysfunction and death are still unclear [4, 5]. As Aβ and tau pathology levels are relatively constant in the symptomatic stages of the disease, they are not considered suitable as progression markers [6–9]. Biomarkers reflecting other aspects of AD pathology are therefore needed. Genome-wide association studies have identified about 30 risk genes with a high proportion related to lipid metabolism, immune response, or both [10, 11]. These findings suggest that dysregulation of lipids and inflammatory proteins play an essential part in the pathogenesis of AD.

Lipids play various roles in the human body, both as structural components of cell membranes and in diverse biochemical processes, including membrane trafficking and cell signaling [12]. A dozen of major lipid classes are found within eukaryotic organisms, each comprising hundreds of individual molecular species [13]. Major classes, including glycerophospholipids, sphingolipids, fatty acids, and cholesterol [14], are abundantly expressed in the brain. These lipids are utilized in different compartments of glial cells and neurons [15]. In recent years, the role of lipid metabolism defects in AD pathogenesis has gained increased attention. Several molecular mechanisms have been identified, which connect membrane lipids to the generation and aggregation of Aβ. Pathological forms of Aβ proteins are formed by proteolytic cleavage of the transmembrane protein AβPP by β- and γ-secretases [16]. Lipid membrane structure and organization can affect the activity of these transmembrane enzymes, and thus AβPP processing and Aβ production [17]. Furthermore, secretases, AβPP, and its derivatives also appear to affect the activity of lipid metabolic enzymes and subcellular trafficking, thereby changing the membrane lipid composition. Lipids might, therefore, play a role in the initiation and progression of AD pathogenesis [16].

Studies have established neuroinflammation as a contributing factor in the pathogenesis and progression of AD and other neurodegenerative diseases [18, 19]. Aβ plaques induce an immune response by activation of microglia and astrocytes [20–22], which in turn is thought to play a role in the formation of NFTs, contributing to neuronal dysfunction and loss [23]. The glial proteins chitinase-3-like-1 protein (YKL-40), calcium-binding protein S100B, and glial fibrillary acidic protein (GFAP) have been associated with AD pathology [24]. All proteins are expressed primarily (YKL-40 and S100B) [25, 26], or exclusively (GFAP) [27] in astrocytes within the central nervous system (CNS). YKL-40, a chitin-binding glycoprotein [28], has been reported to be a promising candidate biomarker of glial activation in AD. Previous studies have detected positive relationships between YKL-40 and the neurodegeneration markers tau [29–32] and neurofilament light (NFL) [33] in cerebrospinal fluid (CSF), demonstrating an association between glial activation and neurodegeneration [34]. NFL is mainly located in myelinated axons, with recent studies indicating a potential for this protein as both a diagnostic and a progression marker in AD and other neurodegenerative diseases [35, 36]. S100B, a calcium-binding protein, exerts both intracellular and extracellular functions and has been found to be upregulated in AD tissues [37, 38]. GFAP is an intermediate filament protein and a marker for astrocyte activation, which has both been associated with amyloid plaque load and the number of NFTs [39–41].

In recent years, a paradigm shift has occurred from clinical to biological definition of AD based on *in vivo* biomarkers measured in CSF or with positron emission tomography (PET) imaging [1]. The most recent research criteria base the diagnosis of AD partly [42] or primarily [1] on signature profiles defined by CSF/PET Aβ, CSF total tau (T-tau), and CSF P-tau levels. The International Working Group (IWG) [42], for example, defines typical AD as a combination of biomarker evidence (decreased CSF/PET $A\beta_{42}$ together with increased CSF T-tau or P-tau) and a specific phenotype (presence of a significant episodic memory impairment). Although the diagnostic accuracies of these core biomarkers are satisfactory [43], there is still a need to examine others. Understanding how biomarkers reflecting other processes could influence AD pathogenesis and severity is critical for the improvement of diagnosis and development of effective pharmacologic treatments. The first aim of

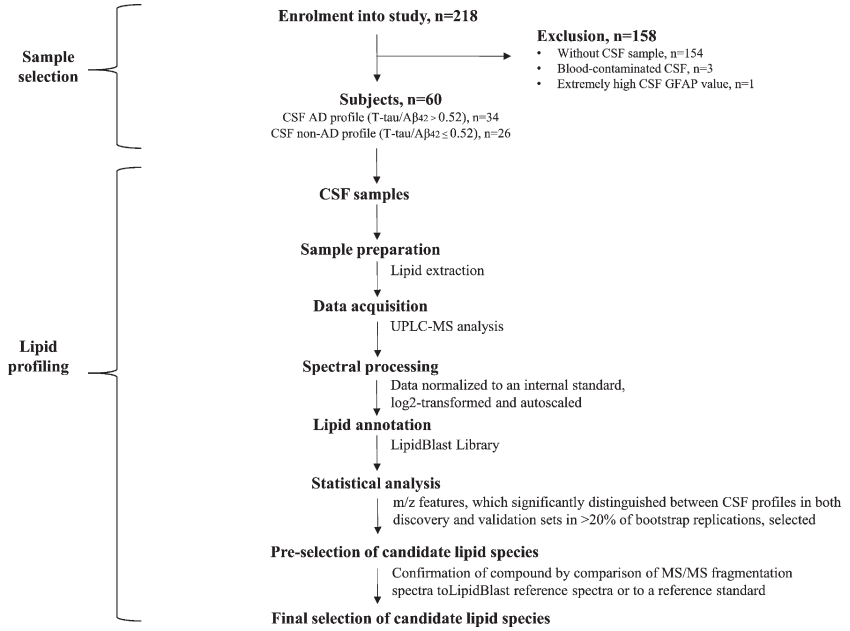

Fig. 1. Flow diagram of sample selection and lipid profiling techniques applied to the analysis of CSF samples.

this study was to identify CSF lipid species associated with CSF profile reflecting signature AD pathology in a cohort of subjects at the pre- and early symptomatic stages of dementia. The second aim was to explore the relationships between candidate lipid species and measures reflecting other AD-associated processes, including neuronal generation, inflammation and impairment in verbal episodic memory.

MATERIALS AND METHODS

Subjects

Subjects from The Icelandic MCI study cohort ($n = 218$), who had undergone lumbar puncture, were selected for this cross-sectional study ($n = 64$). The cohort was comprised of individuals who had been referred to Landspitali University Hospital (LUH) Memory Clinic over a four-year period (Fig. 1). The inclusion criteria for joining the cohort study were: 1) a score between 24–30 on the Mini-Mental State Examination (MMSE) [44] and 2) a score of 4.0 or less on the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) [45]. The exclusion criteria were the following: 1) cognitive impairment caused by a pre-existing condition, 2) difficulties participating due to health or social issues, and 3) residency outside the Reykjavík Capital Area. Each subject underwent various measurements at baseline, which included a medical assessment and a detailed neuropsychological assessment as well as brain magnetic resonance imaging (MRI) for the evaluation of medial temporal lobe atrophy (MTA) and white matter lesions. Lumbar puncture was carried out for the collection of CSF, but the intervention was optional required by the National Bioethics Committee. The final sample included 60 subjects as four were removed due to excessively high CSF GFAP value ($n = 1$) or blood-contamination in CSF samples ($n = 3$). Clinical diagnosis of AD was based on the criteria for probable AD dementia defined by the National Institute on Aging-Alzheimer's Association (NIA-AA) [46], with evidence of AD pathophysiological processes (based on MTA score or/and analysis of core CSF markers). Patients with Lewy body dementia (LBD) were diagnosed based on the consensus criteria of McKeith [47]. The diagnosis of mild cognitive impairment (MCI) required the fulfillment of the Winblad criteria [48]. Those without cognitive impairment were considered to have subjective cognitive impairment (SCI), as they had been referred to the Memory Clinic due to concerns of cognitive decline. Of the 60 participants in this study, 13 were diagnosed with SCI, 23 with MCI, 20 with AD, three with LBD, and one with Parkinson's disease.

The study has been approved by the National Research Ethics Committee of Iceland (VSN-14-028) and all subjects signed an informed consent. The study was conducted in accordance with the Helsinki Declaration latest revision of 2013.

CSF collection and analysis

Collection of CSF was done via lumbar puncture with a 22-gauge spinal needle at the L3/4 or L4/5 interspace. Samples, uncentrifuged, were frozen in 2 ml polypropylene tubes and stored at –80°C. Levels of all proteins were determined using commercially available sandwich enzyme-linked immunosorbent assays (ELISAs) and performed according to manufacturer's instructions. Levels of T-tau (IBL International, Hamburg, Germany), P-tau181 (INNO TEST, Gent, Belgium), and $A\beta_{42}$ (IBL International, Hamburg, Germany), were measured in the ISO 15189 accredited medical laboratory MVZ Labor P.D. Dr. Volkmann und Kollegen GbR (Karlsruhe, Germany). Levels of NFL (Uman Diagnostics, Umeå, Sweden), YKL-40 (Quantikine ELISA Human Chitinase-3–like 1; R&D systems, MN, USA), S100B (BioVendor GmbH, Heidelberg, Germany), and GFAP (BioVendor GmbH, Heidelberg, Germany) were measured in a laboratory at the University of Iceland. All assays had mean Intra-assay CV < 10% and Inter-assay CV < 15%.

Subject grouping based on CSF measures

Each subject was classified based on CSF T-tau and $A\beta_{42}$ values, independently of clinical diagnosis. A cut-off of 0.52 for T-tau/$A\beta_{42}$ ratio was selected based on results from a large memory clinic cohort study [49]. T-tau/$A\beta_{42}$ ratio > 0.52 was defined as a signature CSF AD profile. The CSF AD profile group had a total of 34 subjects (20 with a clinical diagnosis of AD dementia, 10 with MCI, three with SCI and one with Lewy body dementia) while the non-AD profile had a total of 26 subjects (13 with MCI, 10 with SCI, two with LBD, and one with Parkinson's disease). The same ratio cut-off point was also used as a part of the clinical diagnosis of AD, explaining full concordance with the CSF AD profile.

Neuropsychological tests

A detailed neuropsychological assessment, for the evaluation of different cognitive domains, was performed by licensed psychologists under the supervision of a clinical neuropsychologist. A significant impairment in episodic memory is commonly the earliest clinical symptom of AD [50], and therefore of specific interest here. Two tests were used for the evaluation of verbal episodic memory, The Rey Auditory Verbal Learning Test (RAVLT) [51], and a Story test based on the Logical Memory test of the Wechsler Memory Scale-Revised [52]. RAVLT consists of 15 nouns presented across five consecutive trials, with each trial followed by a free-recall test (immediate recall). A score for RAVLT immediate recall was calculated by summing up the number of words recalled from trials 1 through 5 (0 to 75 points). After a 30 min delay, subjects were required to recall the words without being reread the list (delayed recall). A point was given for each correct word (0 to 15 points). The second test was composed of an orally presented story, which included 25 ideas. Right after the presentation, the subject was asked to repeat what they remembered without being given any clues. After a 30 min delay, there was another recall without the story being reread to the subject. For both immediate and delayed recall, a point was given for each idea (0 to 25 points).

Sample preparation and scanning UPLC-MS analysis

CSF sample extraction was based on the method used in Bird et al. [53]. Briefly, C12 ceramide and C17 sphingomyelin (SM d18:1/17:0) were purchased from Avanti Polar Lipids (Alabaster, AL, USA) and added to 30 µl of CSF as internal standards prior to lipid extraction. Dried lipid extracts were resuspended in 300 µL of ACN/IPA/H2O (65:30:5 v/v/v) and stored at –80°C prior to analyses. C18 SM (d18:1/18:0) and C18 ceramide (d18:1/18:0) were run alongside samples for reference as both SMs and ceramides have consistently been associated with AD [54]. Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS) lipidomic analysis was carried out as described in Kotronoulas et al. [55]. The analytical instrumentation used was an ACQUITY UPLC system (UPLC ACQUITY, Waters Corporation, Milford, MA) coupled to a traveling-wave ion mobility (IMS) qTOF mass spectrometer (Synapt G2 HDMS, Waters Corporation, Manchester, UK). The chromatographic gradient separation was performed on an ACQUITY CSH C18 column (2.1 mm × 150 mm, 1.7 µm particle size, Waters Corporation) at 60°C (Supplementary Figure 1). Mobile phase A was H2O/2-isopropanol (80:20) and mobile phase B 2-isopropanol/ACN/H2O (90:9.1),

both with 0.05% of formic acid and 5 mM ammonium formate. Injection volume was 10 μL, flow rate was 0.4 mL/min, and the run time was 17 min. The following gradient pattern (solvent B) was used: 0 min, 40%B; 1 min, 40%B; 3 min, 60%B; 10 min, 100%B; 13.5 min, 100%B; 14 min, 40%B; 17 min, 40 %B. Both positive (+) and negative (-) electrospray ionization (ESI) modes were acquired. The capillary and cone voltage were 2.5 kV and 30 V, respectively. The source and desolvation temperature were 120 and 500°C and the desolvation gas flow was 800 L/h. During High Definition MS^E (HDMSE) experiments, the collision energy in the trap cell was off, and in the transfer cell, it ranged from 20 to 30 eV for the positive mode and from 25 to 40 eV for the negative mode. The resulting MS data were analyzed further within the MS-Dial program.

Spectral processing

Waters RAW files were converted to ABF format using the Reifycs Abf Converter tool. All patient ABF files and pooled samples from either positive or negative controls were loaded into the MS-Dial application [56]. MS-Dial was used for sample alignment and peak detection. Selected mass-to-charge ratio (m/z) peaks, hereafter referred to as m/z features, were manually curated to minimize the effect of sample drift. All m/z features were normalized to the internal standards. M/z feature annotation was performed with MS-Dial and compared to MS/MS spectra in the LipidBlast Library [57]. A total of 1013 m/z features were detected based on their peak mass. M/z features with more than 5% missing values were excluded from the analysis ($n = 5$), leaving 1008 features. The remaining missing data (5% or less) were imputed by the smallest observed value of that particular feature.

Statistical analysis

All CSF measures (detected m/z features and proteins) were log2-transformed to fit a Gaussian distribution. After autoscaling, each CSF measure had an average and a standard deviation of 0 and 1, respectively. Mann-Whitney U non-parametric tests were performed to compare levels of different variables between CSF profile groups. The sample ($n = 60$) was divided into the two sets (discovery and validation) using a bootstrap sampling method [58] for the selection of m/z features best distinguishing between CSF AD and non-AD profile groups. A discovery set was created by drawing a random sample of equal size from the original sample ($n = 60$) with replacement. Approximately 63% of the sample (38 subjects) were selected into the discovery set, and those left out (22 subjects) made up the validation set. The levels of each m/z feature were compared between CSF profile groups in both sets using Mann-Whitney U tests with a significance level of 0.05. This procedure was repeated 1000 times for the purpose of enhancing the robustness of feature selection. The m/z features most frequently significant in both sets (> 20%) were selected for more detailed identification. The cut-off point of 20% was selected based on the distribution of m/z features after 1000 bootstrap replications (Supplementary Figure 2). A cut-off at 20% was considered optimal, as frequencies of significant m/z features started to slowly increase below that point. Pearson's correlations and linear regression models (both unadjusted and adjusted for gender, age, and years of education) were used for estimation of relationships between continuous measures and final selection of lipids. All statistical analyses were performed using R (version 3.6.1, The R Foundation for Statistical Computing).

RESULTS

Table 1 presents the demographic, pathophysiological, and cognitive characteristics of subjects, both within the whole sample and divided by CSF profile (AD and non-AD). No statistical differences ($p > 0.05$) were found between CSF profile groups by gender, age, education, CSF protein levels, or MMSE scores. Subjects with a CSF AD profile performed worse on tests assessing verbal episodic memory, both for immediate (RAVLT: $p = 0.009$; Story: $p = 0.003$) and delayed (RAVLT: $p < 0.001$; Story: $p = 0.002$) recall.

In order to relate changes in lipid species levels to typical AD pathology and related measures, we performed a lipidomic analysis of CSF from patients described in Table 1. The selection of the m/z features best distinguishing between CSF profile groups was done using Mann-Whitney U non-parametric tests. Of the 1008 features detected, eight significantly differed in levels between groups within both the discovery and validation sets in more than 200 of the 1000 (20%) bootstrap replicates (Fig. 2) and were selected for further identification. Eight m/z features reached significance in 10–20% of the replicates, 23 in 2–10% of the replicates and 969 in less than 2%

Table 1
Subject demographics, CSF protein levels and cognitive scores by CSF profile

	CSF profile			All
	Non-AD T-tau/ $A\beta_{42} \leq 0.52$ $n = 26$	AD T-tau/ $A\beta_{42} > 0.52$ $n = 34$	p[a]	$n = 60$
Demographics				
Gender (M/F)	16/10	16/18	0.27	32/28
Age, y	68 (46–85)	70 (51–84)	0.19	70 (46–85)
Education, y	14 (9–20)	13 (6–20)	0.82	13 (6–20)
Clinical diagnosis				
SCI/MCI/AD/LBD/PD	10/13/0/2/1	3/10/20/1/0	N/A[d]	13/23/20/3/1
CSF measures[b]				
$A\beta_{42}$ (pg/ml)	770 (374–2332)	470 (140–977)	N/A[e]	555 (140–2332)
T-tau (pg/ml)	182 (100–722)	429 (132–1086)	N/A[e]	290 (100–1086)
P-tau (pg/ml)	45 (24–77)	84 (30–144)	N/A[e]	58 (24–144)
NFL (ng/ml)	2.0 (0.9–6.5)	2.5 (1.2–5.3)	0.052	2.2 (0.9–6.5)
YKL-40 (ng/ml)	165 (83–399)	189 (124–367)	0.35	183 (83–399)
S100B (pg/ml)	215 (132–335)	240 (129–509)	0.06	228 (129–509)
GFAP (ng/ml)	1.1 (0.1–7.1)	1.3 (0.5–21.3)	0.11	1.3 (0.1–21.3)
Global cognition				
MMSE, score	28 (24–30)	28 (24–30)	0.87	28 (24–30)
Verbal episodic memory				
RAVLT immediate recall, score[c]	34.5 (23–66)	27 (13–58)	0.009	30 (13–66)
RAVLT delayed recall, score[c]	4.5 (0–15)	1 (0–12)	< 0.001	3 (0–15)
Story – immediate recall, score	13.5 (5–17)	8 (1–21)	0.003	10 (1–21)
Story – delayed recall, score	11.5 (1–19)	6 (0–19)	0.002	7 (0–19)

AD, Alzheimer's disease; CSF, Cerebrospinal fluid; LBD, Lewy body dementia; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; N/A, Not applicable; PD, Parkinson's disease; RAVLT, Rey Auditory Verbal Learning Test; SCI, subjective cognitive impairment. Values are shown as median (range) or as numbers per group. [a]Mann-Whitney U non-parametric test used for continuous variables and Chi-Square test for the categorical variable (gender). [b]Raw values (neither log2-transformed nor autoscaled). [c]Analysis based on 59 subjects, one missing value. [d]p-values not applicable for clinical diagnosis due to CSF profiles being part of the diagnostic criteria for AD. [e]p-values not applicable for $A\beta_{42}$ and tau due to their values used for defining CSF profiles.

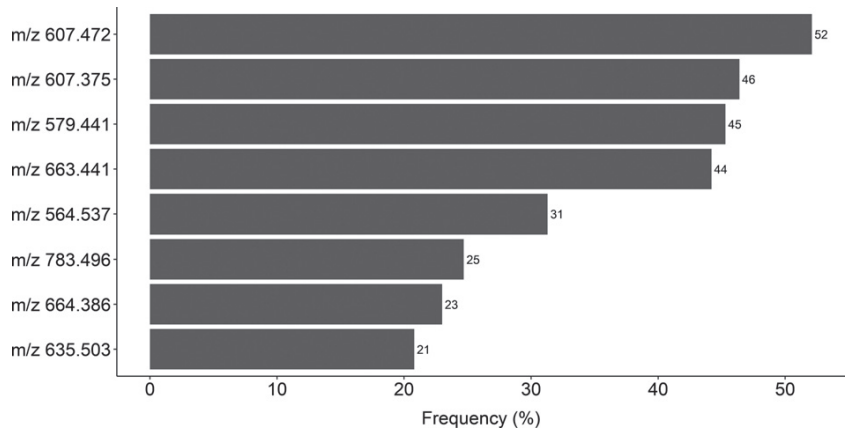

Fig. 2. Frequencies of m/z features significantly distinguishing between CSF profiles (AD and non-AD) in both discovery and validation sets ($p < 0.05$) after 1000 bootstrap replications. A total of eight features with frequencies higher than 200 (20%) were selected for more detailed identification.

of the replicates. The distribution of all m/z features after 1000 boostrap replications is depicted in Supplementary Figure 2.

Table 2 presents the lipid species annotations that were automatically assigned to the eight selected m/z features using the LipidBlast spectral library. It

Table 2
LipidBlast annotations of the eight selected m/z features. Levels for each feature compared between CSF AD and non-AD profile groups

Feature/peak (m/z)	Average RT (min)	Ionization mode	Annotated lipid species	Proposed structure	CSF profile[a]		p[b]
					Non-AD	AD	
607.472	7.74	(-)	FAHFA 40:7	FAHFA (18:5/22:2)	–0.50	0.25	< 0.001
607.375	6.00	(-)	MGDG 20:0	MGDG (10:0/10:0)	–0.54	–0.05	< 0.001
579.441	7.24	(-)	FAHFA 38:7	FAHFA (18:4/20:3)	–0.42	0.27	< 0.001
663.441	6.15	(-)	MGDG 24:0	MGDG (10:0/14:0)	–0.28	0.38	< 0.001
564.537	7.50	(-)	Cer 36:1	C18 Cer (d18:1/18:0)	–0.48	0.31	0.002
783.496	6.65	(-)	PMeOH 42:9	PMeOH (20:3/22:6)	–0.36	0.29	0.003
664.386	6.21	(-)	PE 32:6e	PE (16:23/16:4)	–0.10	0.36	0.003
635.503	8.07	(-)	FAHFA 42:7	FAHFA (20:3/22:4)	–0.56	–0.11	0.005

m/z, mass-to-charge ratio; RT, retention time; Cer, ceramide; FAHFA, fatty acid ester of hydroxyl fatty acid; MGDG, monogalactosyldiacylglycerol; PE, phosphatidylethanolamines; PMeOH, phosphatidyl methanol. [a]Based on normalized peak area arbitrary units (A.U.), log2-transformed and autoscaled before analysis. [b]Mann-Whitney U non-parametric test used.

also includes comparisons in levels of each feature between CSF profiles. Annotations of compounds searching the library are based on similarity matching of elements, including m/z peaks and retention time (RT). The lipid species selected belonged to four different lipid categories; glycerophospholipids (PE 16:23/16:4, PMeOH 20:3/22:6), sphingolipids (ceramide d18:1/18:0), branched fatty acid esters of hydroxy fatty acids (FAHFA 18:5/22:2, 18:4/20:3 and 20:3/22:4), and monogalactosyldiacylglycerol (MGDG 10:0/10:0). Of the eight lipid species annotated via LipidBlast library search, a reference standard was only run for C18 ceramide (d18:1/18:0). A comparison of the MS/MS fragmentation spectra of the C18 ceramide standard confirmed the annotation of C18 ceramide in the CSF samples (Supplementary Figure 3). The other seven annotated lipid species remain unconfirmed, as a comparison of each MS/MS fragmentation spectra to the LipidBlast reference spectra did not clearly confirm the correct chemical structure. All measured m/z features are listed in Supplementary Table 1A (positive ionization mode) and 1B (negative ionization mode).

As the structure for C18 ceramide was confirmed with high confidence, it was selected for further analysis. Figure 3 presents the levels of CSF C18 ceramide by different CSF profiles. As can be observed, levels of C18 ceramide were elevated in the CSF AD profile group compared to the non-AD group ($p = 0.002$).

Linear regression (unadjusted and adjusted for gender, age, and education) was performed to estimate the relationships of established AD ($A\beta_{42}$, T-tau, P-tau), inflammatory (YKL-40, S100B, GFAP) and neuronal degeneration (NFL) markers with CSF C18 ceramide (Table 3). Levels of $A\beta_{42}$

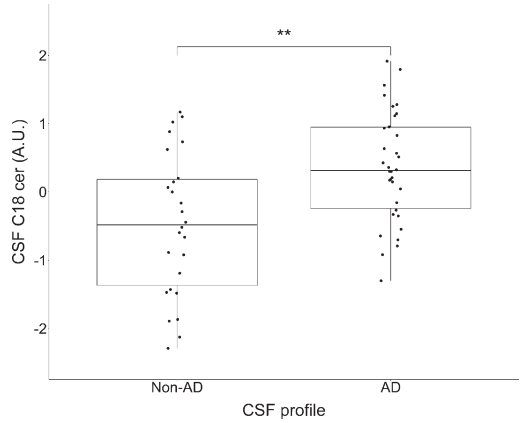

Fig. 3. Comparison in levels of CSF C18 ceramide by CSF profile (non-AD and AD). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ significance according to Mann-Whitney U non-parametric test. Based on normalized peak area arbitrary units (A.U.), log2-transformed and autoscaled before analysis. The lower and upper horizontal lines of the boxplot correspond to the 25th and the 75th centiles and the middle line to the median. Levels of CSF C18 ceramide were significantly higher ($p = 0.002$) among participants with a CSF AD profile compared to those without.

were negatively associated with C18 ceramide when adjusted for age, gender, and education (st. $\beta = –0.36$, $p = 0.007$). T-tau (st. $\beta = 0.41$, $p = 0.005$) and S100B (st. $\beta = 0.51$, $p = 0.001$) positively associated with C18 ceramide when adjusted for the same covariates. Statistically significant, albeit weaker, associations were detected between the same measures when linear regression was not adjusted for demographic variables. Levels of other CSF proteins (NFL, YKL-40, GFAP) and test scores presenting verbal episodic memory (RAVLT, Story) did not significantly associate with levels of C18 ceramide. One subject did

Table 3
Linear regression estimates (unadjusted and adjusted) for the association between various measures and levels of CSF C18 ceramide

	CSF C18 ceramide (A.U.)[a]			
	Unadjusted model		Adjusted model*	
	St. β	*p*	St. β	*p*
Demographics				
Age, y	–0.02	0.902	–0.05	0.765
Education, y	–0.05	0.688	–0.07	0.596
CSF proteins[a]				
$A\beta_{42}$ (pg/ml)	–0.35	0.006	–0.36	0.007
T-tau (pg/ml)	0.30	0.018	0.41	0.005
P-tau (pg/ml)	0.17	0.188	0.21	0.129
T-tau/$A\beta_{42}$	0.44	< 0.001	0.52	< 0.001
NFL (ng/ml)	0.03	0.835	0.05	0.782
YKL-40 (ng/ml)	0.11	0.411	0.25	0.194
S100B (pg/ml)	0.37	0.004	0.51	0.001
GFAP (ng/ml)	0.22	0.087	0.26	0.087
Verbal episodic memory				
RAVLT immediate recall, score[b]	–0.02	0.893	–0.01	0.928
RAVLT delayed recall, score[b]	–0.18	0.339	–0.29	0.095
Story immediate recall, score	–0.13	0.356	–0.14	0.345
Story delayed recall, score	–0.13	0.305	–0.15	0.335

A.U., arbitrary units; RAVLT, Rey Auditory Verbal Learning Test. Numbers present standardized beta coefficients (st.β). *Adjusted for age, gender, and years of education. [a]Values log2-transformed and autoscaled before analysis. [b]Analysis based on 59 subjects, with one missing.

not take the RAVLT test, and therefore the analyses involving RAVLT immediate and delayed recall were only based on 59 subjects. This did not skew comparisons between RAVLT and other regression estimates, as results in Table 3 did not change when calculated again without that subject.

The statistically significant relationships between the levels of proteins and C18 ceramide ($p < 0.05$) from Table 3 are depicted in Fig. 4. The associations were estimated with Pearson's coefficients, which are equal to the unadjusted standardized beta coefficients (st. β) in Table 3. Pearson's coefficients were also calculated for the CSF markers within each CSF profile group (Table 4), but none reached statistical significance ($p > 0.05$).

DISCUSSION

We used an untargeted lipidomic approach to identify CSF lipid species associated with a signature CSF profile reflecting AD pathology. A total of 1008 m/z features were detected, with eight selected as candidate markers. Out of these, one was fully confirmed as corresponding to the lipid species C18 ceramide. Our results showed that C18 ceramide levels were higher among subjects with a CSF AD profile. Relationships were also detected between established AD markers ($A\beta_{42}$, T-tau) and C18 ceramide. Higher levels of C18 ceramide associated with lower levels of $A\beta_{42}$ and higher levels of T-tau. In addition, levels of the inflammatory marker S100B positively related to C18 ceramide. Overall, our results indicate that CSF C18 ceramide levels could increase during pathological changes in the early stages of AD.

Ceramides, the core constituents of sphingolipid metabolism, are composed of a sphingosine backbone linked to a fatty acid chain of varying carbon atom length (C14-C26) [59]. Alterations in sphingolipid metabolism have been observed in healthy aging and in neurodegenerative diseases, including AD [60]. They play essential roles in the structural stability of membranes and as signaling molecules affect differentiation, proliferation, inflammation, and apoptosis [61, 62]. Ceramides are synthesized via two main pathways in eukaryotic cells [63]. In the salvage pathway, sphingomyelin is hydrolyzed through sphingomyelinase (SMase) to produce ceramide, which can be further metabolized to sphingosine by ceramidase. Ceramides can also be generated through the de novo pathway via anabolism of serine and palmitate. Results from cellular and animal studies suggest that both direct and indirect mechanisms by which ceramides can contribute to an increase in Aβ levels and AD pathogenesis [64]. Ceramides stimulate Aβ generation by stabilizing β-secretase enzyme BACE1 and increasing its half-life [65, 66]. Furthermore, soluble and fibrillar forms of Aβ can induce degradation of SM to ceramide by SMases [67, 68] through oxidative stress-mediated mechanisms [69]. This positive loop of ceramide production possibly contributes to immune activation and neuronal loss in AD. Long-chain ceramides, specifically C18 ceramide, have also been linked to tau phosphorylation through modulation of PP2A activity [70–74]. Several postmortem studies have found increased levels of ceramide in brain tissues of AD patients compared to healthy controls [60, 69, 75–77]. Two of the studies [60, 76] examined different ceramide species, with long-chain ceramide levels (C18 and C24) being significantly elevated in the AD group. Han et al. [75] observed the highest elevation of total ceramide in the brains of patients with mild AD, compared to those with severe AD and to controls,

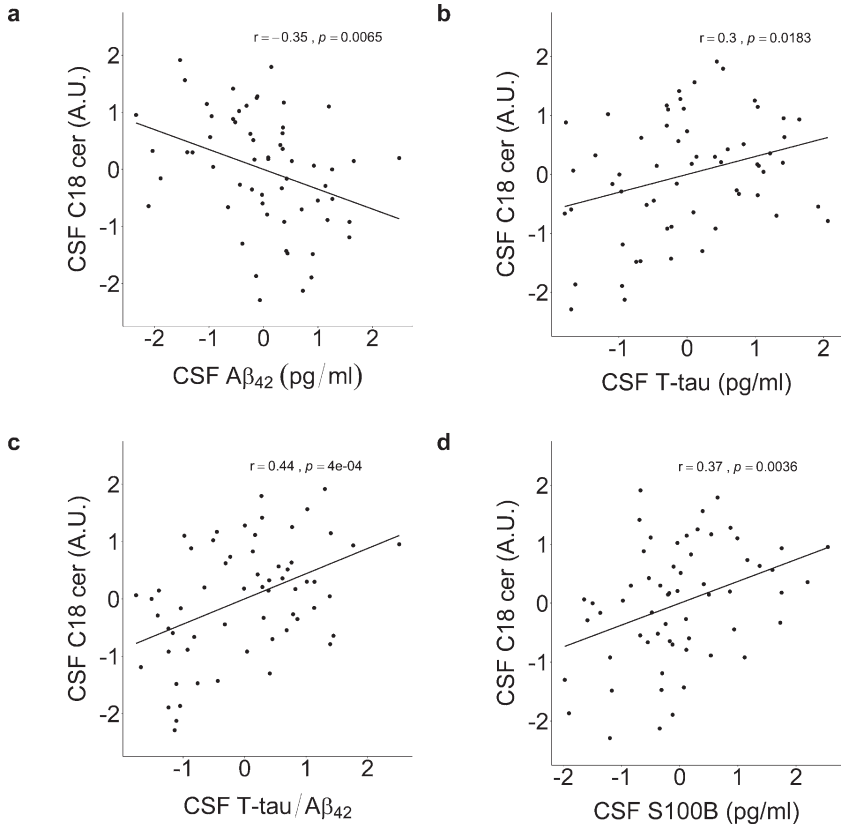

Fig. 4. Pearson's correlations between CSF levels of a) $A\beta_{42}$, b) T-tau, c) T-tau/ $A\beta_{42}$, d) S100B and CSF C18 ceramide. Normalized peak area arbitrary units (A.U.). All CSF measures were log2-transformed and autoscaled before analysis. C18 ceramide levels significantly correlated with levels of the core AD markers $A\beta_{42}$, T-tau, and inflammatory marker S100B.

Table 4
Pearson's correlations between selected CSF markers and levels of CSF C18 ceramide within CSF profiles

	CSF C18 ceramide (A.U.)[a]			
	CSF non-AD profile $n = 26$		CSF AD profile $n = 34$	
	r	p	r	p
CSF proteins[a]				
$A\beta_{42}$ (pg/ml)	–0.04	0.86	–0.25	0.15
T-tau (pg/ml)	0.23	0.25	–0.20	0.25
P-tau (pg/ml)	–0.20	0.34	–0.13	0.47
T-tau/$A\beta_{42}$	0.32	0.11	0.08	0.67
NFL (ng/ml)	–0.09	0.68	–0.07	0.70
YKL-40 (ng/ml)	0.08	0.70	0.02	0.90
S100B (pg/ml)	0.39	0.052	0.21	0.24
GFAP (ng/ml)	0.16	0.43	0.17	0.34

A.U., arbitrary units. [a]Values log2-transformed and autoscaled before analysis.

indicating early changes in the pathological processes of AD. To further confirm the implications of ceramide metabolism in AD, upregulation [78] and increase in activity of enzymes [77] controlling ceramide synthesis have been observed in brain areas (temporal and frontal cortices) affected in the early stages of the disease.

While postmortem studies are essential, *in vivo* studies are the only way to ascertain the role of lipids in early AD pathogenesis and whether these lipids may be indicators or predictors of disease progression. The most informative medium for studying lipid changes in the brain is the CSF, since it is in direct contact with the brain interstitial fluid. Very few CSF studies have been published comparing ceramide levels in AD patients to other groups. Fonteh et al. [79] found slightly higher, albeit not significant, levels of ceramide in AD compared to MCI and control groups in supernatant fluid of CSF. In another study [80], patients with AD had significantly higher levels compared to controls with other neurological condi-

tions. A study by Mielke et al. [81], examined the relationship between different ceramide species in relation to Aβ and tau levels in CSF. A positive correlation was found between C18 ceramide and all Aβ species except $A\beta_{42}$ as well as tau levels in a cohort of cognitively healthy individuals aged 36–69 years ($n = 91$) with a parental history of AD. In contrast with cellular studies, no significant correlation was found between the lipid species and P-tau. The same study also found a negative association between the longer carbon chain species (C20, C22, and C26) and performance on cognitive tests reflecting verbal episodic memory (delayed recall) or working memory. Our results expand upon the findings from this study. The results we present show a significant positive relationship between levels of C18 ceramide and T-tau, but not with P-tau, among the cohort of patients at risk of or at the early stages of dementia. Association between C18 ceramide and verbal episodic memory (immediate or delayed recall) was also not detected. No significant relationship was found with NFL levels, which is interesting considering it is a marker for neurodegeneration and white matter changes, and SM, a precursor of ceramide, is enriched in the myelin sheath of neurons. A possible explanation is CSF C18 ceramide being a marker of apoptosis, rather than degradation of myelinated axons. Evidence supports the role of ceramides in neuronal apoptosis, initiating a cascade of biochemical alterations leading ultimately to neuronal death [64]. In contrast with the study by Mielke et al. [81], we showed higher levels of C18 ceramide associating with lower levels of $A\beta_{42}$, one of the primary toxic species of amyloid [82]. A possible reason could be that all subjects in our study were already at the symptomatic pre- or early stages of dementia, and it is not unlikely that the relationship between the analytes strengthens during the progression of AD. Our study also found a positive association between levels of C18 ceramide and inflammatory marker S100B, but not YKL-40 and GFAP. Our hypothesis is that the relationship could be due to higher concentrations of ceramides and S100B, as both have been associated with the induction of neuronal apoptosis [26, 64]. In high doses, extracellular S100B has been shown to cause neuronal death by activation of the receptor for advanced glycation end products (RAGE). The protein can, by excessive stimulation of RAGE expressed in neurons, hyperactivate the Ras/MEK/ERK pathway, which consequently leads to apoptosis [83, 84]. S100B can also, indirectly, lead to neuronal death through the release of nitric oxide by astrocytes and microglia via RAGE-dependent activation [85, 86].

There are several limitations to our study. First, our sample size was relatively small, with a vast number of features compared to the number of subjects, resulting in little power and a considerable risk of type-II errors. Corrections for multiple comparisons were, therefore, not performed when comparing levels of features between groups in the discovery set. A more significant validation set would also have allowed for evaluation of accuracy in distinguishing between CSF profile groups using multiple lipid species simultaneously as predictors. Second, our study did not include a healthy control group or patients with moderate to severe AD. It also included very few participants with other dementias. It would be of interest to examine the relationships between ceramides and AD-related markers in CSF in a more diverse cohort, for a better evaluation of C18 ceramide as a marker of AD progression and severity, preferably in a longitudinal study. Third, information about the *APOE* genotype of subjects was not available, and therefore, its potential effect on results could not be adjusted for [87].

In summary, our results indicate that CSF C18 ceramide levels associate with established markers of AD pathology ($A\beta_{42}$ and T-tau) and inflammation (S100B) at the symptomatic pre- and early stages of dementia. These findings suggest that ceramide metabolism could influence the pathophysiological processes during the early stages of AD. Furthermore, ceramides could potentially serve as therapeutic targets, with strategies aiming at reducing ceramide levels to slow down the progression of the disease. Longitudinal studies are, however, needed to validate the pathological implications of these results.

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SUPPLEMENTARY MATERIAL

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REFERENCES

[1] Jack CR, Jr., Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* **14**, 535-562.

[2] Glenner GG, Wong CW (1984) Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun* **120**, 885-890.

[3] Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI (1986) Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci U S A* **83**, 4913-4917.

[4] Selkoe DJ, Hardy J (2016) The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* **8**, 595-608.

[5] Chen XQ, Mobley WC (2019) Alzheimer disease pathogenesis: Insights from molecular and cellular biology studies of oligomeric Abeta and tau species. *Front Neurosci* **13**, 659.

[6] Perrin RJ, Fagan AM, Holtzman DM (2009) Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. *Nature* **461**, 916-922.

[7] Zhou B, Teramukai S, Yoshimura K, Fukushima M (2009) Validity of cerebrospinal fluid biomarkers as endpoints in early-phase clinical trials for Alzheimer's disease. *J Alzheimers Dis* **18**, 89-102.

[8] Jack CR, Jr., Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* **9**, 119-128.

[9] Lewczuk P, Riederer P, O'Bryant SE, Verbeek MM, Dubois B, Visser PJ, Jellinger KA, Engelborghs S, Ramirez A, Parnetti L, Jack CR Jr, Teunissen CE, Hampel H, Lleó A, Jessen F, Glodzik L, de Leon MJ, Fagan AM, Molinuevo JL, Jansen WJ, Winblad B, Shaw LM, Andreasson U, Otto M, Mollenhauer B, Wiltfang J, Turner MR, Zerr I, Handels R, Thompson AG, Johansson G, Ermann N, Trojanowski JQ, Karaca I, Wagner H, Oeckl P, van Waalwijk van Doorn L, Bjerke M, Kapogiannis D, Kuiperij HB, Farotti L, Li Y, Gordon BA, Epelbaum S, Vos SJB, Klijn CJM, Van Nostrand WE, Minguillon C, Schmitz M, Gallo C, Lopez Mato A, Thibaut F, Lista S, Alcolea D, Zetterberg H, Blennow K, Kornhuber J; Members of the WFSBP Task Force Working on this Topic: Peter Riederer, Carla Gallo, Dimitrios Kapogiannis, Andrea Lopez Mato, Florence Thibaut (2018) Cerebrospinal fluid and blood biomarkers for neurodegenerative dementias: An update of the Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry. *World J Biol Psychiatry* **19**, 244-328.

[10] Jones L, Holmans PA, Hamshere ML, Harold D, Moskvina V, Ivanov D, Pocklington A, Abraham R, Hollingworth P, Sims R, Gerrish A, Pahwa JS, Jones N, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schurmann B, Heun R, Kolsch H, van den Bussche H, Heuser I, Peters O, Kornhuber J, Wiltfang J, Dichgans M, Frolich L, Hampel H, Hull M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Singleton AB, Guerreiro R, Muhleisen TW, Nothen MM, Moebus S, Jockel KH, Klopp N, Wichmann HE, Ruther E, Carrasquillo MM, Pankratz VS, Younkin SG, Hardy J, O'Donovan MC, Owen MJ, Williams J (2010) Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. *PLoS One* **5**, e13950.

[11] Matarin M, Salih DA, Yasvoina M, Cummings DM, Guelfi S, Liu W, Nahaboo Solim MA, Moens TG, Paublete RM, Ali SS, Perona M, Desai R, Smith KJ, Latcham J, Fulleylove M, Richardson JC, Hardy J, Edwards FA (2015) A genome-wide gene-expression analysis and database in transgenic mice during development of amyloid or tau pathology. *Cell Rep* **10**, 633-644.

[12] van Meer G, Voelker DR, Feigenson GW (2008) Membrane lipids: Where they are and how they behave. *Nat Rev Mol Cell Biol* **9**, 112-124.

[13] Shevchenko A, Simons K (2010) Lipidomics: Coming to grips with lipid diversity. *Nat Rev Mol Cell Biol* **11**, 593-598.

[14] Hussain G, Schmitt F, Loeffler JP, Gonzalez de Aguilar JL (2013) Fatting the brain: A brief of recent research. *Front Cell Neurosci* **7**, 144.

[15] Zarrouk A, Debbabi M, Bezine M, Karym EM, Badreddine A, Rouaud O, Moreau T, Cherkaoui-Malki M, El Ayeb M, Nasser B, Hammami M, Lizard G (2018) Lipid biomarkers in Alzheimer's disease. *Curr Alzheimer Res* **15**, 303-312.

[16] Walter J, van Echten-Deckert G (2013) Cross-talk of membrane lipids and Alzheimer-related proteins. *Mol Neurodegener* **8**, 34.

[17] Hartmann T, Kuchenbecker J, Grimm MO (2007) Alzheimer's disease: The lipid connection. *J Neurochem* **103 Suppl 1**, 159-170.

[18] Ardura-Fabregat A, Boddeke E, Boza-Serrano A, Brioschi S, Castro-Gomez S, Ceyzeriat K, Dansokho C, Dierkes T, Gelders G, Heneka MT, Hoeijmakers L, Hoffmann A, Iaccarino L, Jahnert S, Kuhbandner K, Landreth G, Lonnemann N, Loschmann PA, McManus RM, Paulus A, Reemst K, Sanchez-Caro JM, Tiberi A, Van der Perren A, Vautheny A, Venegas C, Webers A, Weydt P, Wijasa TS, Xiang X, Yang Y (2017) Targeting neuroinflammation to treat Alzheimer's disease. *CNS Drugs* **31**, 1057-1082.

[19] Calsolaro V, Edison P (2016) Neuroinflammation in Alzheimer's disease: Current evidence and future directions. *Alzheimers Dement* **12**, 719-732.

[20] Medeiros R, LaFerla FM (2013) Astrocytes: Conductors of the Alzheimer disease neuroinflammatory symphony. *Exp Neurol* **239**, 133-138.

[21] El Khoury JB, Moore KJ, Means TK, Leung J, Terada K, Toft M, Freeman MW, Luster AD (2003) CD36 mediates the innate host response to beta-amyloid. *J Exp Med* **197**, 1657-1666.

[22] Steardo L, Jr., Bronzuoli MR, Iacomino A, Esposito G, Steardo L, Scuderi C (2015) Does neuroinflammation turn

on the flame in Alzheimer's disease? Focus on astrocytes. *Front Neurosci* **9**, 259.

[23] Heppner FL, Ransohoff RM, Becher B (2015) Immune attack: The role of inflammation in Alzheimer disease. *Nat Rev Neurosci* **16**, 358-372.

[24] Carter SF, Herholz K, Rosa-Neto P, Pellerin L, Nordberg A, Zimmer ER (2019) Astrocyte biomarkers in Alzheimer's disease. *Trends Mol Med* **25**, 77-95.

[25] Bonneh-Barkay D, Bissel SJ, Kofler J, Starkey A, Wang G, Wiley CA (2012) Astrocyte and macrophage regulation of YKL-40 expression and cellular response in neuroinflammation. *Brain Pathol* **22**, 530-546.

[26] Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, Tubaro C, Giambanco I (2009) S100B's double life: Intracellular regulator and extracellular signal. *Biochim Biophys Acta* **1793**, 1008-1022.

[27] Yang Z, Wang KK (2015) Glial fibrillary acidic protein: From intermediate filament assembly and gliosis to neurobiomarker. *Trends Neurosci* **38**, 364-374.

[28] Rehli M, Niller HH, Ammon C, Langmann S, Schwarzfischer L, Andreesen R, Krause SW (2003) Transcriptional regulation of CHI3L1, a marker gene for late stages of macrophage differentiation. *J Biol Chem* **278**, 44058-44067.

[29] Alcolea D, Vilaplana E, Pegueroles J, Montal V, Sánchez-Juan P, González-Suárez A, Pozueta A, Rodríguez-Rodríguez E, Bartrés-Faz D, Vidal-Piñeiro D, González-Ortiz S, Medrano S, Carmona-Iragui M, Sánchez-Saudinós M, Sala I, Anton-Aguirre S, Sampedro F, Morenas-Rodríguez E, Clarimón J, Blesa R, Lleó A, Fortea J (2015) Relationship between cortical thickness and cerebrospinal fluid YKL-40 in predementia stages of Alzheimer's disease. *Neurobiol Aging* **36**, 2018-2023.

[30] Alcolea D, Carmona-Iragui M, Suarez-Calvet M, Sanchez-Saudinos MB, Sala I, Anton-Aguirre S, Blesa R, Clarimon J, Fortea J, Lleo A (2014) Relationship between beta-Secretase, inflammation and core cerebrospinal fluid biomarkers for Alzheimer's disease. *J Alzheimers Dis* **42**, 157-167.

[31] Antonell A, Mansilla A, Rami L, Llado A, Iranzo A, Olives J, Balasa M, Sanchez-Valle R, Molinuevo JL (2014) Cerebrospinal fluid level of YKL-40 protein in preclinical and prodromal Alzheimer's disease. *J Alzheimers Dis* **42**, 901-908.

[32] Craig-Schapiro R, Perrin RJ, Roe CM, Xiong C, Carter D, Cairns NJ, Mintun MA, Peskind ER, Li G, Galasko DR, Clark CM, Quinn JF, D'Angelo G, Malone JP, Townsend RR, Morris JC, Fagan AM, Holtzman DM (2010) YKL-40: A novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol Psychiatry* **68**, 903-912.

[33] Melah KE, Lu SY, Hoscheidt SM, Alexander AL, Adluru N, Destiche DJ, Carlsson CM, Zetterberg H, Blennow K, Okonkwo OC, Gleason CE, Dowling NM, Bratzke LC, Rowley HA, Sager MA, Asthana S, Johnson SC, Bendlin BB (2016) Cerebrospinal fluid markers of Alzheimer's disease pathology and microglial activation are associated with altered white matter microstructure in asymptomatic adults at risk for Alzheimer's disease. *J Alzheimers Dis* **50**, 873-886.

[34] Querol-Vilaseca M, Colom-Cadena M, Pegueroles J, San Martin-Paniello C, Clarimon J, Belbin O, Fortea J, Lleo A (2017) YKL-40 (Chitinase 3-like I) is expressed in a subset of astrocytes in Alzheimer's disease and other tauopathies. *J Neuroinflammation* **14**, 118.

[35] Olsson B, Lautner R, Andreasson U, Ohrfelt A, Portelius E, Bjerke M, Holtta M, Rosen C, Olsson C, Strobel G, Wu E, Dakin K, Petzold M, Blennow K, Zetterberg H (2016) CSF and blood biomarkers for the diagnosis of Alzheimer's disease: A systematic review and meta-analysis. *Lancet Neurol* **15**, 673-684.

[36] Olsson B, Portelius E, Cullen NC, Sandelius A, Zetterberg H, Andreasson U, Hoglund K, Irwin DJ, Grossman M, Weintraub D, Chen-Plotkin A, Wolk D, McCluskey L, Elman L, Shaw LM, Toledo JB, McBride J, Hernandez-Con P, Lee VM, Trojanowski JQ, Blennow K (2019) Association of cerebrospinal fluid neurofilament light protein levels with cognition in patients with dementia, motor neuron disease, and movement disorders. *JAMA Neurol* **76**, 318-325.

[37] Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, White CL, 3rd, Araoz C (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci U S A* **86**, 7611-7615.

[38] Mrak RE, Sheng JG, Griffin WS (1996) Correlation of astrocytic S100 beta expression with dystrophic neurites in amyloid plaques of Alzheimer's disease. *J Neuropathol Exp Neurol* **55**, 273-279.

[39] Hanzel DK, Trojanowski JQ, Johnston RF, Loring JF (1999) High-throughput quantitative histological analysis of Alzheimer's disease pathology using a confocal digital microscanner. *Nat Biotechnol* **17**, 53-57.

[40] Muramori F, Kobayashi K, Nakamura I (1998) A quantitative study of neurofibrillary tangles, senile plaques and astrocytes in the hippocampal subdivisions and entorhinal cortex in Alzheimer's disease, normal controls and non-Alzheimer neuropsychiatric diseases. *Psychiatry Clin Neurosci* **52**, 593-599.

[41] Vehmas AK, Kawas CH, Stewart WF, Troncoso JC (2003) Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. *Neurobiol Aging* **24**, 321-331.

[42] Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, DeKosky ST, Gauthier S, Selkoe D, Bateman R, Cappa S, Crutch S, Engelborghs S, Frisoni GB, Fox NC, Galasko D, Habert MO, Jicha GA, Nordberg A, Pasquier F, Rabinovici G, Robert P, Rowe C, Salloway S, Sarazin M, Epelbaum S, de Souza LC, Vellas B, Visser PJ, Schneider L, Stern Y, Scheltens P, Cummings JL (2014) Advancing research diagnostic criteria for Alzheimer's disease: The IWG-2 criteria. *Lancet Neurol* **13**, 614-629.

[43] Ferreira D, Perestelo-Perez L, Westman E, Wahlund LO, Sarria A, Serrano-Aguilar P (2014) Meta-review of CSF core biomarkers in Alzheimer's disease: The state-of-the-art after the new revised diagnostic criteria. *Front Aging Neurosci* **6**, 47.

[44] Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189-198.

[45] Jorm AF (2004) The Informant Questionnaire on cognitive decline in the elderly (IQCODE): A review. *Int Psychogeriatr* **16**, 275-293.

[46] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263-269.

[47] McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor J-P, Weintraub D, Aarsland D, Galvin J, Attems J, Ballard CG, Bayston A, Beach TG, Blanc F, Bohnen N, Bonanni L, Bras J, Brundin P, Burn D, Chen-Plotkin A, Duda JE, El-Agnaf O, Feldman H, Ferman TJ, ffytche D, Fujishiro H, Galasko D, Goldman JG, Gomperts SN, Graff-Radford NR, Honig LS, Iranzo A, Kantarci K, Kaufer D, Kukull W, Lee VMY, Leverenz JB, Lewis S, Lippa C, Lunde A, Masellis M, Masliah E, McLean P, Mollenhauer B, Montine TJ, Moreno E, Mori E, Murray M, O'Brien JT, Orimo S, Postuma RB, Ramaswamy S, Ross OA, Salmon DP, Singleton A, Taylor A, Thomas A, Tiraboschi P, Toledo JB, Trojanowski JQ, Tsuang D, Walker Z, Yamada M, Kosaka K (2017) Diagnosis and management of dementia with Lewy bodies. Fourth consensus report of the DLB Consortium. *Neurology* **89**, 88-100.

[48] Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, Nordberg A, Bäckman L, Albert M, Almkvist O, Arai H, Basun H, Blennow K, de Leon M, DeCarli C, Erkinjuntti T, Giacobini E, Graff C, Hardy J, Jack C, Jorm A, Ritchie K, van Duijn C, Visser P, Petersen RC (2004) Mild cognitive impairment–beyond controversies, towards a consensus: Report of the International Working Group on Mild Cognitive Impairment. *J Intern Med* **256**, 240-246.

[49] Duits FH, Teunissen CE, Bouwman FH, Visser PJ, Mattsson N, Zetterberg H, Blennow K, Hansson O, Minthon L, Andreasen N, Marcusson J, Wallin A, Rikkert MO, Tsolaki M, Parnetti L, Herukka SK, Hampel H, De Leon MJ, Schroder J, Aarsland D, Blankenstein MA, Scheltens P, van der Flier WM (2014) The cerebrospinal fluid "Alzheimer profile": Easily said, but what does it mean? *Alzheimers Dement* **10**, 713-723.e712.

[50] Petersen RC, Smith GE, Ivnik RJ, Kokmen E, Tangalos EG (1994) Memory function in very early Alzheimer's disease. *Neurology* **44**, 867-867.

[51] Lezak MD, Howieson DB, Bigler ED, Tranel D (2012) *Neuropsychological assessment, 5th ed*, Oxford University Press, New York, NY, US.

[52] Wechsler D (1987) *WMS-R : Wechsler Memory Scale-Revised : Manual*, Harcourt Brace Jovanovich, San Antonio.

[53] Bird SS, Marur VR, Sniatynski MJ, Greenberg HK, Kristal BS (2011) Serum lipidomics profiling using LC-MS and high-energy collisional dissociation fragmentation: Focus on triglyceride detection and characterization. *Anal Chem* **83**, 6648-6657.

[54] Haughey NJ, Bandaru VV, Bae M, Mattson MP (2010) Roles for dysfunctional sphingolipid metabolism in Alzheimer's disease neuropathogenesis. *Biochim Biophys Acta* **1801**, 878-886.

[55] Kotronoulas A, Jonasdottir HS, Sigurethardottir RS, Halldorsson S, Haraldsson GG, Rolfsson O (2020) Wound healing grafts: Omega-3 fatty acid lipid content differentiates the lipid profiles of acellular Atlantic cod skin from traditional dermal substitutes. *J Tissue Eng Regen Med* **14**, 441-451.

[56] Tsugawa H, Cajka T, Kind T, Ma Y, Higgins B, Ikeda K, Kanazawa M, VanderGheynst J, Fiehn O, Arita M (2015) MS-DIAL: Data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat Methods* **12**, 523-526.

[57] Kind T, Liu KH, Lee DY, DeFelice B, Meissen JK, Fiehn O (2013) LipidBlast in silico tandem mass spectrometry database for lipid identification. *Nat Methods* **10**, 755-758.

[58] Efron B, Gong G (1983) A leisurely look at the bootstrap, the jackknife, and cross-validation. *Am Stat* **37**, 36-48.

[59] Fanani ML, Maggio B (2017) The many faces (and phases) of ceramide and sphingomyelin I - single lipids. *Biophys Rev* **9**, 589-600.

[60] Stranahan AM, Cutler RG, Button C, Telljohann R, Mattson MP (2011) Diet-induced elevations in serum cholesterol are associated with alterations in hippocampal lipid metabolism and increased oxidative stress. *J Neurochem* **118**, 611-615.

[61] Cuvillier O (2002) Sphingosine in apoptosis signaling. *Biochim Biophys Acta* **1585**, 153-162.

[62] Gomez-Munoz A, Presa N, Gomez-Larrauri A, Rivera IG, Trueba M, Ordonez M (2016) Control of inflammatory responses by ceramide, sphingosine 1-phosphate and ceramide 1-phosphate. *Prog Lipid Res* **61**, 51-62.

[63] Hannun YA, Obeid LM (2008) Principles of bioactive lipid signalling: Lessons from sphingolipids. *Nat Rev Mol Cell Biol* **9**, 139-150.

[64] Jazvinscak Jembrek M, Hof PR, Simic G (2015) Ceramides in Alzheimer's disease: Key mediators of neuronal apoptosis induced by oxidative stress and Abeta accumulation. *Oxid Med Cell Longev* **2015**, 346783.

[65] Kalvodova L, Kahya N, Schwille P, Ehehalt R, Verkade P, Drechsel D, Simons K (2005) Lipids as modulators of proteolytic activity of BACE: Involvement of cholesterol, glycosphingolipids, and anionic phospholipids *in vitro*. *J Biol Chem* **280**, 36815-36823.

[66] Puglielli L, Ellis BC, Saunders AJ, Kovacs DM (2003) Ceramide stabilizes beta-site amyloid precursor protein-cleaving enzyme 1 and promotes amyloid beta-peptide biogenesis. *J Biol Chem* **278**, 19777-19783.

[67] Grimm MO, Grimm HS, Patzold AJ, Zinser EG, Halonen R, Duering M, Tschape JA, De Strooper B, Muller U, Shen J, Hartmann T (2005) Regulation of cholesterol and sphingomyelin metabolism by amyloid-beta and presenilin. *Nat Cell Biol* **7**, 1118-1123.

[68] Lee JT, Xu J, Lee JM, Ku G, Han X, Yang DI, Chen S, Hsu CY (2004) Amyloid-beta peptide induces oligodendrocyte death by activating the neutral sphingomyelinase-ceramide pathway. *J Cell Biol* **164**, 123-131.

[69] Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, Troncoso JC, Mattson MP (2004) Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proc Natl Acad Sci U S A* **101**, 2070-2075.

[70] Chalfant CE, Kishikawa K, Mumby MC, Kamibayashi C, Bielawska A, Hannun YA (1999) Long chain ceramides activate protein phosphatase-1 and protein phosphatase-2A. Activation is stereospecific and regulated by phosphatidic acid. *J Biol Chem* **274**, 20313-20317.

[71] Dobrowsky RT, Kamibayashi C, Mumby MC, Hannun YA (1993) Ceramide activates heterotrimeric protein phosphatase 2A. *J Biol Chem* **268**, 15523-15530.

[72] Goedert M, Jakes R, Qi Z, Wang JH, Cohen P (1995) Protein phosphatase 2A is the major enzyme in brain that dephosphorylates tau protein phosphorylated by proline-directed protein kinases or cyclic AMP-dependent protein kinase. *J Neurochem* **65**, 2804-2807.

[73] Gong CX, Grundke-Iqbal I, Iqbal K (1994) Dephosphorylation of Alzheimer's disease abnormally phosphorylated tau by protein phosphatase-2A. *Neuroscience* **61**, 765-772.

[74] Mukhopadhyay A, Saddoughi SA, Song P, Sultan I, Ponnusamy S, Senkal CE, Snook CF, Arnold HK, Sears RC, Hannun YA, Ogretmen B (2009) Direct interaction between the inhibitor 2 and ceramide via sphingolipid-protein binding is involved in the regulation of protein phosphatase 2A activity and signaling. *FASEB J* **23**, 751-763.

[75] Han X, M. Holtzman D, W. McKeel Jr D, Kelley J, Morris JC (2002) Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: Potential role in disease pathogenesis. *J Neurochem* **82**, 809-818.

[76] Filippov V, Song MA, Zhang K, Vinters HV, Tung S, Kirsch WM, Yang J, Duerksen-Hughes PJ (2012) Increased ceramide in brains with Alzheimer's and other neurodegenerative diseases. *J Alzheimers Dis* **29**, 537-547.

[77] He X, Huang Y, Li B, Gong CX, Schuchman EH (2010) Deregulation of sphingolipid metabolism in Alzheimer's disease. *Neurobiol Aging* **31**, 398-408.

[78] Katsel P, Li C, Haroutunian V (2007) Gene expression alterations in the sphingolipid metabolism pathways during progression of dementia and Alzheimer's disease: A shift toward ceramide accumulation at the earliest recognizable stages of Alzheimer's disease? *Neurochem Res* **32**, 845-856.

[79] Fonteh AN, Ormseth C, Chiang J, Cipolla M, Arakaki X, Harrington MG (2015) Sphingolipid metabolism correlates with cerebrospinal fluid Beta amyloid levels in Alzheimer's disease. *PLoS One* **10**, e0125597.

[80] Satoi H, Tomimoto H, Ohtani R, Kitano T, Kondo T, Watanabe M, Oka N, Akiguchi I, Furuya S, Hirabayashi Y, Okazaki T (2005) Astroglial expression of ceramide in Alzheimer's disease brains: A role during neuronal apoptosis. *Neuroscience* **130**, 657-666.

[81] Mielke MM, Haughey NJ, Bandaru VVR, Zetterberg H, Blennow K, Andreasson U, Johnson SC, Gleason CE, Blazel HM, Puglielli L, Sager MA, Asthana S, Carlsson CM (2014) Cerebrospinal fluid sphingolipids, beta-amyloid, and tau in adults at risk for Alzheimer's disease. *Neurobiol Aging* **35**, 2486-2494.

[82] Sengupta U, Nilson AN, Kayed R (2016) The role of amyloid-β oligomers in toxicity, propagation, and immunotherapy. *EBioMedicine* **6**, 42-49.

[83] Huttunen HJ, Kuja-Panula J, Sorci G, Agneletti AL, Donato R, Rauvala H (2000) Coregulation of neurite outgrowth and cell survival by amphoterin and S100 proteins through receptor for advanced glycation end products (RAGE) activation. *J Biol Chem* **275**, 40096-40105.

[84] Fanò G, Mariggiò MA, Angelella P, Nicoletti I, Antonica A, Fulle S, Calissano P (1993) The S-100 protein causes an increase of intracellular calcium and death of PC12 cells. *Neuroscience* **53**, 919-925.

[85] Hu J, Ferreira A, Van Eldik LJ (1997) S100beta induces neuronal cell death through nitric oxide release from astrocytes. *J Neurochem* **69**, 2294-2301.

[86] Adami C, Sorci G, Blasi E, Agneletti AL, Bistoni F, Donato R (2001) S100B expression in and effects on microglia. *Glia* **33**, 131-142.

[87] Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G (2013) Apolipoprotein E and Alzheimer disease: Risk, mechanisms and therapy. *Nat Rev Neurol* **9**, 106-118.

Paper III

Phenotypic displays of cholinergic enzymes associate with markers of inflammation and neurodegeneration in a memory clinic cohort

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Keywords: Alzheimer's disease, Cerebrospinal fluid, Acetylcholinesterase, Butyrylcholinesterase, inflammation, neurodegeneration

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ABSTRACT

Objectives

To evaluate the relationship between cholinergic enzyme activity, markers of amyloidosis, neurodegeneration and inflammation in cerebrospinal spinal fluid (CSF) and verbal episodic memory performance among subjects from a memory clinic cohort.

Methods

In this cross-sectional study, 46 cholinergic drug-free subjects (median age=71, 54% female, median MMSE=28) were recruited from an Icelandic memory clinic cohort targeting early stages of cognitive impairment. Subjects were classified into three profiles based on the CSF levels of the core AD markers amyloid-$\beta_{1\text{-}42}$ ($A\beta_{42}$), total tau (T-tau) and phosphorylated tau (P-tau). Each profile was divided into two categories according to a cut-off point; positive (abnormal) or a negative (normal) levels. The activities of the cholinergic enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) as well as levels of neurofilament light (NFL), YKL-40, S100 calcium-binding protein B (S100B) and glial fibrillary acidic protein (GFAP) were also measured in CSF. Verbal episodic memory was assessed by the use of Rey Auditory Verbal Learning (RAVLT) and Story tests.

Results

No significant differences were found in AChE or BuChE activity between positive and negative CSF $A\beta_{42}$ profile groups (p>0.05). In contrast, AChE activity was significantly higher among subjects with positive T-tau or P-tau profiles, compared to those with negative profiles. In accordance, T-tau (r=0.46, p=0.001) and P-tau (r=0.45, p=0.002) levels significantly correlated with AChE. Inflammation markers S100B and YKL-40 both significantly correlated with AChE (S100B: r=0.43, p=0.003; YKL-40: r=0.32, p=0.03) and BuChE (S100B: r=0.47, p<0.001; YKL-40: r=0.38, p=0.009) activity. Weak correlation was detected between AChE activity and composite score reflecting verbal episodic memory (r=-0.34, p=0.02). LASSO regression analyses with a stability approach were performed for the selection of a set of measures best predicting cholinergic activity and verbal episodic memory score. S100B was the predictor with the highest model selection frequency for both AChE (68%) and BuChE (73%) activity. Age (91%) was the

most reliable predictor for verbal episodic memory, with selection frequency of both cholinergic enzymes below 10%.

Conclusions

These results indicate a relationship between higher activity of the ACh-degrading cholinergic enzymes with increased neurodegeneration and inflammation in the stages of pre- and early symptomatic dementia, independent of CSF $A\beta_{42}$ levels.

INTRODUCTION

The neuropathological changes in AD include the accumulation of beta-amyloid (Aβ) in plaques and hyper-phosphorylated tau protein in neurofibrillary tangles (NFT), leading to loss of synapses, dendrites, and eventually neurons. The most consistent neuronal loss through the progression of AD is found within the cholinergic system of the basal forebrain [1]. The cholinergic neurons of this region are the major source of cholinergic innervation to the cerebral cortex and hippocampus, playing a pivotal role in cognitive functions including memory, learning and attention [2]. Due to the critical role of the neurotransmitter acetylcholine (ACh) in cognitive functions, most of the approved pharmacological treatments for AD are cholinesterase inhibitors (ChEIs). ChEIs increase the availability of ACh at synapses in the brain and are among the few drugs that have been proven clinically useful in the treatment of AD, thus validating the cholinergic system as an important therapeutic target in the disease [3].

According to the classical view of cholinergic signaling [4], the neurotransmitter ACh is synthesized in the cytoplasm of cholinergic neurons by the enzyme choline acetyltransferase (ChAT). ACh molecules are released into the synaptic clefts for initiating or propagating a neurotransmission. The transmission is subsequently terminated by the cleavage of ACh by cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Notably, ACh is not restricted to neurons and synapses. The cholinergic signaling system has also been associated with inflammation, both generally and in neurodegenerative diseases like AD. ACh is hypothesized to act as a suppressor on non-excitable cholinoceptive cells, including astrocytes and microglia, inhibiting cytokine release through activation of nicotinic α7-ACh receptors [5, 6]. Recent research demonstrated that astrocytes secrete the ACh synthesizing enzyme ChAT, suggesting that the physiological function of extracellular ChAT is to maintain steady-state equilibrium of hydrolysis and synthesis of ACh [7].

Extracellular accumulation of Aβ in AD may cause imbalances in cholinergic signaling, which facilitates increased degradation of ACh and enhanced cytokine expression and release. Such an exaggerated inflammatory microenvironment could, in turn, have consequential neurodegenerative effects [8]. BuChE may also have a particularly critical role in the dynamic control of levels of extracellular ACh, via its ACh hydrolyzing activity [9], as the primary source of it in the CNS is attributed to non-excitable cells such as astrocytes and microglia [10, 11].

In recent years, a paradigm shift has occurred from clinical to biological definition of AD based on in vivo biomarkers measured in cerebrospinal fluid (CSF) or with positron emission tomography (PET) imaging. In 2018, the National Institute on Aging and Alzheimer's Association (NIA-AA) [12] created a research framework for AD diagnosis, defining AD based on biomarker evidence of pathology. Understanding how biomarkers reflecting different biological processes could influence AD pathogenesis and severity is critical for the improvement of diagnosis and development of effective pharmacologic treatments. It is essential for the evaluation of novel biomarkers to examine their relationship with signature AD pathology, independent of diagnosis. Such an approach could both enhance understanding of the underlying pathology of AD and the sequence of events leading to cognitive impairment. The aim of this study was to examine the association between the activity of cholinergic enzymes and CSF markers reflecting the the state of brain amyloidosis ($A\beta_{42}$), neurodegeneration (T-tau, P-tau, NFL) and inflammation (YKL-40, S100B, GFAP) among subjects at the pre-and early symptomatic stages of dementia. The second aim was to explore the relationships of same enzymes with the loss of verbal episodic memory.

MATERIALS AND METHODS

Subjects

The current study cohort and the selection criteria have been described earlier [13]. Subjects were recruited from the Icelandic MCI study cohort (n=165). The cohort was comprised of individuals who had been referred to Landspitali University Hospital (LUH) Memory Clinic over a four-year period. The inclusion criteria for joining the cohort study were: i) a score between 24-30 on the Mini-Mental State Examination (MMSE) [14] and 2) a score of 4.0 or less on the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) [15]. The exclusion criteria were the following: i) cognitive impairment caused by a pre-existing condition, ii) difficulties participating due to health or social issues, and iii) residency outside the Reykjavík Capital Area. Each subject underwent various measurements at baseline, which included a medical assessment and a detailed neuropsychological assessment as well as brain magnetic resonance imaging (MRI) for the evaluation of medial temporal lobe atrophy (MTA), global cortical atrophy (GCA) and white matter lesions (Fazekas). Lumbar puncture was carried out for the collection of CSF, but the intervention was optional by the requirement of the National Bioethics Committee. For this cross-sectional study, the final sample included 46 subjects (Fig. 1) . Only those who underwent lumbar puncture were selected from the Icelandic MCI study cohort, excluding 113 subjects. In addition, six other subjects were removed due to excessively high CSF P-tau (n=1), GFAP (n=1), AChE and BuChE (n=1) values or blood-contamination in CSF samples (n=3). Clinical diagnosis of AD was based on the criteria for probable AD dementia defined by the National Institute on Aging-Alzheimer's Association (NIA-AA) [16], with evidence of AD pathophysiological processes (based on MTA score or/and analysis of core CSF markers). Patients with Lewy body dementia (LBD) were diagnosed based on the consensus criteria of McKeith [17]. The diagnosis of MCI required the fulfillment of the Winblad criteria [18]. Those without cognitive impairment were considered to have subjective mild cognitive impairment (SCI), as they had been referred to the Memory Clinic due to concerns of cognitive decline. Of the 46 subjects in this study, 15 were diagnosed with SCI or MCI and remained stable after a two year follow-up. One subject converted from a diagnosis of MCI to AD after a two year follow-up and another one from MCI to cortico-basilar degeneration (CBD) after a one year follow-up. A total of 18 subjects were diagnosed with AD, three with LBD and one with Parkinson's disease (PD) at baseline. Seven of the subjects were diagnosed with SCI and MCI at baseline but left the study before one year og two year follow-up. None of the subjects had been prescriped cholinergic drugs before entering the study.

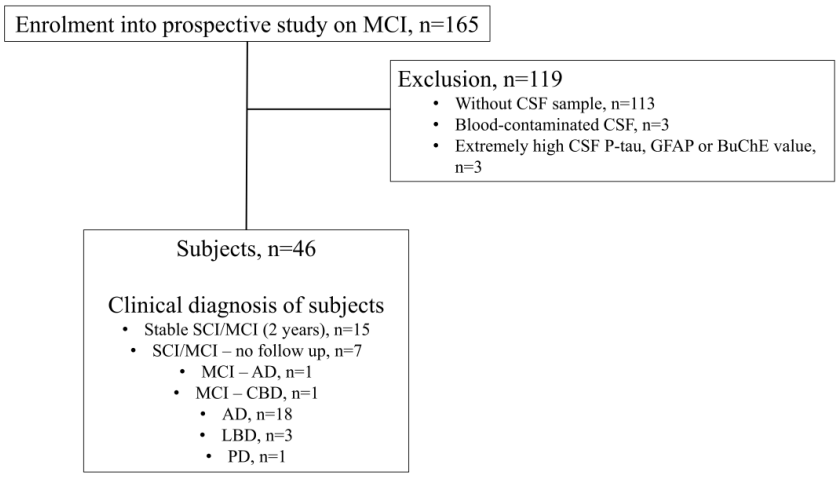

Figure 1. Flow chart illustrating the inclusion/exclusion criteria for the study.

CSF collection and analysis

Collection of CSF was done via lumbar puncture with a 22-gauge spinal needle at the L3/4 or L4/5 interspace. Samples, uncentrifuged, were frozen in 2 ml polypropylene tubes and stored at -80 °C. Levels of all proteins were determined using commercially available sandwich enzyme-linked immunosorbent assays (ELISAs) and performed according to manufacturer's instructions. Levels of T-tau (IBL International, Hamburg, Germany), P-tau181 (INNOTEST, Gent, Belgium) and $A\beta_{42}$ (IBL International, Hamburg, Germany), were measured in the ISO 15189 accredited medical laboratory MVZ Labor P.D. Dr. Volkmann und Kollegen GbR (Karlsruhe, Germany). Levels of NFL (Uman Diagnostics, Umeå, Sweden), YKL-40 (Quantikine ELISA Human Chitinase-3–like 1; R&D systems, MN, USA), S100B (BioVendor GmbH, Heidelberg, Germany) and GFAP (BioVendor GmbH, Heidelberg, Germany) were measured in a

laboratory at the University of Iceland. All assays had mean Intra-assay CV <10% and Inter-assay CV <15%. The activity of BuChE and AChE in CSF was measured as described in Darreh-Shori et al. [19, 20].

Subject grouping based on CSF measures

Three different CSF biomarker profiles were created, each based on the levels of one core CSF biomarker ($A\beta_{42}$ T-tau and P-tau). The CSF profile was divided into two categories, abnormal (positive) or normal (negative). The specific cut-off points were established by MVZ Labor P.D. Dr. Volkmann und Kollegen GbR , the laboratory performing the ELISAs. Abnormal values were defined as $A\beta_{42}$ < 375 pg/ml, T-tau > 445 pg/ml and P-tau > 61 pg/ml.

Neuropsychological tests

A detailed neuropsychological assessment was carried out by licensed psychologists under the guidance of a clinical neuropsychologist. Two tests were used for the evaluation of verbal episodic memory, The Rey Auditory Verbal Learning Test (RAVLT) [21], and a Story test based on the Logical Memory test of the Wechsler Memory Scale-Revised [22]. RAVLT requires the subject to learn 15 nouns presented across five consecutive trials. Each trial is followed by a free-recall test (immediate recall). The sum of the number of words recalled from trials 1 through 5 (0 to 75 points) is calculated for the score of RAVLT immediate recall. After a 30 min delay, the subject is asked to recall as many words as possible without hearing them again (delayed recall). The score is a sum of correctly recalled words (0-15 points). Lastly, a list containing the previous 15 nouns as well as 30 new ones, is read to the subject whose task is to recognize the nouns from the list, with number of false positives subtracted from the score (-30 to 15 points). The second test is composed of a brief story, presented orally by the examiner. The story includes 25 story ideas, where the subject is required to repeat it immediately after presentation without any clues given. A point is given for the number of story ideas correctly recalled (0 to 25 points).

After a 30 minute delay, the subject is asked to recall the story again without it being orally repeated again.

Statistical analysis

Descriptive group comparisons were performed using Mann-Whitney U tests or Kruskal-Wallis H tests for continuous variables and chi-square tests for categorical variables. The composite score for verbal episodic memory was calculated by averaging the z-scores of each neuropsychological tests and subsequently converting those scores into z-scores. Raw values of CSF proteins ($A\beta_{42}$, P-tau, T-tau, NFL, YKL-40, S100B, GFAP) were naturally log-transformed to account for a non-normal distribution. Pearson's correlations and scatter plots were used for estimation and visualization of relationships between continuous variables. Stability selection was employed in combination with least absolute shrinkage and selection operator (LASSO) regression for the purpose of identifying stable predictors in multivariable models [49]. Stability selection was performed by the use of the function stabsel in the R package stabs, implementing the package glmnet for LASSO model fitting [50, 51]. Cut-off value for stable selection was set to 75% (the percentage of times a variable was selected into a model) and per-family error rate (PFER) to 1 for all analyses. Each subsample was half the size of the original one, with 100 subsamples being drawn. All statistical analyses were performed using R (version 3.6.1, The R Foundation for Statistical Computing).

RESULTS

Study cohort characteristics

Table 1 shows the demographic, pathophysiological and clinical characteristics of the cohort by CSF $A\beta_{42}$ and T-tau profiles. No statistical difference ($p > 0.05$) was found in characteristics between subjects with abnormally low CSF $A\beta_{42}$ levels (positive profile; +) and those with normal $A\beta_{42}$ levels (negative profile; -). In contrast, statistical difference was found between the CSF T-tau profile groups. Subjects with abnormally high CSF T-tau levels (+) were statistically higher in age (p=0.02), CSF AChE activity (p=0.008), CSF NFL levels (p<0.001) and CSF YKL-40 levels (p=0.02) compared to those with normal T-tau levels (-). The T-tau+ group did also score significantly lower on all neuropsychological subtests reflecting verbal episodic memory (p<0.01).

Table 1. Subject demographics, CSF marker levels and cognitive scores by CSF $A\beta_{42}$ and T-tau profiles

	CSF $A\beta_{42}$ profile			CSF T-tau profile		
	$A\beta_{42}$-, $A\beta_{42}$ > 375 pg/ml (n=33)	**$A\beta_{42}$+, $A\beta_{42}$ < 375 pg/ml (n=13)**	***p* value [a]**	**T-tau-, T-tau < 445 pg/ml (n=29)**	**T-tau+, T-tau > 445 pg/ml (n=17)**	***p* value [a]**
Demographics						
Gender (M/F)	13/20	8/5	0.18	14/15	7/10	0.64
Age, years	71 (46-85)	70 (51-84)	0.75	70 (46-85)	77 (51-84)	0.02
Education, years	13 (6-19)	13 (6-20)	0.77	13 (6-19)	13 (6-20)	0.75
Clinical diagnosis						
Stable SCI or MCI/AD[b]/ OD[c]/SCI or MCI – no follow-up	12/11/5/5	3/8/0/2	N/A[d]	15/4/3/7	0/15/2/0	N/A[d]
CSF measures						
$A\beta_{42}$ (pg/ml)	671 (404-1434)	254 (140-335)	N/A	594 (167-1434)	490 (140-977)	0.27
T-tau (pg/ml)	294 (106-1886)	397 (132-916)	0.09	253 (106-438)	643 (475-1086)	N/A
P-tau (pg/ml)	57 (33-144)	83 (30-125)	0.40	49 (30-87)	106 (70-144)	N/A
AChE activity (nmol/min/ml)	12.2 (4.4-16.3)	10.5 (8.0-16.0)	0.35	11.1 (4.4-15.5)	13.5 (8.0-16.3)	0.008
BuChE activity (nmol/min/ml)	6.3 (4.2-10.9)	6.4 (4.7-11.5)	0.92	6.2 (4.2-9.4)	7.0 (4.7-11.5)	0.11
NFL (ng/ml)	2.1 (1.0-5.0)	2.3 (1.2-5.3)	0.60	2.0 (1.0-3.6)	3.0 (1.6-5.3)	< 0.001
YKL-40 (ng/ml)	194 (83-367)	177 (124-351)	0.87	160 (83-365)	235 (124-367)	0.02
S100B (pg/ml)	233 (143-458)	240 (175-509)	0.89	228 (143-382)	243 (175-509)	0.15
GFAP (ng/ml)	1.2 (0.2-21.3)	1.4 (0.8-5.8)	0.14	1.3 (0.2-6.3)	1.1 (0.5-21.3)	0.59
Global cognition						
MMSE, score	28 (24-30)	27 (24-30)	0.32	28 (24-30)	27 (24-30)	0.25
Verbal episodic memory						
RAVLT immediate recall, score[e]	30 (13-65)	26 (15-51)	0.27	31 (16-65)	25 (13-39)	0.009
RAVLT delayed recall, score[e]	3 (0-15)	2 (0-12)	0.13	4 (0-15)	1 (0-8)	0.003
RAVLT recognition-fp, score[f]	7 (-3-15)	6 (0-15)	0.61	7 (2-15)	4 (-3-9)	0.001
Story immediate recall, score	9 (1-21)	6 (2-18)	0.21	11 (2-21)	6 (1-14)	0.003
Story delayed recall, score	7 (0-19)	6 (0-16)	0.30	8 (0-19)	3 (0-12)	0.002
Composite z-score[f]	-0.1 (-1.5-2.3)	-0.5 (-1.2-1.9)	0.13	0.1 (-1.0-2.3)	-0.7 (-1.5- -0.2)	< 0.001

Abbreviations: *AD* Alzheimer's disease, *CSF* Cerebrospinal fluid, *MCI* Mild Cognitive Impairment, *MMSE* Mini-Mental State – Examination, *N/A* Not applicable, *OD* Other dementia, *RAVLT* Rey Auditory-Verbal Learning Test, *SCI* Subjective Cognitive Impairment.

Values are shown as median (range) or as numbers per group, [a]Mann-Whitney U non-parametric tests used for continuous variables and Chi-Square tests for categorical variables. [b]The AD category included one subject who converted from MCI to AD. [c]The OD category included one subject who converted from MCI to OD. *P*-values not applicable for [d]clinical diagnosis due to CSF $A\beta_{42}$ and Tau values being a part of the diagnostic criteria for AD. Analysis based on 45[e] or 44[f] subjects.

Distributions in cholinergic activity (AChE and BuChE) between CSF profiles ($A\beta_{42}$, T-tau and P-tau) and clinical diagnosis are visualized with boxplots in Figure 2. As presented in Table 1, no significant differences were deteted in AChE (Fig. 2a, p=0.35) or BuChE activity (Fig. 2b, p=0.92) between the Aβ+ and Aβ- profile groups. AChE activity was significantly higher among subjects with T-tau+ profile compared to those with a T-tau- profile (Fig. 2c, p=0.008). No differences were detected between BuChE activity between the same groups (Fig. 2d, p=0.11). The same pattern was observed as well for P-tau, with higher AChE activity found among the positive group compared to the negative one (Fig. 2e), and no difference between groups in regards to BuChE activity (Fig. 2f). No significant difference (p>0.05) was observed in AChE (Fig. 2g) or BuChE activity (Fig. 2h) between different diagnostic groups.

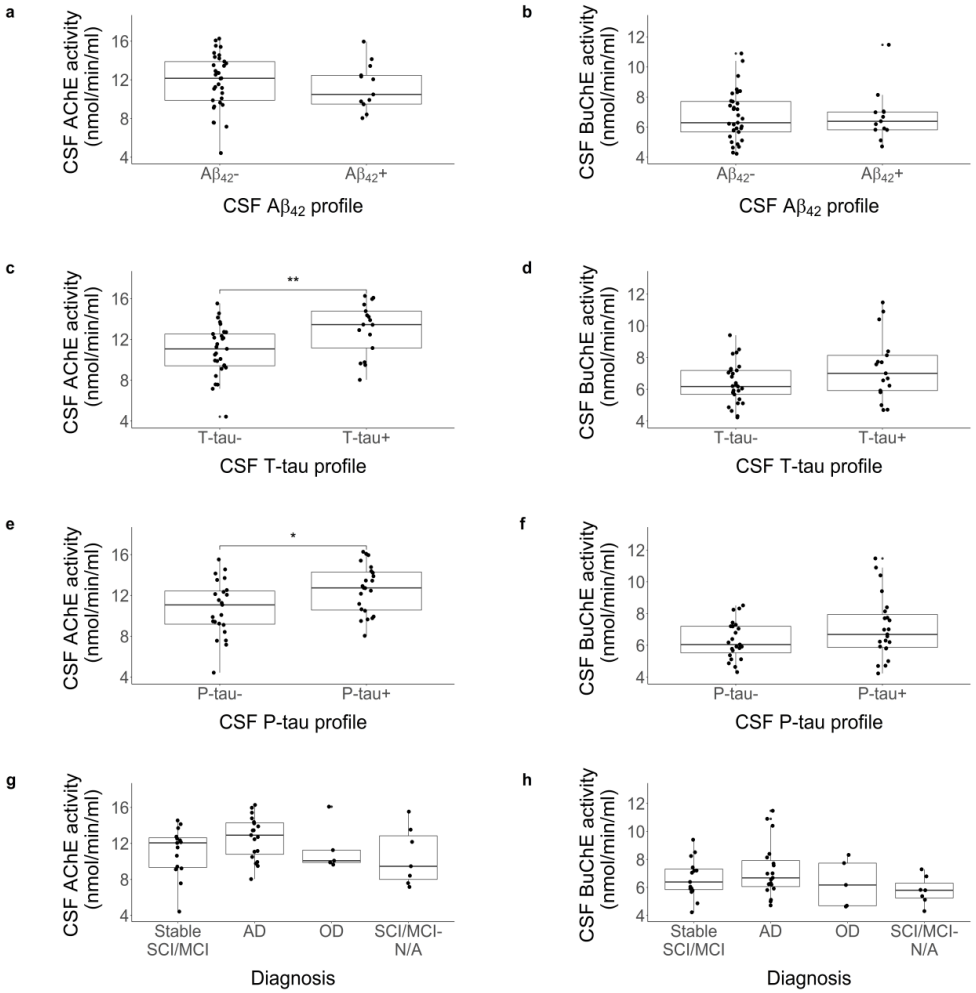

Figure 2. Comparison in activity of CSF AChE and BuChE enzymes by CSF $A\beta_{42}$ profile (a, b), T-tau profile (c, d), P-tau profile (e, f) and clinical diagnosis (g, h), *p<0.05, **p<0.01, ***p<0.001. The lower and upper horizontal lines of the boxplot correspond to the 25th centile and 75th centile and the middle line to the median. CSF AChE activity was significantly higher among subjects with a CSF T-tau+ or P-tau+ profiles compared to those without.

Correlation matrix between CSF markers, age and education

Pearson's correlations between the CSF markers, age and education are presented in Figure 3. Inflammatory markers YKL-40 and S100B and neurodegeneration markers NFL, P-tau and T-tau all correlated positively and significantly (p<0.05) with each other. GFAP did only significantly correlate with the CSF markers S100B (r=0.69, p<0.001) and NFL (r=0.36, p=0.01). No CSF marker correlated significantly with $A\beta_{42}$. All the CSF markers, except for $A\beta_{42}$ and the cholinergic enzymes, correlated positively with age (p<0.05). AChE activity correlated with levels of neurodegeneration markers P-tau and T-tau as well as inflammatory markers YKL-40 and S100B. BuChE activity correlated significantly with levels of YKL-40 and S100B, but not with the neurodegeneration markers.

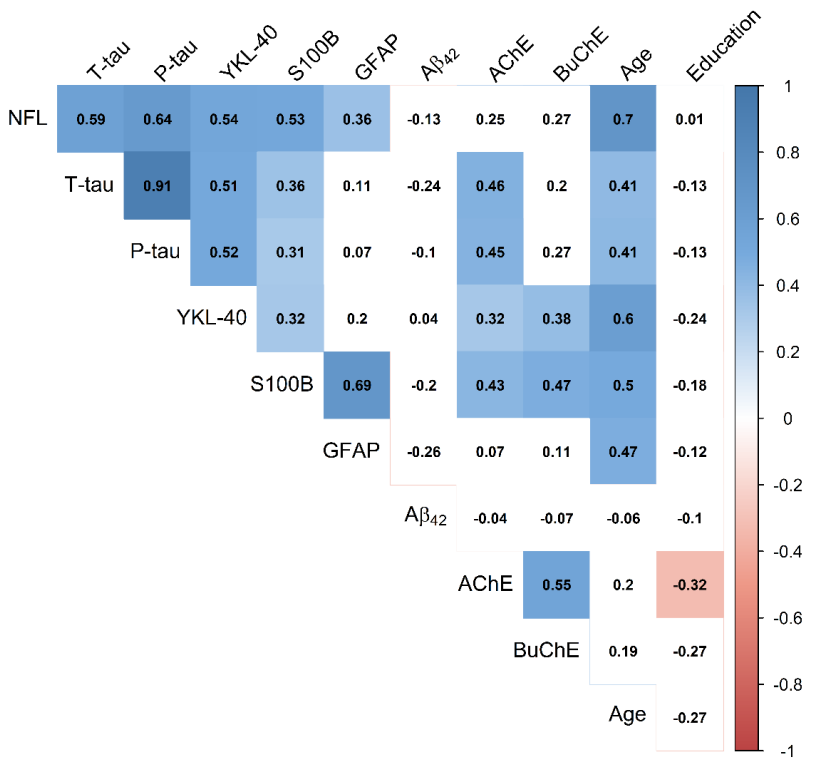

Figure 3. Pearson's correlation matrix between CSF cholinergic enzymes, CSF protein levels, age and education. Colored squares indicate statistical significance (p<0.05). Values of CSF proteins ($A\beta_{42}$, T-tau, P-tau, NFL, YKL-40, S100B and GFAP) were natural log-transformed.

Significant relationships of CSF AChE and BuChE activities with CSF markers from Figure 3 are visualized by scatter plots in Figure 4. Moderately strong, positive correlations were detected between AChE activity and levels of a) P-tau (r=0.46, *p*=0.001), b) P- tau (r=0.45, *p*=0.002) and c) S100B (r=0.43, *p*=0.003). Weak correlation was found between AChE activity and d) YKL-40 levels (r=0.32, *p*=0.03). As with AChE, BuChE also showed moderately strong, positive correlation with levels of e) S100B (r=0.47, *p*<0.001) and f) YKL-40 (r=0.47, *p*<0.001).

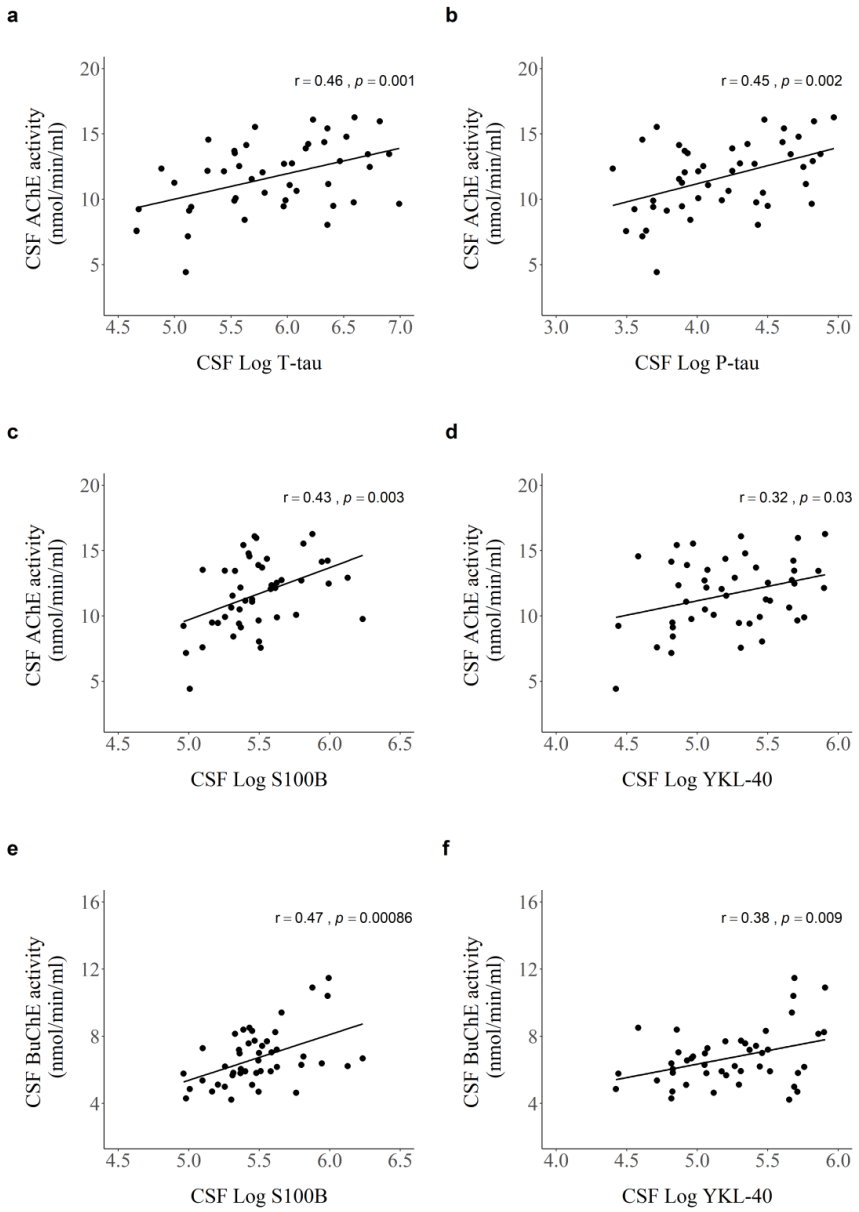

Figure 4. Pearson's correlations between AChE activity and levels of a) T-tau, b) P-tau, c) S100B, d) YKL-40 and BuChE activity and levels of e) S100B and f) YKL-40 in CSF. Values of CSF proteins (T-tau, P-tau, YKL-40 and S100B) were natural log-transformed.

Selection of best predictors for cholinergic enzyme activity

LASSO linear regression with a stability selection was performed for the identification of a set of variables predicting cholinergic enzyme activity with the highest consistency (Fig. 5). Variables with stability selection above 75% were considered reliable predictors. Two analyses were performed, one for each enzyme (AChE and BuChE) as a dependent variable. Ten possible predictors could be selected for each analysis, five CSF markers ($A\beta_{42}$, T-tau, P-tau, NFL, YKL-40, S100B, GFAP) and three demographic measures (gender, age and length of education).

No variables reached the selection criteria for prediction of AChE activity (Fig. 5a). S100B was the predictor with the highest selectiong frequency (68%), by far the highest compared to other measures. The next predictors in order of selection frequency were education (68%), T-tau (45%) and P-tau (40%). All other possible predictors had much lower selection frequency (≤ 25%).

S100B was also the measure most often selected into a model (73%) for the best prediction of BuChE activity (Fig. 5b). The CSF marker almost reached the selection criteria of 75%. Gender (64%) and YKL-40 (61%) both had selection frequency over 60%. Education had the fourth highest frequency (38%). All other possible predictors had far lower selection frequency (≤ 15%).

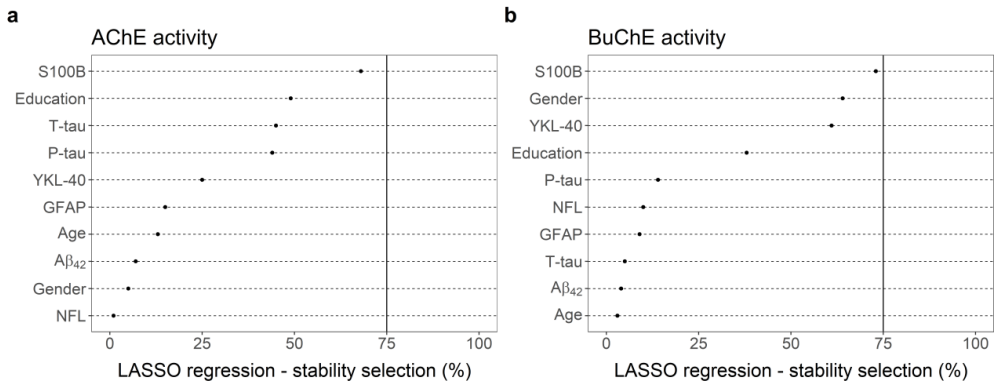

Figure 5. LASSO linear regression—stability selection analyses for prediction of CSF enzyme activities of a) AChE and b) BuChE. The cut-off selection value was set at 75% and the per-family error rate (PFER) at 1.

Association between CSF markers and verbal episodic memory

CSF activity of AChE showed a weak, negative correlation with the composite z-score reflecting verbal episodic memory (r=-0.34, *p*=0.02), while BuChE did not reach significance (r=-0.19, *p*=0.21). The analysis was based on 44 subjects as two subjects did not take the RAVLT test. Correlations between the cholinergic enzymes and verbal episodic memory are presented in Supplementary figure 1.

LASSO linear regression with a stability selection was also applied for identifying a set of variables (CSF markers and demographic variables) predicting verbal episodic memory composite score (Supplementary figure 2). Only age was selected as a reliable predictor, with a selection frequency of 91%. Neurodegeneration markers NFL (61%) and P-tau (55%) both had a selection frequencies above 50%. All other measurements had selection frequencies below 30%, including AChE (8%) and BuChE (0%).

DISCUSSION

We explored the association of cholinergic enzyme activity with CSF markers reflecting amyloidosis, neurodegeneration and inflammation as well as with loss of verbal episodic memory among subjects in a memory clinic cohort. Our results indicated no difference in AChE and BuChE activity between individuals with abnormal and normal $A\beta_{42}$ levels. In contrast, individuals with abnormally high T-tau or P-tau levels had increased AChE activity compared to those with normal levels. Higher levels of inflammatory markers S100B and YKL-40 associated with higher activity of AChE and BuChE. Interestingly, S100B was also the most reliable predictor of both AChE and BuChE activity in comparison to other CSF markers and demographic measures. Weak association was detected between AChE activity and verbal episodic memory performance, although the enzyme did not prove to be a reliable predictor when other measures had also been accounted for. Overall, these results indicate a relationship between higher activity of the ACh-degrading cholinergic enzymes and increased inflammation and neurodegeneration in the stages of pre- and early symptomatic dementia, independent of CSF $A\beta_{42}$ levels.

The relationship between Aβ protein and cholinergic dysfunction has long been established through in vitro and post-mortem studies. AChE activity is consistently increased in regions around Aβ plaques and NFTs at all stages of the disease, although overall AChE activity decreases in the AD brain [23-25]. Studies have also reported about 40–50% reduction in ACh synthesis in cultured cholinergic neurons upon exposure to a high nanomolar concentration of $A\beta_{42}$ [26-29]. More recent research indicate that Aβ peptides act directly as allosteric modulators of cholinergic signaling by forming highly stable and soluble complexes with apolipoprotein E (ApoE) and cholinesterases [30-33]. A better understanding of the functional relationship between Aβ and the cholinergic system is though needed, as the biochemical environment in vivo is complex. Results regarding association between $A\beta_{42}$ levels and AChE activity in CSF have, for example, been inconsistent [19, 34, 35]. Neither Johansson et al. [34] nor Darreh-Shori [19] detected a relationship between $A\beta_{42}$ levels and AChE activity among AD patients. In contrast, García-Ayllón et al. [35] did observe an association between the proteins within same patient group. Johansson et al. [34] also did find a positive relationship between the proteins, but only within a whole cohort of demented cases and healthy controls. Neither Darreh-Shori et al.[19] nor Gabriel et al. [36] detected relationship between BuChE activity and $A\beta_{42}$ levels among all AD patients. Gabriel et al. [36] did, however, detect a positive association among those patients carrying the ApoE-ε4 allele. In this current study, relationships between the activities of the cholinergic enzymes and $A\beta_{42}$ levels were not detected.

Previous post-mortem studies have shown that the loss of cortical cholinergic innervation is associated with, and potentially caused by, NFT in the nucleus basalis of Meynert (NBM) of the basal forebrain [37-39], with degeneration already present at the very early stages of AD [40, 41]. Silveyra et al. [42] reported that P-tau could be an important regulator of AChE expression. Over-expression of P-tau in transgenic mice (Tg-VLW) led to an increase in the activity of the AChE, suggesting that increase in AChE expression around NFTs could be a consequence of disturbed tau phosphorylation. Very few studies have, however, examined the relations between CSF tau levels and cholinsterase activity in CSF. Our study found a correlation between T-tau and P-tau with AChE activity. This is in accordance with a study by Johansson et al. [34], where a positive association between T-tau and P-tau levels with AChE activity was detected in CSF among demented cases and healthy controls. We did not find a correlation between T-tau or P-tau levels and BuChE activity. The results are in line with results from a study by Gabriel et al. [36], which did not find correlations between CSF T-tau and P-tau with BuChE activity among AD patients. One possible explanation for the difference in results between the enzymes in regards to the neurodegeneration markers, could be due to localization. BuChE activity is mainly localized to glial cells while AChE is predominantly located within neurons and axons [43, 44]. Interestingly, we did not find a relationship between NFL, a marker of both neurodegeneration and white matter changes, and the activity of the cholinergic enzymes. To the best of our knowledge, NLF has not been researched in regards to AChE and BuChE activity in CSF samples from a dementia cohort. A study by Aeinehband et al. [45] among patients with multiple sclerosis (MS) found a positive correlation between NFL levels and BuChE activity, but not AChE activity.

The neurotransmitter ACh is not only restricted to neurons, but can also act on regions distal from synaptic sites [46]. It has anti-inflammatory effects, inhibiting pro-inflammatory responses by acting on α7 nicotinic ACh receptors expressed on non-excitable cholinoceptive cells like astrocytes and microglia [46]. Extracellular ACh is therefore hypothesized to play a key role in homeostatic functions that include neuronal support, the maintenance of myelin, synaptic function and plasticity, Aβ clearance, and maintenance of the blood–brain barrier [47]. Both AChE and BuChE enzymes play a major dynamic role in this homeostatis as they degrade ACh, with BuChE being specifically important for the function of glial cells [47]. S100B and GFAP are two commonly used CSF markers of astroglial reactivity. A study by Darreh-Shori et al. [48] found that BuChE activity positively associated with S100B and GFAP in CSF among AD patients. A possible explanation could be that lower levels of extracellular ACh associate with

higher BuChE activity, enhancing the reactivity of glia. This heightened function can be protective, but prolonged glial activation may gradually lead to degeneration and eventually loss of neurons, further triggering inflammatory responses [49]. No relationship was detected between AChE activity and levels of S100B and GFAP in the same study. Our study found a positive relationship between S100B, and to lesser extent YKL-40, with activity of both enzymes. To the best of our knowledge, YKL-40 has not been explored before in regards to cholinergic enzymes. YKL-40 is widely used as a glial activation marker, although the cellular source of expression in brain remains uncertain. In AD, studies show a variable pattern of YKL-40 expression that includes astrocytes, microglia or, on rare occasions, neurons [50]. Interestingly, we did not find association between GFAP levels with either enzyme. Different patterns of association between the enzymes with different inflammatory markers could possibly be explained by different cellular functions. Both YKL-40 [51] and S100B [52] have extracellular functions while GFAP [53] is an intracellular protein. Increased GFAP levels in CSF may therefore be a later event in the pathogenic cascade.

Although all ChEIs enhance synaptic transmission, they exert their effects on cholinesterases differently. The drugs donepezil and galantamine are reversible inhibitors of AChE while rivastigmine is a pseudo-irreversible inibitor of both AChE and BuChE [54]. The difference in pharmacological properties affects both the ability of breaking down extrasynaptic ACh as well as the expression of extracellular AChE [55, 56]. Therefore, different ChEIs could alter glial-neuronal interactions in different ways [49]. A larger, longitudinal study might be able to detect the effect of each ChEI on CSF markers reflecting cholinergic activity, inflammation and neurodegeneration. Measures of those markers, in combination with factors including age, gender and genotype, could potentially be of use in predicting which patients could benefit from a treatment with a specific ChEI.

It is unclear to what extent episodic memory dysfunction relates to structural and functional changes in the cholinergic system at the symptomatic pre-dementia stages (SCI or MCI) [57]. The basal forebrain undergoes severe neurofibrillary degeneration and neuronal loss in patients with moderate to severe AD, most prominent in the NBM. In comparison, changes at the MCI stage are thought to be characterized by alterations in cholinergic functions rather than by cholinergic neuronal loss [58]. In accordance with this, histological studies have revealed that cognitive deficits are not evident before at least 30% of the basal forebrain cholinergic neurons have degenerated [59] and individuals with MCI show only around 15% volume loss compared to healthy controls [60]. Our results are in line with those

findings as only a weak assocation was found between AChE activity and verbal episodic memory performance. When other factors (e.g. age) were simultaenously considered, neither of the cholinergic enzymes did prove to be a good predictor for the performance on verbal episodic memory. Previous studies have revealed NFL to be a promising progression marker, associating with cognitive impairment in both AD and LBD [61, 62]. In our study, the colinergic enzymes did not correlate with levels of NFL, emphasizing further the lack of contribution of the cholinergic system to episodic memory dysfunction at the very early stages of dementia.

This study has several limitations. First, the sample was relatively small, and hence present findings need to be validated in a larger study. The sample did not include healthy controls, which could underestimate associations between the studied variables. Another limitation of the study is the lack of information about the ApoE and BuChE genotypes. A reduction of the BuChE activity in CSF of AD patients carrying both the K variant and the ApoE-ε4 allele has previously been found [63]. However, the influence of the BuChE-K variant in the levels of $A\beta_{42}$, T-tau or P-tau in the CSF is still unknown.

Our findings suggest that the activity of ACh-degrading cholinergic enzymes at the pre- early symptomatic stages of dementia relate to inflammatory and neurodegenerative processes, but not to loss in verbal episodic memory. A better understanding of the cholinergic system and its relations to both pathology and cognitive functions are critical, given that it is the main target of current symptomatic treatment of AD. Further studies are needed for validation of these results, preferably with larger samples and access to genotype information.

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ETHICS APPROVAL

The study has been approved by the National Research Ethics Committee of Iceland (VSN-14-028) and all subjects signed an informed consent. The study was conducted in accordance with the Helsinki Declaration latest revision of 2013.

DISCLOSURE STATEMENT

The authors have no conflict of interest to report.

REFERENCES

[1] Mufson EJ, Ginsberg SD, Ikonomovic MD, DeKosky ST (2003) Human cholinergic basal forebrain: chemoanatomy and neurologic dysfunction. *J Chem Neuroanat* **26**, 233-242.

[2] Auld DS, Kornecook TJ, Bastianetto S, Quirion R (2002) Alzheimer's disease and the basal forebrain cholinergic system: relations to beta-amyloid peptides, cognition, and treatment strategies. *Prog Neurobiol* **68**, 209-245.

[3] Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, Khachaturian AS, Vergallo A, Cavedo E, Snyder PJ, Khachaturian ZS (2018) The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain : a journal of neurology* **141**, 1917-1933.

[4] Adem A (1992) Putative mechanisms of action of tacrine in Alzheimer's disease. *Acta Neurologica Scandinavica* **85**, 69-74.

[5] Pavlov VA, Parrish WR, Rosas-Ballina M, Ochani M, Puerta M, Ochani K, Chavan S, Al-Abed Y, Tracey KJ (2009) Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. *Brain Behav Immun* **23**, 41-45.

[6] van Westerloo DJ, Giebelen IA, Florquin S, Daalhuisen J, Bruno MJ, de Vos AF, Tracey KJ, van der Poll T (2005) The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis. *J Infect Dis* **191**, 2138-2148.

[7] Vijayaraghavan S, Karami A, Aeinehband S, Behbahani H, Grandien A, Nilsson B, Ekdahl KN, Lindblom RPF, Piehl F, Darreh-Shori T (2013) Regulated Extracellular Choline Acetyltransferase Activity- The Plausible Missing Link of the Distant Action of Acetylcholine in the Cholinergic Anti-Inflammatory Pathway. *PloS one* **8**, e65936-e65936.

[8] Malmsten L, Vijayaraghavan S, Hovatta O, Marutle A, Darreh-Shori T (2014) Fibrillar β-amyloid 1-42 alters cytokine secretion, cholinergic signalling and neuronal differentiation. *Journal of cellular and molecular medicine* **18**, 1874-1888.

[9] Darreh-Shori T, Forsberg A, Modiri N, Andreasen N, Blennow K, Kamil C, Ahmed H, Almkvist O, Långström B, Nordberg A (2011) Differential levels of apolipoprotein E and butyrylcholinesterase

show strong association with pathological signs of Alzheimer's disease in the brain in vivo. *Neurobiology of Aging* **32**, 2320.e2315-2320.e2332.
[10] Minghetti L, Carnevale D, Simone RD (2007) Microglia-Neuron Interaction in Inflammatory and Degenerative Diseases: Role of Cholinergic and Noradrenergic Systems. *CNS & Neurological Disorders - Drug Targets* **6**, 388-397.
[11] Shytle RD, Mori T, Townsend K, Vendrame M, Sun N, Zeng J, Ehrhart J, Silver AA, Sanberg PR, Tan J (2004) Cholinergic modulation of microglial activation by alpha 7 nicotinic receptors. *J Neurochem* **89**, 337-343.
[12] Jack CR, Jr., Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* **14**, 535-562.
[13] Teitsdottir UD, Jonsdottir MK, Lund SH, Darreh-Shori T, Snaedal J, Petersen PH (2020) Association of glial and neuronal degeneration markers with Alzheimer's disease cerebrospinal fluid profile and cognitive functions. *Alzheimer's Research & Therapy* **12**, 92.
[14] Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189-198.
[15] Jorm AF (1994) A short form of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): development and cross-validation. *Psychol Med* **24**, 145-153.
[16] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263-269.
[17] McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, Aarsland D, Galvin J, Attems J, Ballard CG, Bayston A, Beach TG, Blanc F, Bohnen N, Bonanni L, Bras J, Brundin P, Burn D, Chen-Plotkin A, Duda JE, El-Agnaf O, Feldman H, Ferman TJ, Ffytche D, Fujishiro H, Galasko D, Goldman JG, Gomperts SN, Graff-Radford NR, Honig LS, Iranzo A, Kantarci K, Kaufer D, Kukull W, Lee VMY, Leverenz JB, Lewis S, Lippa C, Lunde A, Masellis M, Masliah E, McLean P, Mollenhauer B, Montine TJ, Moreno E, Mori E, Murray M, O'Brien JT, Orimo S, Postuma RB, Ramaswamy S, Ross OA, Salmon DP, Singleton A, Taylor A, Thomas A, Tiraboschi P, Toledo JB, Trojanowski JQ, Tsuang D, Walker Z, Yamada M, Kosaka K (2017) Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. *Neurology* **89**, 88-100.
[18] Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, Nordberg A, Bäckman L, Albert M, Almkvist O, Arai H, Basun H, Blennow K, de Leon M, DeCarli C, Erkinjuntti T, Giacobini E, Graff C, Hardy J, Jack C, Jorm A, Ritchie K, van Duijn C, Visser P, Petersen RC (2004) Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *J Intern Med* **256**, 240-246.
[19] Darreh-Shori T, Brimijoin S, Kadir A, Almkvist O, Nordberg A (2006) Differential CSF butyrylcholinesterase levels in Alzheimer's disease patients with the ApoE epsilon4 allele, in relation to cognitive function and cerebral glucose metabolism. *Neurobiol Dis* **24**, 326-333.
[20] Darreh-Shori T, Kadir A, Almkvist O, Grut M, Wall A, Blomquist G, Eriksson B, Långström B, Nordberg A (2008) Inhibition of acetylcholinesterase in CSF versus brain assessed by 11C-PMP PET in AD patients treated with galantamine. *Neurobiol Aging* **29**, 168-184.
[21] Lezak MD, Howieson DB, Bigler ED, Tranel D (2012) *Neuropsychological assessment*.
[22] Wechsler D (1987) *WMS-R : Wechsler Memory Scale--Revised : manual*, Psychological Corp. : Harcourt Brace Jovanovich, San Antonio.

[23] Perry RH, Blessed G, Perry EK, Tomlinson BE (1980) Histochemical observations on cholinesterase activities in the brains of elderly normal and demented (Alzheimer-type) patients. *Age Ageing* **9**, 9-16.
[24] Mesulam MM, Asuncion Morán M (1987) Cholinesterases within neurofibrillary tangles related to age and Alzheimer's disease. *Ann Neurol* **22**, 223-228.
[25] Ulrich J, Meier-Ruge W, Probst A, Meier E, Ipsen S (1990) Senile plaques: staining for acetylcholinesterase and A4 protein: a comparative study in the hippocampus and entorhinal cortex. *Acta Neuropathol* **80**, 624-628.
[26] Pedersen WA, Kloczewiak MA, Blusztajn JK (1996) Amyloid beta-protein reduces acetylcholine synthesis in a cell line derived from cholinergic neurons of the basal forebrain. *Proc Natl Acad Sci U S A* **93**, 8068-8071.
[27] Hoshi M, Takashima A, Murayama M, Yasutake K, Yoshida N, Ishiguro K, Hoshino T, Imahori K (1997) Nontoxic amyloid beta peptide 1-42 suppresses acetylcholine synthesis. Possible role in cholinergic dysfunction in Alzheimer's disease. *J Biol Chem* **272**, 2038-2041.
[28] Kar S, Issa AM, Seto D, Auld DS, Collier B, Quirion R (1998) Amyloid beta-peptide inhibits high-affinity choline uptake and acetylcholine release in rat hippocampal slices. *J Neurochem* **70**, 2179-2187.
[29] Nunes-Tavares N, Santos LE, Stutz B, Brito-Moreira J, Klein WL, Ferreira ST, de Mello FG (2012) Inhibition of choline acetyltransferase as a mechanism for cholinergic dysfunction induced by amyloid-β peptide oligomers. *J Biol Chem* **287**, 19377-19385.
[30] Darreh-Shori T, Forsberg A, Modiri N, Andreasen N, Blennow K, Kamil C, Ahmed H, Almkvist O, Långström B, Nordberg A (2011) Differential levels of apolipoprotein E and butyrylcholinesterase show strong association with pathological signs of Alzheimer's disease in the brain in vivo. *Neurobiol Aging* **32**, 2320.e2315-2332.
[31] Darreh-Shori T, Modiri N, Blennow K, Baza S, Kamil C, Ahmed H, Andreasen N, Nordberg A (2011) The apolipoprotein E ε4 allele plays pathological roles in AD through high protein expression and interaction with butyrylcholinesterase. *Neurobiol Aging* **32**, 1236-1248.
[32] Vijayaraghavan S, Karami A, Aeinehband S, Behbahani H, Grandien A, Nilsson B, Ekdahl KN, Lindblom RP, Piehl F, Darreh-Shori T (2013) Regulated Extracellular Choline Acetyltransferase Activity- The Plausible Missing Link of the Distant Action of Acetylcholine in the Cholinergic Anti-Inflammatory Pathway. *PLoS One* **8**, e65936.
[33] Kumar R, Nordberg A, Darreh-Shori T (2016) Amyloid-β peptides act as allosteric modulators of cholinergic signalling through formation of soluble BAβACs. *Brain* **139**, 174-192.
[34] Johansson P, Almqvist EG, Johansson JO, Mattsson N, Andreasson U, Hansson O, Wallin A, Blennow K, Zetterberg H, Svensson J (2013) Cerebrospinal fluid (CSF) 25-hydroxyvitamin D concentration and CSF acetylcholinesterase activity are reduced in patients with Alzheimer's disease. *PLoS One* **8**, e81989.
[35] García-Ayllón MS, Silveyra MX, Sáez-Valero J (2008) Association between acetylcholinesterase and beta-amyloid peptide in Alzheimer's cerebrospinal fluid. *Chem Biol Interact* **175**, 209-215.
[36] Gabriel AJ, Almeida MR, Ribeiro MH, Durães J, Tábuas-Pereira M, Pinheiro AC, Pascoal R, Santana I, Baldeiras I (2017) Association between butyrylcholinesterase and cerebrospinal fluid biomarkers in Alzheimer's disease patients. *Neuroscience Letters* **641**, 101-106.
[37] Geula C, Mesulam MM (1995) Cholinesterases and the pathology of Alzheimer disease. *Alzheimer Disease and Associated Disorders* **9**, 23-28.
[38] Braak H, Del Tredici K (2013) Reply: the early pathological process in sporadic Alzheimer's disease. *Acta Neuropathol* **126**, 615-618.
[39] Mesulam MM (2013) Cholinergic circuitry of the human nucleus basalis and its fate in Alzheimer's disease. *J Comp Neurol* **521**, 4124-4144.

[40] Mesulam M, Shaw P, Mash D, Weintraub S (2004) Cholinergic nucleus basalis tauopathy emerges early in the aging-MCI-AD continuum. *Ann Neurol* **55**, 815-828.
[41] Mufson EJ, Counts SE, Perez SE, Ginsberg SD (2008) Cholinergic system during the progression of Alzheimer's disease: therapeutic implications. *Expert review of neurotherapeutics* **8**, 1703-1718.
[42] Silveyra MX, García-Ayllón MS, de Barreda EG, Small DH, Martínez S, Avila J, Sáez-Valero J (2012) Altered expression of brain acetylcholinesterase in FTDP-17 human tau transgenic mice. *Neurobiol Aging* **33**, 624.e623-634.
[43] Giacobini E (2003) Cholinesterases: New Roles in Brain Function and in Alzheimer's Disease. *Neurochemical Research* **28**, 515-522.
[44] Wright CI, Geula C, Mesulam MM (1993) Neurological cholinesterases in the normal brain and in Alzheimer's disease: relationship to plaques, tangles, and patterns of selective vulnerability. *Ann Neurol* **34**, 373-384.
[45] Aeinehband S, Lindblom RPF, Al Nimer F, Vijayaraghavan S, Sandholm K, Khademi M, Olsson T, Nilsson B, Ekdahl KN, Darreh-Shori T, Piehl F (2015) Complement component C3 and butyrylcholinesterase activity are associated with neurodegeneration and clinical disability in multiple sclerosis. *PloS one* **10**, e0122048-e0122048.
[46] Nizri E, Hamra-Amitay Y, Sicsic C, Lavon I, Brenner T (2006) Anti-inflammatory properties of cholinergic up-regulation: A new role for acetylcholinesterase inhibitors. *Neuropharmacology* **50**, 540-547.
[47] Lane RM, He Y (2013) Butyrylcholinesterase genotype and gender influence Alzheimer's disease phenotype. *Alzheimer's & Dementia* **9**, e17-e73.
[48] Darreh-Shori T, Vijayaraghavan S, Aeinehband S, Piehl F, Lindblom RP, Nilsson B, Ekdahl KN, Långström B, Almkvist O, Nordberg A (2013) Functional variability in butyrylcholinesterase activity regulates intrathecal cytokine and astroglial biomarker profiles in patients with Alzheimer's disease. *Neurobiol Aging* **34**, 2465-2481.
[49] Lane RM, Darreh-Shori T (2015) Understanding the beneficial and detrimental effects of donepezil and rivastigmine to improve their therapeutic value. *J Alzheimers Dis* **44**, 1039-1062.
[50] Querol-Vilaseca M, Colom-Cadena M, Pegueroles J, San Martín-Paniello C, Clarimon J, Belbin O, Fortea J, Lleó A (2017) YKL-40 (Chitinase 3-like I) is expressed in a subset of astrocytes in Alzheimer's disease and other tauopathies. *Journal of Neuroinflammation* **14**, 118.
[51] Bonneh-Barkay D, Bissel SJ, Kofler J, Starkey A, Wang G, Wiley CA (2012) Astrocyte and macrophage regulation of YKL-40 expression and cellular response in neuroinflammation. *Brain Pathol* **22**, 530-546.
[52] Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, Tubaro C, Giambanco I (2009) S100B's double life: intracellular regulator and extracellular signal. *Biochim Biophys Acta* **1793**, 1008-1022.
[53] Yang Z, Wang KK (2015) Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. *Trends Neurosci* **38**, 364-374.
[54] Grossberg GT (2003) Cholinesterase inhibitors for the treatment of Alzheimer's disease:: getting on and staying on. *Curr Ther Res Clin Exp* **64**, 216-235.
[55] Darreh-Shori T, Soininen H (2010) Effects of cholinesterase inhibitors on the activities and protein levels of cholinesterases in the cerebrospinal fluid of patients with Alzheimer's disease: a review of recent clinical studies. *Curr Alzheimer Res* **7**, 67-73.
[56] Nordberg A, Darreh-Shori T, Peskind E, Soininen H, Mousavi M, Eagle G, Lane R (2009) Different cholinesterase inhibitor effects on CSF cholinesterases in Alzheimer patients. *Current Alzheimer research* **6**, 4-14.
[57] Peter J, Lahr J, Minkova L, Lauer E, Grothe MJ, Teipel S, Köstering L, Kaller CP, Heimbach B, Hüll M, Normann C, Nissen C, Reis J, Klöppel S (2016) Contribution of the Cholinergic System

to Verbal Memory Performance in Mild Cognitive Impairment. *Journal of Alzheimer's disease : JAD* **53**, 991-1001.
[58] Schliebs R, Arendt T (2011) The cholinergic system in aging and neuronal degeneration. *Behav Brain Res* **221**, 555-563.
[59] Schliebs R, Arendt T (2006) The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. *J Neural Transm (Vienna)* **113**, 1625-1644.
[60] Peter J, Lahr J, Minkova L, Lauer E, Grothe MJ, Teipel S, Köstering L, Kaller CP, Heimbach B, Hüll M, Normann C, Nissen C, Reis J, Klöppel S (2016) Contribution of the Cholinergic System to Verbal Memory Performance in Mild Cognitive Impairment. *J Alzheimers Dis* **53**, 991-1001.
[61] Olsson B, Portelius E, Cullen NC, Sandelius Å, Zetterberg H, Andreasson U, Höglund K, Irwin DJ, Grossman M, Weintraub D, Chen-Plotkin A, Wolk D, McCluskey L, Elman L, Shaw LM, Toledo JB, McBride J, Hernandez-Con P, Lee VM, Trojanowski JQ, Blennow K (2019) Association of Cerebrospinal Fluid Neurofilament Light Protein Levels With Cognition in Patients With Dementia, Motor Neuron Disease, and Movement Disorders. *JAMA Neurol* **76**, 318-325.
[62] Zetterberg H, Skillbäck T, Mattsson N, Trojanowski JQ, Portelius E, Shaw LM, Weiner MW, Blennow K (2016) Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression. *JAMA Neurol* **73**, 60-67.
[63] Darreh-Shori T, Siawesh M, Mousavi M, Andreasen N, Nordberg A (2012) Apolipoprotein ε4 modulates phenotype of butyrylcholinesterase in CSF of patients with Alzheimer's disease. *J Alzheimers Dis* **28**, 443-458.