Biochemical markers in dementia

Exploring Swedish registry data and the human proteome

Tobias Skillbäck

Institute of Neuroscience and Physiology
Department of Psychiatry and Neurochemistry
The Sahlgrenska Academy at the University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2019

Cover illustration:
Self Reflected
22K micro etching under white light
2014 - 2016
Greg Dunn and Brian Edwards

Biochemical markers in dementia - Exploring Swedish registry data and the human proteome

tobias.skillback@gu.se

ISBN 978-91-7833-524-4 (Print)
ISBN 978-91-7833-525-1 (PDF)
http://hdl.handle.net/2077/60288

Printed in Gothenburg, Sweden 2019 by BrandFactory AB

Abstract

Cerebrospinal fluid (CSF) biomarkers of neurodegenerative diseases have a wide scope of applications in diagnostics, prognosis assessment, disease staging, treatment evaluation and more. In this PhD project we aimed to expand the understanding of the properties of known CSF biomarkers of Alzheimer's disease (AD) and other neurodegenerative diseases, including the most prevalent dementia disorders.

In study I, we explored CSF concentrations of three hallmark biomarkers of AD (amyloid β 1-42 [$A\beta_{1-42}$], total tau [T-tau] and phosphorylated tau [P-tau]) in samples collected in clinical routine from 5676 patients. We found that the most clear-cut AD-like biomarker pattern was found in patients diagnosed with AD, but that large proportions of patients with other dementia disorders also had an AD-like profile. However, this was less often seen in the frontotemporal dementia (FTD) group.

In study II, we studied CSF concentrations of neurofilament light (NfL), a biomarker of general neurodegeneration, in 3356 patients with different dementia diagnoses. We found that CSF NfL is especially high in dementias with vascular engagement, but also in frontotemporal dementia. We also found that high CSF NfL concentrations are linked to short survival, which supports the notion that high CSF NfL indicates more aggressive disease processes.

In study III, the biomarkers T-tau and P-tau were evaluated as biomarkers of Creutzfeldt-Jakob disease (CJD), a rare rapid neurodegenerative disease. We could conclude that the combination of increased T-tau levels and increased T-tau/P-tau ratios in patients with CJD has a very high specificity against important differential diagnoses to CJD. We further concluded that CJD patients exhibit rising T-tau concentrations as the disease progresses.

In study IV, we developed a new strategy for analyzing data output from explorative mass spectrometry. We used a clustering algorithm to allow for higher efficiency and were able to prove the validity of this concept by identifying and validating a new biomarker of AD, a 16 amino acids long peptide from the protein pleiotrophin ($PTN_{151-166}$). We concluded that quantification-driven proteomics aided by clustering is a viable way of hypothesis generation in biomarker discovery studies. We further concluded that $PTN_{151-166}$ is a promising AD biomarker candidate that our data indicates to be AD specific and able to discriminate AD from other dementia pathologies at an early stage of disease.

In conclusion, the results from the studies in this thesis demonstrate the diagnostic, prognostic and investigative properties of CSF biomarkers.

Populärvetenskaplig sammanfattning

Demenssjukdomar är vanliga och är på väg att bli ännu mer vanliga. Detta beror främst på att sociala, ekomomiska och medicinska framsteg har gjort att vi blir allt äldre. Denna utveckling är naturligtvis glädjande, men baksidan är att åldersrelaterade sjukdomar, såsom demenssjukdomar, blir vanligare. Stora resurser har lagts på att utveckla läkemedel mot demenssjukdomar under de senaste decennierna, men besvikelserna har varit många. Det finns ännu ingen bot eller effektiv behandling mot någon demenssjukdom. Studierna i denna avhandling är inriktade på att undersöka så kallade biomarkörer för demenssjukdomar. Biomarkörer är substanser eller egenskaper hos en individ som indikerar förekomst av ett tillstånd eller en sjukdom. Biomarkörer kan t.ex. användas i den kliniska vardagen för att avgöra om någon har en viss sjukdom, eller i en läkemedelsstudie för att avgöra om en nyutvecklad medicin har effekt på en sjukdom. Syftet med studierna i denna avhandling har varit att öka kunskapen om biomarkörer för demenssjukdomar.

I de två första studierna i denna avhandling sammankopplade vi det svenska demensregistret (Svedem) med laboratoriedatabasen på Sahlgrenska sjukhuset. Genom detta kunde vi samla tusentals mätningar av biomarkörer relaterade till den vanligaste demenssjukdomen, Alzheimer's sjukdom ($A\beta_{1-42}$, T-tau och P-tau), och allmän nervcellsdöd (NfL). I den första studien fann vi i en population omfattande 5676

individer att biomarkörerna $A\beta_{1\text{-}42}$, T-tau och P-tau tillsammans utmärker Alzheimer's sjukdom från andra demenser, men att förhöjda nivåer av dessa markörer ofta kan ses även i andra demenssjukdomar. I den andra studien, som innefattade en population om 3356 individer, såg vi att markören NfL är förhöjd i alla demenssjukdomar representerade i vårt material jämfört med friska kontroller, samt att patienter med höga nivåer av denna biomarkör hade kortare överlevnad.

I den tredje studien undersökte vi två varianter av proteinet tau (T-tau och P-tau) som biomarkörer för den ovanliga och snabbt framskridande demensen Creutzfeldt-Jakobs sjukdom. Vi fann att patienter med denna sjukdom hade mycket höga nivåer av tau och att detta effektivt kunde skilja dem från patienter med andra demenssjukdomar. Vidare fann vi allt högre nivåer i patienter ju längre sjukdomen framskred, vilket tyder på att nervcellsdöden i Creutzfeldt-Jakobs sjukdom accelerar med tiden, och att tau kan användas för att mäta sjukdomens intensitet.

Den fjärde studien syftade till att leta nya biomarkörer för Alzheimer's sjukdom. Vi utvecklade ett nytt sätt att analysera data från mass spektrometri. Mass spektrometri är en teknik som kan användas för att analysera protein-innehållet i t.ex. cerebrospinalvätskan, dvs den vätska som omger hjärnan. Med den nya metoden kunde vi ta vara på den stora mängd data som produceras vid en sådan analys på ett mycket effektivare sätt än vad som tidigare varit möjligt. Vi kunde även bevisa att den nya metoden fungerade genom att identifiera och validera en helt ny och tidigare okänd biomarkör för Alzheimer's sjukdom, $PTN_{151\text{-}166}$.

List of papers

This thesis is based on the following studies, referred to in text by their roman numerals.

I. **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 and Zetterberg H. *Cerebrospinal fluid tau and amyloid–β_{1-42} in patients with dementia*. Brain 2015, 138; 2716-2731

II. **Skillbäck T**, Farahmand B Y, 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, and Zetterberg H. *CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival.* Neurology, 2014, 83:1945-1953

III. **Skillbäck T**, Rosén C, Asztely F, Mattsson N, Blennow K and Zetterberg H. *Diagnostic performance of cerebrospinal fluid total tau and phosphorylated tau in Creutzfeldt-Jakob disease – Results from the Swedish mortality registry*. JAMA Neurology 2014, 71(4):476-483

IV. **Skillbäck T**, Mattson N, Hansson K, Mirgorodskaya E, Dahlén R, van der Flier W, Scheltens P, Duits F, Hansson O, Teunissen C, Blennow K, Zetterberg H and Gobom J. *A novel quantification-driven proteomic strategy identifies an endogenous peptide of pleiotrophin as a new biomarker of Alzheimer's disease*. Scientific reports 2017, 7:13333

Table of Contents

Abbreviations

AChE	Acetylcholineesterase
AD	Alzheimer's disease
ADAD	Autosominal dominant Alzheimer's disease
ADAS-Cog	Alzheimer's Disease Assessment Scale-cognitive subscale
AICD	APP intracellular domain
APP	Amyloid precursor protein
AUC	Area under the curve
Aβ	Amyloid β
$A\beta_{1-40}$	Amyloid β amino acid sequence 1-40
$A\beta_{1-42}$	Amyloid β amino acid sequence 1-42
BBB	Blood-brain-barrier
bvFTD	Behavioural variant FTD
CBD	Corticobasal degeneration
CID	Collision induced dissociation
CJD	Creutzfeldt-Jakob disease
CNS	Central nervous system
CSF	Cerebrospinal fluid
DLB	Dementia with Lewy bodies
EAD	Early onset Alzheimer's disease
ELISA	Enzyme linked immunosorbent array
ESI	Electrospray ionisation
FDA	Federal drugs administration
FTLD	Frontotemporal lobar degeneration
FTD	Frontotemporal dementia
FTD-MND	Frontotemporal dementia with motor neuron disease
FTDP-17	Frontotemporal dementia and parkinsonism linked to chromosome 17
FUS	Fused in sarcoma
HPLC	High pressure/performance liquid chromatography
HSV-1	Herpes simplex virus 1
ICD-10	International Statistical Classification of Diseases and Related Health
IWG	International working group
LAD	Late onset Alzheimer's disease
LC	Liquid chromatography
LRP	LDL receptor-related protein

LP	Lumbar puncture
m/z	Mass-to-charge-ratio
MALDI	Matrix-assisted laser desorption/ionisation
MAPT	Microtubule-associated protein tau
MCI	Mild cognitive impairment
MMSE	Mini mental state examination
MND	Motor neuron disease
MRI	Magnetic resonance imaging
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
Nf(L/M/H)	Neurofilament [light/medium/heavy] chain
NFT	Neurofibrillary tangle
nfvPPA	Nonfluent variant primary progressive aphasia
Ng	Neurogranin
NIA-AA	US National Institute on Aging-Alzheimer's Association
ROC	Receiver Operating Characteristics
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PET	Positron emission tomography
PRM	Parallel reaction monitoring
PSP	Progressive supranuclear palsy
P-tau	Total concentration of phosphorylated protein tau
PTN	Pleiotrophin
$PTN_{151-166}$	Pleiotrophin amino acid sequence 155-166
PTPRZ	Chondroitin sulfate proteoglycan receptor-type protein tyrosine
SAD	Sporadic Alzheimer's disease
SRM	Single reaction monitoring
SSRI	Selective serotonin reuptake inhibitor
sPDGFRβ	Platelet-derived growth factor receptor-β
SPECT	Single photon emission computer tomography
svPPA	Semantic variant of primary progressive aphasia
TBI	Traumatic brain injury
TDP-43	TAR DNA-binding protein 43
TMT	Tandem Mass Tag
T-tau	Total concentration of protein tau
VaD	Vascular dementia
YKL-40	Chitinase-3-like protein 1

"Excessive reservations and paralyzing despondency have not helped the sciences to advance nor are they helping them to advance, but a healthy optimism that cheerfully searches for new ways to understand, as it is convinced that it will be possible to find them."

Alois Alzheimer

Introduction

Neurodegenerative disease and dementia

Steady progress across several areas including medicine, technology and economy has helped increase living standards and life expectancies across the globe over the past 70 years [1-3]. This undeniably positive development has however brought new challenges as decreased mortality rates are followed by a growing elderly population, and a growing incidence of age-related disease [4]. One of the disease groups that have seen such an incidence surge is dementia, leading to the fear of a growing dementia epidemic being discussed in the field of dementia research around the world [5-7].

Primary concepts

Dementia is a syndrome and a general term describing a group of pathologic disorders with the common denominator of permanent

decline in the patients' cognitive and functional abilities [8]. Dementia symptoms may arise in a variety of different disorders characterized by many pathological processes. The most common symptom associated with dementia is short term memory loss, but dementia symptomatology is broad and can include many different cognitive, behavioral or emotional symptoms including impairment in communication, language and visual perception, focus and attention, difficulties with reasoning and judgment, anxiety and depression. The symptom spectra of the different dementia disorders vary greatly. Dementias result in severe suffering for the affected patient and relatives, and are generally progressive and lead to increasing disability and ultimately death. Alzheimer's disease (AD), the most common dementia disorder, is often called a family disease due to the tremendous toll it takes on the relatives watching the personality of a spouse, parent, sister, brother or friend slowly fade away.

Age is the most important risk factor for developing most dementia disorders [9]. Some studies suggest that the dementia risk might be decreasing among older adults due to a number of factors, such as better cardiovascular prevention and healthier lifestyles, leading to lower risks of developing vascular dementia and higher education levels generating better cognitive reserves [10-12]. However, the overall prevalence of dementia is still expected to grow rapidly in the ageing world population [4]. Additionally, there are currently no disease-modifying treatments available for any of the most prevalent dementia disorders. In sum, dementia is a growing health concern and is projected to pose a great social and economic burden in the relatively near future [13, 14].

Alzheimer's disease (AD)

Alzheimer's disease is named after the German psychiatrist and neuropathologist Alois Alzheimer (1864-1915), who met a 51-year-old patient with memory-loss and behavioral symptoms at the Frankfurt asylum in 1901. Her name was Auguste Deter. He was intrigued by her symptoms and observed her over the following years. When she died five years later he had her brain neuropathologically examined. He found it atrophied and riddled with protein aggregates (later dubbed amyloid plaques and neurofibrillary tangles, collectively referred to as "AD pathology" below), and described his findings at meetings and in papers over the following years, although initially failing to spark much attention within the scientific community [15, 16]. However, interest slowly caught on in the following years and in 1910 his mentor Emil Kraepelin coined the name Alzheimer's disease and described the syndrome in the 8th edition of his *Handbook of psychiatry*.

AD is now known to be the most common form of dementia, accounting for approximately 60 – 70 % of all dementia cases [17]. AD is mainly a disease of the aging brain and has a marked increase in incidence with a doubling every fifth year after the age of 65. The approximate prevalence of AD in a population over 60 years old is 5 % [18]. AD pathology affects the cerebral cortex and certain subcortical regions. The entorhinal cortex and hippocampus are affected early on in the disease process leading to the most characteristic symptom of AD,

short term memory loss. Though the majority of AD cases are sporadic and have a late onset, a small minority of AD patients have causative genetic mutations. This form of the disease is called autosomal dominant AD (ADAD) and often manifests clinically as early-onset AD (defined as AD with symptom onset before 65 year of age). Most AD patients lack such dominant mutations, and are therefore said to have a sporadic form of the disease (SAD). Most patients with SAD have late onset of symptoms, after 65 years of age, although SAD can also debut early, and most early-onset patients do not have ADAD.

The amyloid cascade hypothesis

AD pathology is characterized by an accumulation of extracellular plaques in the brain, containing the aggregated form of the amyloid β (Aβ) peptide, and intraneuronal neurofibrillary tangles (NFTs) consisting of aggregates of the hyperphosphorylated form of the tau protein [19, 20]. Following the identification of Aβ and the genetic variants linked to autosomal dominant forms of the disease (all in genes involved in Aβ metabolism), *the amyloid cascade hypothesis* was introduced stating that an imbalance in the production or clearance of Aβ is the instigating event in AD leading to subsequent formation of amyloid plaques, tau tangles, oxidative stress, and microglial activation resulting in neuronal death (figure 1) [21, 22]. While there are other hypotheses for the underlying pathological mechanisms of AD (discussed in a later section), the amyloid cascade hypothesis is the most prominent and one that has sparked extensive research into the cause of abnormal production and clearance of Aβ peptides, and especially the highly

aggregation prone and potentially toxic 42 amino acid long Aβ peptide ($A\beta_{1-42}$), and the development of drugs targeting the production, aggregation and clearance of Aβ peptides [23-25]. $A\beta_{1-42}$ is the main component of amyloid plaques in AD. Different lengths of Aβ peptides are cleaved from the membrane embedded amyloid precursor protein (APP) by the enzymes β- and γ-secretase. In AD, shedding of Aβ is for unknown reasons shunted into more $A\beta_{1-42}$ (rather than shorter isoforms including $A\beta_{1-40}$), which appears to lead to amyloid plaque build-up [26]. Amyloid plaque accumulation precedes the formation of wide-spread NFTs in AD, but the link between the two remains to be explained. Unproven theories postulate that Aβ might induce phosphorylation of tau by directly altering the phosphorylation of tau, by interacting with APP or by inducing kinases to modify tau [27].

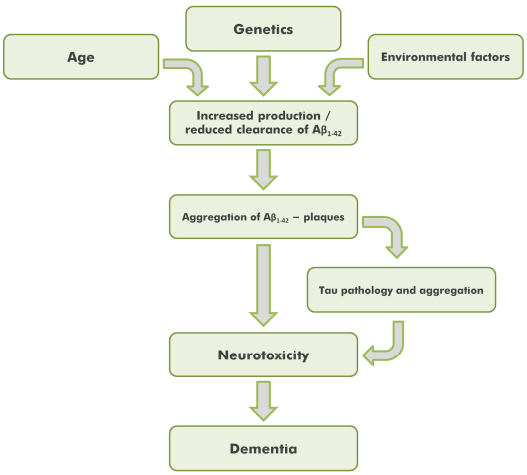


Figure 1. The amyloid cascade hypothesis schematic.

The amyloid cascade hypothesis is backed up by several lines of evidence. The brains of AD patients' exhibit hallmark post-mortem signs: amyloid plaques, NFTs and atrophy. Studies of ADAD have shown that mutations in the amyloid precursor protein gene (*APP*), or in the presenilin-1 (*PSEN1*) and presenilin-2 genes (*PSEN2*), the key

catalytic subunits of γ–secretase, cause AD [28, 29]. Transgenic mice expressing familial human *APP* and *PSEN* mutations also develop syndromes that mirror certain aspects of AD [30]. But there are also challenges to the amyloid cascade hypothesis [31]:

- At autopsy about 20-40% of cognitively intact elderly subjects meet some neuropathological criteria for AD, and in CSF biomarker or PET imaging studies in cognitively healthy individuals, biomarker signs of Aβ deposition increase with age and is particularly elevated in about 20% of adults aged 60 and over [32, 33]. This is not readily reconciled with the notion of Aβ aggregates as the instigating factor in AD [26, 34, 35].
- Amyloid plaque and NFT burden and clinical measures of cognitive health does generally not correlate well [32, 35]. If amyloid and tangles are the sole cause of neurodegeneration in AD, this correlation should be clear.
- Drug trials targeting the obvious culprit in the amyloid cascade paradigm, i.e. Aβ aggregates and associated proteins, have broadly failed. Although having in several cases successfully cleared Aβ plaques and shown signs of reversing AD symptoms in mice, these properties have not translated well into human treatments [36]. Some treatments have shown effects on Aβ pathology in humans but nonetheless been unsuccessful in stopping cognitive decline or neurodegeneration [37-39]. Roche's anti Aβ antibody gantenerumab removed Aβ plaques in patients to mean levels below 24 centiloid (a radiological threshold for evidence of Aβ pathology) in 1-2 years, but still failed to halt

cognitive decline [40]. When writing this, the news of another failed drug trial has just been released. In 2016, study results from a phase 1B study were published that showed that aducanumab reduced Aβ plaque load and indicated better cognitive results in treated patients; but the subsequent phase III has now been shut down due to falling short of their primary endpoint [37, 41].

- Brainstem and medial temporal lobe NFTs are seen in subjects without Aβ depositions in all age categories, which don't seem to support the idea of Aβ plaque formation as an upstream feature of AD pathogenesis [42, 43].
- Aβ is expressed fairly equally throughout the AD brain, while neurodegeneration initially affects specific parts of the brain, namely the hippocampus and entorhinal cortex (figure 2) [44]. This phenomenon is not explained by the amyloid cascade hypothesis.
- Although Aβ build up is clearly an important feature of AD-like pathology, the exact biochemical mechanisms for the propagation of the adverse effects of Aβ remain elusive [45].
- Amyloid plaques and NFTs can occur alone in some disorders. NFTs develop without the presence of amyloid plaques in tangle-only dementia, and amyloid plaques accumulate without subsequent NFTs in hereditary cerebral hemorrhage with amyloidosis of the Dutch type [46, 47]. While this doesn't directly contradict the validity of the amyloid cascade hypothesis, it suggests a complex relationship between amyloid plaques and NFTs that remain to be elucidated. Further, transgenic mice

harboring the APP or PSEN mutations develop Aβ plaques, but not NFTs [48].

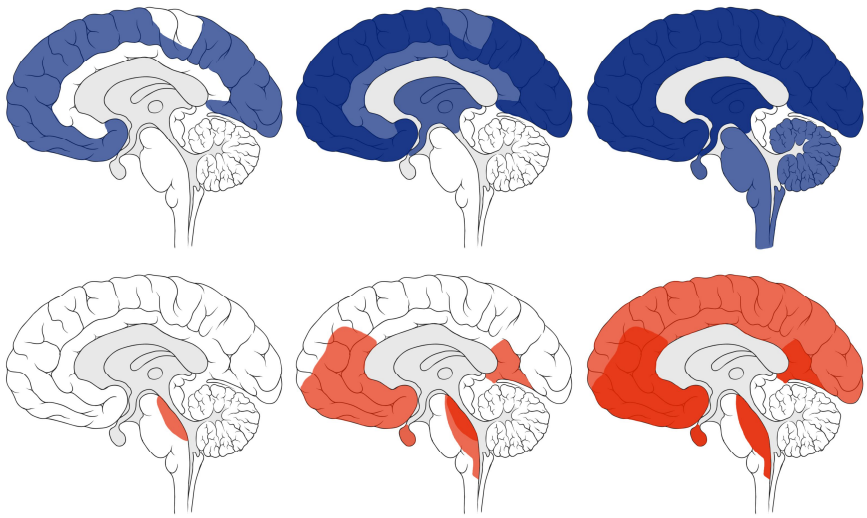


Figure 2. Propagation of pathology in the brain of AD patients follows a defined pattern. Aβ plaque pathology (top row) engages cerebral regions relatively uniformly and subsequently propagates to deeper regions, while NFTs (bottom row) initially build up in the entorhinal cortex and from there spread to cerebral regions as the disease progresses.

Alternative hypotheses of AD pathology

In spite of the above mentioned weaknesses, the amyloid cascade hypothesis might still be valid after some tweaking, or to explain heritable AD. Being designed after findings in animal models of ADAD, it might be the assumption that the hypothesis can be extrapolated onto all AD that is not accurate. This is the case in other diseases, for example skin blistering disorders, where early- and late-onset forms clinically

resemble each other but have different etiological bases. Early-onset forms (epidermolysis) have a genetic basis, while late-onset forms (pemphigoids) are autoimmune diseases, leaving widely different options for treatment of the two forms [49]. Diabetes type I and II are also examples of diseases with common symptomatology, but different etiology.

Alois Alzheimer noted another histopathological hallmark of AD that has not garnered nearly as much attention as the others: lipoid granules [50]. The identification of *APOE* as the strongest genetic risk factor of AD points to a link between lipid metabolism and AD, as *APOE* is a regulator of cholesterol metabolism in the CNS. Epidemiological studies also support a role of cholesterol in AD pathogenesis [51]. Statin treatment in animal models leads to decreased levels of Aβ, and retrospective epidemiological studies have suggested a reduced risk of AD in statin treated patients [52]. Physiological differences in plasma lipid metabolism could also explain the higher incidence of AD in women [53]. Lipids might regulate the pathogenic potential of other agents by regulating cell membrane integrity, and could also influence the aggregation of these agents. Growing evidence suggest that the amyloidogenic processing of APP occur mainly in so called lipid rafts, lipid rich membrane domains that cluster receptors and signaling molecules [54]. In a scenario where changes in lipid metabolism is the instigating factor in AD pathogenesis, Aβ-aggregation would merely be a side effect due to increased occurrence of lipid rafts in cell membranes, which in turn would explain the lack of success of drugs targeting Aβ-plaques, BACE1 and Aβ oligomers.

Evidence have also been put fourth that support a major role of contagions in AD pathogenesis. *Herpes simplex 1* (HSV-1) encephalitis primarily affects the entorhinal cortex and the hippocampus, the same anatomical regions where neurofibrillary tangles gain foothold [55]. Further, HSV-1 kinase has been implicated in tau hyperphosphorylation, and neuropathological studies have shown a strong correlation between the presence of HSV-1 DNA in human brains and the likelihood of AD [55, 56]. Reactivated HSV-1 in the brains of elderly and more susceptible brains could be the trigger factor in AD pathogenesis. In this theory, again, Aβ and tau aggregation would only be side effects of another pathological process. Other pathogens have also been implicated in AD pathogenesis. Recently, toxic proteases from the bacteria *Porphyromonas gingivalis*, a common oral pathogen, were identified in the brains of AD patients, and found to correlate with tau pathology, and *P. gingivalis* infection in mice resulted in increased production of intracerebral $A\beta_{1-42}$ [57].

Diagnosis and diagnostic challenges

A definitive diagnosis of AD cannot be reached without post-mortem neuropathological examination of the patients' brain. Due to practical limitations to this method, diagnostic criteria and tools have been developed to aid diagnostics in clinical practice and research. According to ICD-10 criteria [58], AD is characterized by:

A. The development of multiple cognitive deficits manifested by both:

1. Memory impairment
2. One or more of:
    a) Aphasia
    b) Apraxia
    c) Agnosia
    d) Disturbance in executive functioning

B. Cognitive deficits in A1 and A2 each cause significant impairment in social functioning.

C. Symptoms appear with gradual onset and continuing decline.

D. Symptoms in A1 and A2 cannot be explained by other diseases or substance-intake.

E. Symptoms do not occur exclusively during delirium.

F. Symptoms cannot be better be explained by depression, schizophrenia or similar conditions.

The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria is also commonly used [59].

In the research setting, The International Working Group (IWG) has put forward diagnostic criteria for AD, which were updated in 2014 and dubbed the IWG-2 criteria [60, 61]. In the new revision neurochemical and neuroimaging diagnostic markers were introduced. Low concentrations of $A\beta_{1\text{-}42}$ and high concentrations of total tau (T-tau) and phosphorylated tau (P-tau) in the cerebrospinal fluid (CSF) indicate plaque pathology and neuronal damage respectively. Increased tracer retention of amyloid PET is also considered *in vivo* evidence of AD pathology. The IWG-2 criteria are as of yet mainly recommended for

research purposes and CSF and imaging biomarkers are thus not yet fully implemented in diagnostic criteria for the clinical setting. However, many European countries, including Germany, have recently issued recommendations to include CSF biomarkers as a supplement to clinical evaluation in dementia diagnostics [62, 63].

Diagnostics in AD and dementia in general can be challenging. Cognitive decline is a progressive and often slow process, and it can be difficult to distinguish specific traits in clinical presentations. Early diagnosis is especially challenging (and preclinical AD, prior to any symptoms, can by definition not be detected by clinical testing alone). Additionally, co-morbidities are common, blurring the lines between specific disorders. In post-mortem AD brains, Lewy body pathology associated with dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) have been shown to occur in more than 50% of cases, and signs of vascular dementia might be even more common [64-66]. Further, neither amyloid plaques nor neurofibrillary tangles are specific for AD [66-68]. NFTs are found in many other neurodegenerative diseases, such as prion disease, metabolic diseases, some brain tumors and also in cognitively normal aging subjects [42]. Amyloid plaques are, as previously mentioned, found in many cognitively intact elderly subjects, and are also prevalent in DLB and PDD [69]. Mixed pathologies and presence of subclinical pathologies in dementia lead to variations in both clinical presentation and uncertainties in biomarker read outs.

Current treatment of AD

Despite the significant effort put into the search for disease modifying treatments of AD, none other than symptomatic treatments have as of yet been found [70]. There are two strategies of treating the symptoms of AD available today, the first being acetylcholineesterase (AChE) inhibitors like donepezil, galantamine or rivastigmine. By inhibiting the enzyme AChE, the rate of degradation of acetylcholine in the synaptic cleft is reduced, thus potentiating the level and duration of action of the neurotransmitter. The aim of this treatment is to slow cognitive decline and ease memory difficulties. Effects of the different agents in this group on the market are similar and generally considered moderate [70-72]. Response rates vary, and about one third of the patients experience no benefit, while one third doesn't tolerate the treatment due to side effects [70].

The second strategy of AD treatment is to block NMDA receptors by NMDA receptor antagonists like memantine. The aim of this strategy is to hinder neuronal excitotoxicity and by that exert neuroprotection [73]. Memantine was first synthesized in the 60s and marketed as a potential diabetes treatment. The NMDA receptor blocking properties of the drug was first discovered and applied in AD treatment in the 1980s [74]. Memantine is generally better tolerated than AChE inhibitors and is especially used for treatment of AD patients that don't tolerate or have contraindications for AChE inhibitor use, or patients with more than mild symptoms. Memantine might also have beneficial effects in combination with an AChE inhibitor [73]. However,

although memantine therapy improve cognition and global function in AD, the efficacy is limited as is evidence of clinical benefit [75].

Vascular dementia (VaD) and mixed dementia

VaD, the second most common dementia, accounts for about 10 - 20% of all dementia cases. Subtypes of VaD include multi-infarct dementia, caused by series of minor ischemic or hemorrhagic strokes leading to stepwise cognitive decline; strategic infarct dementia, caused by ischemic lesions involving specific sites in the brain; and subcortical dementia, caused by small vessel disease leading to lacunar infarcts and diffuse white matter lesions [76, 77]. Symptoms of VaD vary depending of which regions of the brain are affected; Cortical lesions can cause aphasia, apraxia and epileptic seizures, while subcortical lesions lead to bradyphrenia, executive dysfunction, gait changes, urinary incontinence and parkinsonism [78]. VaD patients also often exhibit focal neurologic signs such as hemiparesis, bradykinesia or hyperreflexia. The clinical distinction between AD and VaD can be challenging, and AD and VaD pathologies often coexist in a condition called mixed dementia. Neuropathological studies indicate that this might be very common [79, 80].

Management of VaD includes addressing risk factors of cardiovascular health, including tobacco use, hypertension, atrial fibrillation, diabetes and high cholesterol to provide protection against strokes and vascular pathology. As progress has been made in stroke and

vascular disease prevention over the last decades the incidence of VaD is declining [81].

Frontotemporal dementia (FTD)

FTD is a group of clinical syndromes with a common feature of progressive neurodegeneration of mainly the frontal and anterior temporal lobes, leading to personality and behavioral changes or difficulties with language. FTD has a strong genetic component with about 40 % of cases having a family history of dementia, psychiatric disease or motor symptoms [82]. FTD also has an earlier onset than other dementias and symptoms usually occur in between ages 45 to 65 [83]. FTD is commonly divided into three main subtypes: behavioral variant FTD (bvFTD) is the most common one accounting for about half of the FTD cases, while semantic variant of primary progressive aphasia (svPPA) and nonfluent variant primary progressive aphasia (nfvPPA) are rarer. BvFTD engage mainly the paralimbic areas including the medial frontal, orbital frontal, anterior cingulate and frontoinsular cortices [84]. Right hemisphere atrophy is associated more with behavior changes, and affected patients often display apathy, become socially withdrawn, rigid in their thinking and might behave socially inappropriate [85-87]. SvPPA and nfvPPA are characterized by anterior temporal lobe atrophy, and clinically feature language problems with loss of meaning of words in svPPA and problems with producing speech in nfvPPA [88, 89]. When the left temporal lobe is engaged, language

functions are mostly impaired, and when the right temporal lobe is engaged the symptoms are mainly behavioral. Over time, both temporal lobes become affected, and subsequently also the frontal lobes leading to symptoms of bvFTD. Memory problems are not a key feature of FTD. There are also conditions that are considered closely related to FTD, and are collected under the frontotemporal lobar degeneration (FTLD) umbrella term, but engage partly different anatomical regions, including frontotemporal dementia with motor neuron disease (FTD-MND), progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). There is further a logopenic variant of PPA that has been correlated predominantly with AD pathology.

As in AD, protein aggregation is a major pathological feature of FTD and FTLD. FTLD is sub classified according to immunohistochemical staining for specific protein accumulations into four main subtypes, each with several sub classifications of their own [90]. In FTLD-tau, like in AD, the protein tau is accumulated; although tau inclusions in FTLD-tau differ from AD in that they primarily contain one or two of the six tau isoforms and not all six. FTLD-tau can be divided into 4R tauopathies, including CBD, PSP, and 3R tauopathies, including Pick's disease, depending on which isoform of tau is predominantly deposited. Pick's disease clinically most commonly presents as bvFTD, but can sometimes also be seen as the nfvPPA or svPPA phenotypes [84]. Specific mutations in *MAPT*, the tau gene, cause dominantly inherited FTD and Parkinsonism linked to chromosome 17 (FTDP-17) or familial FTLD-tau [91].

FTLD-TDP is characterized by four types (A-D) of TAR DNA-binding protein 43 (TDP-43) aggregation and related pathologic properties. TDP-43 is involved in mRNA processing, but its exact biological function is unknown. FTLD-TDP clinically typically presents as svPPA (type C), but bvFTD, nfvPPA and CBS can also be seen (type A, B). Aggregates of TDP-43 is also a main feature of motor neuron disease (MND), and mutations in the gene *C90RF72* is the most common genetic cause of both FTD and MND [92]. Additionally, about 10-15% of patients with FTD also subsequently develop MND (FTD-MND) symptoms, and inversely about 50% of the patients that debut with MND later in the disease progression develop cognitive impairment and 15% meet criteria for FTD [93].

The third immunohistochemical sub classification of FTLD, FTLD-FET, account for 5-10 % of the total FTLD cases, a group that is both tau and TDP-43 negative. In 2009 links between the fused in sarcoma (FUS) gene and MND was found [94]. The known overlap of FTD and MND sparked an investigation of the relation between FTD and FUS, where FUS inclusions were found in FTD but mutations in FUS showed no link to FTD [95]. FUS is a member of the FET protein family, and an RNA/DNA binding protein just like TDP-43, implying abnormal RNA metabolism as an important event in FTLD-FET pathology.

The fourth and last sub group of FTLD is FTLD-UPS, caused by a rare mutation in the *CHMP2B* gene found in a Danish family. FTLD-UPS exhibit inclusions of ubiquitin, but are negative for tau, TDP-43 and FET.

There are no specific treatments of FTD, although symptoms might sometimes be relieved by antidepressants and antipsychotics [96]. The average survival time from diagnosis is between 3-12 years depending of which subpopulation of patients is studied; Patients with bvFTD and concomitant motor neuron disease average 3 years, while svPPA patients live 12 years from diagnosis on average [97].

Dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD)

DLB and PDD are both characterized by the formation of α-synuclein containing deposits in the brain and peripheral nervous system called Lewy bodies [69]. In both diseases Lewy bodies can be found in the frontal and temporal cortex, however, there is a higher cortical Lewy body load as well as more frequent and severe hippocampus and amygdala load in DLB [69]. There is also convergent influence of Aβ and tau pathology in both DLB and PDD, but higher degrees of Aβ and tau loads in the cortex and striatum can be seen in DLB [69].

Both diseases feature impaired cognition, sleep disorders, visual hallucinations, depression and parkinsonism, i.e. muscular rigidity, bradykinesia, postural instability [98-101]. The distinguishing factor between the two disorders is the order in which symptoms appear. In PDD, a diagnosis of PD precedes the onset of cognitive decline, while in DLB cognitive symptoms debut simultaneously or before the symptoms of parkinsonism. Some studies suggest PDD and DLB are part of a

continuum and that it might be meaningful to separate them clinically but still recognize their common pathophysiological mechanisms in a research context [69, 102, 103].

There are no disease modifying treatments for DLB and PDD. Parkinsonism is treated with L-dopa just like in PD without dementia, and, like in AD, memory and attention deficits can be alleviated by AChE inhibitors like rivastigmine, galantamine and donezepil or NMDA receptor antagonists like memantine. Depressive symptoms can be managed by SSRI treatment, and hallucinations can be treated (very carefully and with low doses) with neuroleptics like quetiapine and clozapine. However, effective treatment of hallucinations in DLB and PDD is rare and adverse effects like worsened parkinsonism and increased risks of stroke and sudden cardiac death often outweigh the benefits [104].

Creutzfeldt–Jakob disease (CJD)

Sporadic CJD is a rare neurodegenerative disease that affects about 1/1 000 000 people per year worldwide, and is unlike the more common forms of dementias in that it is known to be transmittable [105]. CJD is caused by endogenous intracellularly misfolded proteins called prions, first discovered in the 1960s [106]. CJD is characterized by massive and escalating neuronal death, and the first symptom is usually rapidly progressive memory loss and dementia. Myoclonus, anxiety, depression and psychosis is also common but clinical presentations vary

greatly [107]. While the sporadic forms of prion disease occur spontaneously, and are the most common forms accounting for about 85% of all cases, there are also familial disorders caused by mutations in the *PRNP* gene encoding for the PrP protein, including familial CJD, fatal familial insomnia, Gerstmann-Sträussler-Scheinker syndrome and Kuru [108-110]. A small part of prion disease cases are also caused by infection from external sources such as transplants contaminated by prions or by ingestion of meat infected with prions [111, 112]. All known prion disease start with the conformational change of the endogenous membrane protein PrP^{C} into the disease associated PrP^{Sc}. By this change PrP^{Sc} acquires protease resistance and the ability to induce transformation of other PrP^{C} proteins into PrP^{Sc}. PrP^{Sc} is prone to aggregation and form neurodegenerative amyloid fibrils [113]. All prion disease is fatal and no disease modifying treatments exist. There are also several prion diseases affecting other mammals all involving the same well preserved *PRNP* gene and PrP protein. Scrapie in sheep, bovine spongiform encephalopathy in bovines and chronic wasting syndrome in deer and moose all stem from the same transformation of host genome encoded PrP^{C} into PrP^{Sc} [114, 115].

The physiological function of PrP^{C} is not clear, and initial reports of PrP^{C} knockout mice revealed no apparent phenotype abnormalities. However, more recent studies reveal adult-onset demyelination of the peripheral nervous system (PNS) in PrP^{C} knockout mice, and further studies have corroborated a role for PrP^{C} in myelin maintenance and cellular differentiation [116]. PrP^{C} reportedly also acts as an inhibitor of

BACE1, thereby reducing the amount of Aβ produced with a potential protective effect against AD pathology [117].

Protein misfolding occurs in a number of other diseases: AD, PD, Huntington's disease, MND and more all feature aggregation of different endogenous proteins. Analogies and similarities between prion disease and other conditions involving protein aggregation have been found. For instance, evidence suggests that both tau and Aβ pathology in AD, as well as α–synuclein in PD might propagate through prion like mechanisms [118, 119]. This concept is discussed further in chapter 2.2.5 and 2.2.6.

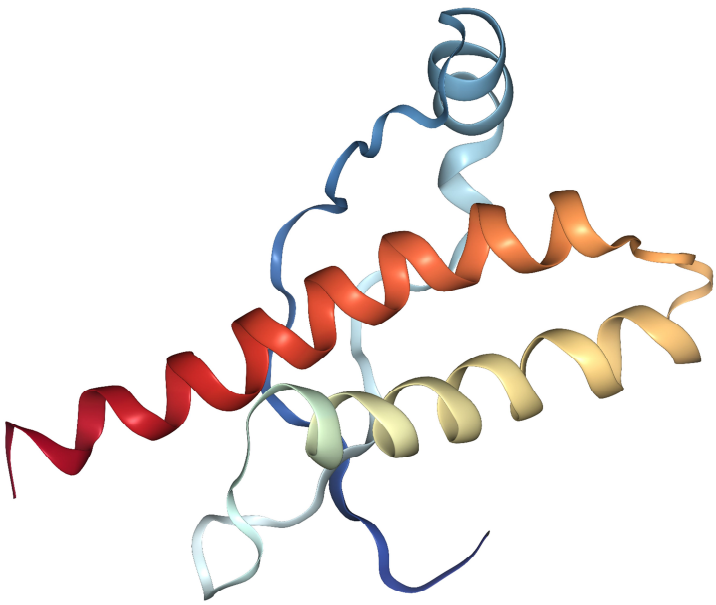


Figure 3. PRP^{C}, the normal and non-pathological strain of PRP.

Biomarkers of AD and neurodegeneration

While the clinical presentation in concert with cognitive, neurological and neuropsychological testing still forms the basis of the diagnostic process in dementia investigation, laboratory and radiological tests have been developed, and are increasingly used in clinical and research settings. In recent years, these tests have been included as recommended methods for supporting clinical evaluations in dementia diagnostics in several countries [62, 63].

The value of biomarkers

The ability to readily identify and discern different causes of dementia as early as possible in the course of disease is essential in order to be able to provide optimal care and to enable administration of correct treatment. It is further important to identify means to be able to monitor disease progression and treatment effects. A host of drug trials aimed at treating AD have failed over the past few years. In fact, no new medications specifically aimed at treating AD have been approved by the FDA since 2003 (memantine being the latest). However, there is hope that this long dry spell may be nearing an end. In the most recent

assessment there were 112 agents tested in 135 separate clinical trials underway, and in different stages of completion [38]. The principle focal points of drug development have sprung from the amyloid cascade hypothesis and aim at development and administration of antibodies targeting Aβ or related peptides to facilitate their removal, limit their production or hinder aggregation. An example of a highlight in this field is the antibody BAN2401, that binds to Aβ protofibrils and that has shown promising results in early phases or trial [120, 121]. A phase II clinical study on MCI patients who were administered BAN2401 was able to show not only dose-dependent reduction in amyloid plaques and slowed cognitive decline as measured by ADAS-Cog, but also increased CSF Aβ and reduced T-tau concentrations [122]. There is cause for optimism and keeping up hope that one of the many paths taken will one day lead to successful treatment of AD.

Efficient biomarkers can provide aid in clinical trials by identifying suitable subjects for inclusion. It is likely that AD pathology must be targeted as early in the disease process as possible in order to prevent irreversible neuronal damage. It has been argued that the failure of some of the clinical trials in AD over the years can in part be attributed to treatment being administered to late in the course of the disease [36]. Signs of AD, including amyloid plaque build-up, have been shown to precede clinical symptoms by decades [34, 123, 124]. Using well characterized biomarkers can help find patients at an early enough stage of disease to be eligible for treatment, and help secure presence of AD pathology. Further, biomarkers can be used to monitor treatment effects in clinical studies. For instance, neurofilament light protein (NfL),

the biomarker of interest in paper II of this thesis, can be considered a measure of rate of neurodegeneration in AD and other neurodegenerative diseases, and might be used to evaluate the efficacy of a given treatment or to compare dosages [125, 126].

Another difficulty in treating dementia is the multifactorial nature of dementia disorders, and the difficulty in mapping out the disease processes present in the individual patient's CNS [70]. Pure Alzheimer-type pathology is rare, especially in the elderly [66]. There might also be as of yet unknown sub-classifications present in the spectrum of dementia disorders that have therapeutic significance. In the future, biomarkers might be used to obtain detailed information on the influence of different pathologies in the individual patient's brains, and inform tailored treatment.

There are several different modalities of biomarkers with a potential to allow for early and dependable diagnosis and prognosis as well as measures of rates of ongoing disease processes.

Imaging biomarkers

Structural magnetic resonance imaging (MRI) is the most widely used neuroimaging technique to investigate anatomical changes of neurodegeneration *in vivo,* and has contributed significantly to the understanding of different dementia disorders [127, 128]. In positron emission tomography (PET) and single photon emission CT (SPECT),

radioactive ligands are used to image structures, metabolism and perfusion of the brain, allowing for quantification of functional markers of neurodegeneration and specific neuropathological features of disease, such as amyloid plaques and neurofibrillary tangles in AD [128, 129]. PET and SPECT adds important information in the diagnostic process, and in the prognosis and management of dementia disorders in the clinical setting, and can reveal information on disease specific mechanisms of pathogenesis in the research setting [129]. The use of MRI in differential diagnosis is however limited due to lack of specificity for underlying pathology, as atrophy patterns might overlap across several dementia syndromes, and since the normal variability for structural measures is large [128]. Concordance between neuroimaging and CSF biomarkers of AD pathology is generally considered excellent [130-132].

CSF biomarkers

CSF – Function and characteristics

The cerebrospinal fluid envelopes the brain and provides buoyancy and a buffer zone protecting the brain from physical trauma, while also removing metabolic waste by diffusing it out into the blood stream [133]. About 125-150 mL of CSF is in circulation at any given time, and the turnover rate is about 25 mL / hour [134]. Pathological processes in the brain leave traces in the CSF, which may thereby serve

as a biochemical window into the brain and a valuable source of information for investigation of the biochemistry of the CNS. To use CSF biomarkers optimally a detailed understanding of their distribution and dynamics is required. Many different aspects might influence a biomarker's concentration beside its relation to clinical pathology, such as age, sex, concomitant pathologies, genetic differences, rate of degradation of the analyte etc. AD is the most common and prominent dementia disorder and also the one where CSF biomarker research has been most fruitful. In this thesis we focus on exploring the large amount of data gathered in clinical routine, where assays for biomarkers in dementia have been available for several years. The biomarkers in our data include $A\beta_{1-42}$, T-tau, P-tau, which all reflect different aspects of AD pathology, and NfL which is considered a more general biomarker of neuronal decay.

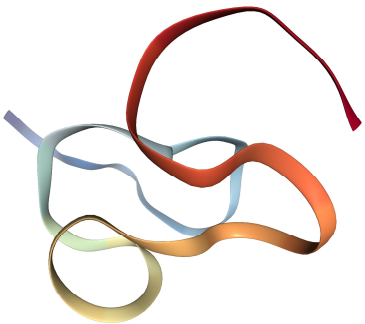
Lumbar puncture

CSF is sampled by means of a lumbar puncture (LP). An LP is performed by introducing a needle into the subarachnoid space of the lumbar spinal column below the termination of the spinal cord, usually between vertebrae L3/L4 or L4/L5 [134]. For dementia biomarker analysis a volume of about 12 mL of CSF is normally collected and put in polypropylene tubes before further processing. Lumbar puncture is a safe procedure with little side effects, the most commonly reported being post-LP headache, a benign condition that typically resolve within a week and that occur in about 10% of patients when atraumatic needles are used [134].

Biomarkers of AD pathology

There are several established CSF biomarkers of AD correlating to different characteristics of AD pathology. A classical, but somewhat disputed, interpretation of the three major AD biomarkers are that low levels of $A\beta_{1-42}$ correlate with senile plaque load, levels of T-tau increase with higher rates of neuronal death, and levels of P-tau correlate with neurofibrillary tangle pathology [135]. Various composite biomarkers has also been suggested and evaluated. For example, the P-tau/$A\beta_{1-42}$ ratio has been shown to have particularly good discriminatory power in AD towards other dementias, presumably because it integrates information about amyloid and tau pathology, the core hallmarks of AD [136-138]. Another prominent composite biomarker is the $A\beta_{1-42}/A\beta_{1-40}$ ratio, where the dynamic of low $A\beta_{1-42}$ concentrations in contrast to unchanged concentrations of the $A\beta_{1-40}$ in AD is employed [139]. This ratio is probably superior since it adjusts for the between-person variability in overall amyloid peptide metabolism. In patients with a clinical AD like presentation (also in pre-dementia stages) a pattern of low levels of $A\beta_{1-42}$ in combination with elevated levels of T-tau and P-tau should strengthen the suspicion on AD. However, as previously discussed, other common dementia disorders might overlap both in clinical symptoms and CSF characteristics, and mixed pathologies are common [140-144].

$A\beta_{1-42}$



We use the term Aβ to refer to peptides that are derived from the amyloid precursor protein (APP). APP is a membrane bound protein that can be cleaved by three enzymes, α-, β-, and γ-secretase. Cleavage by γ- and β-secretase (BACE1) sheds several Aβ-isoforms, including $A\beta_{1-40}$ and $A\beta_{1-42}$, 40 and 42 amino acids long respectively. $A\beta_{1-42}$ is produced by BACE1 and γ-secretase cleavage and prone to aggregation, while residues produced by α-secretase cleavage are not (figure 4). $A\beta_{1-40}$ is also produced by BACE1 and γ-secretase cleavage but does not contribute to aggregation at the same rate as $A\beta_{1-42}$. High concentrations of intracerebral $A\beta_{1-42}$ or increased $A\beta_{1-42}/A\beta_{1-40}$ ratios lead to amyloid plaque build-up. In sporadic AD, production of Aβ is thought to be shifted into higher rates of $A\beta_{1-42}$, or alternatively the clearance of Aβ is reduced [145]. The physiological roles of APP and Aβ are also not clearly mapped out. APP knock-out mice exhibit growth and brain weight deficits, reduced grip strength, agenesis of

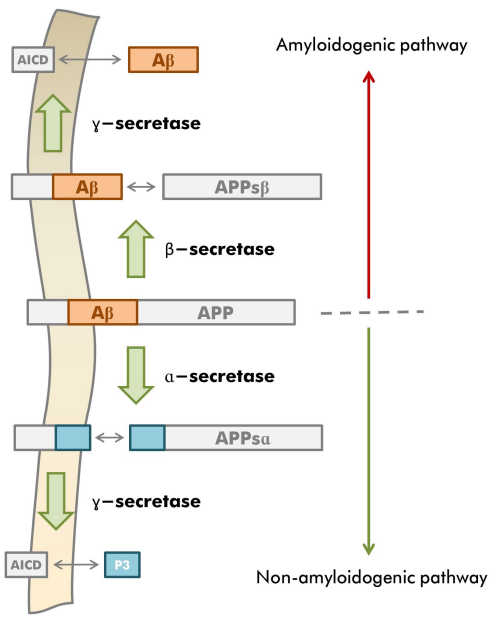


Figure 4. APP processing by α-, β- and γ-secretase.

the corpus callosum and several other abnormal traits [146]. Mutations in APP at the BACE1 cleavage site in humans increase $A\beta_{1-42}$ production and are associated with ADAD [147]. BACE1 knock out mice don't produce $A\beta_{1-42}$ and are healthy and fertile but exhibit memory and behavioral deficits [148]. Presenilin is the sub-component of γ-secretase that is responsible for APP cleavage. Mutations in the presenilin genes PSEN1 and PSEN2 are the most common causes of familiar early onset AD in humans [149]. Most mutations in presenilin do not increase the amounts of Aβ produced but shunts production into more $A\beta_{1-42}$ at the cost of less $A\beta_{1-40}$ [150, 151].

AD pathology leads to lower concentrations of $A\beta_{1-42}$ in CSF as compared to healthy controls [135]. The most commonly accepted explanation for this is that intracerebral $A\beta_{1-42}$ aggregation prohibits $A\beta_{1-42}$ clearance into CSF. This has been corroborated by autopsy studies finding correlations between low $A\beta_{1-42}$ in ventricular CSF and high numbers of amyloid plaques in the neocortex and hippocampus [152]. Cerebral Aβ aggregation is an early event in AD and might precede clinical symptoms by decades. Amyloid positivity in subjects with normal cognition has been shown to be associated with observable clinical symptoms 10-15 years before they emerge [153]. After it was concluded that the main component of amyloid plaques in AD was Aβ, and that Aβ was a

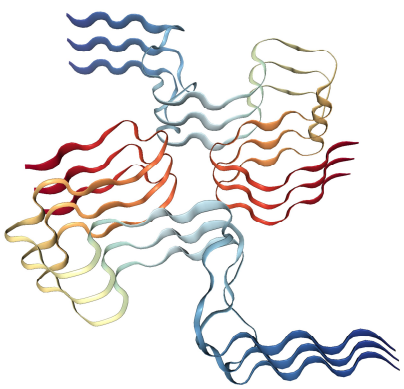


Figure 5. Aggregated $A\beta_{1-42}$ in AD

soluble peptide secreted by a variety of cell types, the search for means of measuring Aβ in CSF begun [154]. The first ELISAs developed measured total Aβ levels and failed to discriminate different Aβ isoforms, and thus also AD patients from healthy controls [155]. It was later found that several different forms of Aβ existed and that $A\beta_{1-42}$ was the predominating form deposited in amyloid plaques [156, 157]. In light of these discoveries immunoassays targeting $A\beta_{1-42}$ were developed and shown to identify lower concentrations of CSF $A\beta_{1-42}$ in AD patients as compared to healthy controls [155, 158, 159]. A commercial sandwich ELISA assay (INNOTEST® β-amyloid$_{1-42}$) was used for CSF $A\beta_{1-42}$ measurements in paper I of this thesis.

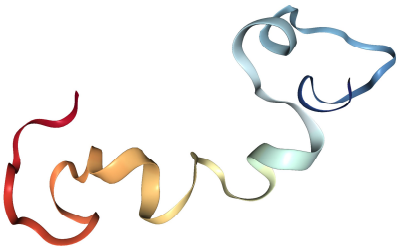
Amyloid plaques are not exclusive to AD. For instance, in DLB, amyloid plaque formation is an early feature, and PD patients who develop PDD also show heightened amyloid burden [160, 161]. These overlaps might indicate presence of Aβ in non-AD pathology, but might also indicate comorbidities.

It has long been assumed that the insoluble amyloid plaques in AD are the instigating factor in AD pathogenesis [21]. However, this has been disputed by a growing body of evidence supporting the importance of the prefibrillar stage of amyloid plaques, soluble Aβ oligomers, in inducing synapse loss and neurotoxicity in AD [162]. Studies have shown Aβ oligomers to be more cytotoxic than fibrillary Aβ plaques in general and to inhibit long-term potentiation of synapses both *in vivo* and in *in vitro* [163, 164]. The so called Arctic mutation in the APP gene causes a form of ADAD, and was discovered in a Swedish family in the early 2000s [165]. However, the Arctic mutation cause increased

formation of large soluble Aβ oligomers and protofibrils, and the brains of diseased patients with the mutation don't exhibit amyloid plaques in the classical sense. Interestingly, NFTs occur at the same rate as in sporadic AD, further supporting the idea of Aβ oligomers being important in AD pathology [166].

As previously mentioned, evidence has been put forth to support a prion like propagation of Aβ pathology. Several research groups have injected brain tissue from deceased AD patients into the brains of transgenic human APP mice and could then observe Aβ plaques develop and propagate from the injection site throughout the rodents' brains [119, 167]. The degree of Aβ seeding in the mouse brain has been found to be in direct proportion to the concentration of the injected brain extract [168]. Evidence promotes a propagation of Aβ pathology through axonally connected brain areas, unlike PrP^{Sc} that spread to anatomically adjacent brain areas via the brain interstitial fluid [169, 170].

Tau



Tau proteins are most abundant in neurons, but are also expressed in other cells in humans. Under normal conditions their main function is to stabilize microtubules and primarily do so in non-myelinated axons [155]. There are six isoforms of tau encoded by the same gene (*MAPT*) but results of alternative splicing. The tau isoforms are distinguished by their number of binding domains and their resulting performance in microtubule stabilization. Tau is a phosphoprotein with more than 30 potential phosphorylation sites and the tubule binding power of tau is regulated by a host of kinases [68]. Phosphorylated tau disrupts microtubule organization and leads to increased neurofibrillary plasticity or degeneration [171, 172]. Hyperphosphorylated tau of all isoforms have severely reduced affinity for microtubules and is prone to aggregation leading to formation of intracellular NFTs, thereby rendering a normally soluble protein resistant to degradation and clearance [173]. NFTs are neurotoxic and mediate neuronal death and cognitive decline in AD. Tau inclusions are not specific to AD, but key components of the pathology in a group of diseases called tauopathies, i.e. neurodegenerative diseases associated with neurofibrillary or glial fibrillary tangles. However, tau aggregates differ across tauopathies in their composition and locale. Astrocytic tufts form in PSP, astrocytic plaques in CBD and Pick bodies in FTD [174].

The precise role of tau in AD and neurodegeneration is unclear and has been debated. Evidence suggests that tau is needed for Aβ neurotoxicity in AD, as neurons from tau knockout mice, unlike those from normal mice, are resistant to exposure to Aβ [175]. Tau dysfunction might cause neuronal damage in two different ways, by loss of function and by gain of cytotoxicity. Data indicates that increased levels of intracellular Aβ cause tau to hyperphosphorylate and detach from microtubules, impairing axonal transport and leading to synaptic dysfunction. Tau is then deposited in the neuron's somatodendritic departments [176]. Hyperphosphorylated tau has a tendency to self-aggregate into filaments that ultimately form NFTs, a classical neuropathological hallmark sign of AD pathology, and long considered neurotoxic. However, it could also be that the NFTs are the end-product of a process where an intermediary product is the neurotoxic agent, i.e. the NFTs themselves don't propagate neurotoxicity. Some studies indicate that soluble, hyperphosphorylated tau is closer related to synapse loss and neuronal decay than NFTs by showing that these destructive events occur in cell models in the presence of mutated tau independent of NFT formation, indicating that NFTs are merely a side effect of neurodegeneration [177, 178].

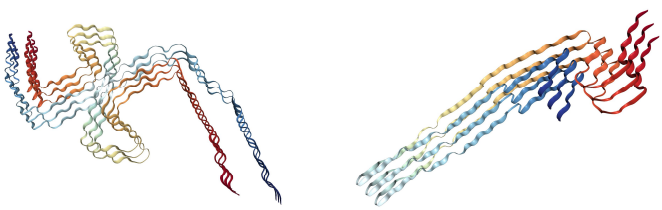


Figure 6. Tau aggregations in a NFT in AD (left) and a narrow Pick filament in FTD (right).

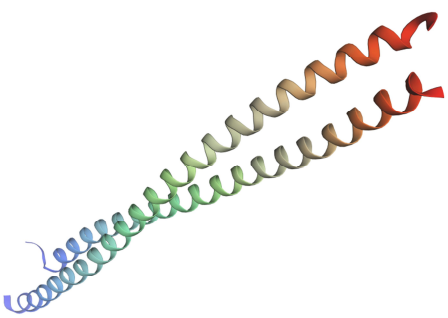
The T-tau concentration in CSF has historically been considered a biomarker of neurodegeneration. However, recent evidence suggests that the increase in CSF tau concentrations arise due to ramped up phosphorylation, and is released as a response to Aβ exposure [179]. In any case, T-tau is increased in AD and can effectively discriminate AD patients from healthy controls [180]. In some other tauopathies, including FTD, CBD, and PSP, CSF T-tau concentrations are surprisingly not distinguishable from healthy controls [181]. In most non-AD dementias, such as DLB, PDD and VaD, T-tau concentrations are also normal or close to normal [182]. However, T-tau concentrations are not exclusively increased in AD. The most dramatic increase in CSF T-tau concentrations can be seen in CJD, where nearly exponential increases can be seen as the neurodegeneration spread through-out the affected brain, as studied in paper III of this thesis [183]. In stroke and traumatic brain injury (TBI), CSF T-tau concentrations also increase [184]. In conclusion T-tau is a biomarker reflecting the intensity of neurodegeneration in several disorders, and is considered one of the hallmark biomarkers of AD, where elevated concentrations in CSF might be a response to Aβ exposure.

Tau is encoded by a single gene, *MAPT*. No known *MAPT* mutations are known in AD, but rare familial cases of non-AD tauopathies have been linked to *MAPT* mutations. About 100 families with *MAPT* mutations have been reported. Mutated tau has reduced ability to bind to microtubules and lead to tauopathies like PSP, CBD, Pick's disease (a form of FTD) and the rare autosomal dominant disease

frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) [184].

As with Aβ accumulation, evidence have been put forth to support a prion-like propagation of tau aggregation. Defining features of prion-like behavior include a protein or protein aggregate gaining insolubility and protease resistance, neurotoxicity and the ability to propagate these traits to proteins in adjacent cells, inducing a wild fire like spread [185]. Mounting evidence suggest that tau might fulfill these criteria. As previously described tau aggregates are neurotoxic and insoluble. Studies have also shown uptake of tau by cells through specific mechanisms, notably by interaction with heparin sulfate proteoglycans that also interact with pleiotrophin, the subject of interest in paper IV of this thesis [186, 187]. In addition, studies have shown that tau pathology in AD do not distribute randomly but spread following neuronal networks throughout the brain, possibly implying connectivity as a key for propagation [188, 189]. Several studies have further shown seeding, i.e., the induction of aggregation of soluble tau by abnormal tau [190-192]. Introduction of synthetic tau fibrils into the brains of mice induce build-up of NFT-like inclusions that propagate from the injection site into connected brain regions [118].

NfL



Another CSF biomarker of importance in dementia and neurodegeneration in general is NfL, which is part of a family of proteins, neurofilaments, consisting of three members: neurofilament light, medium and heavy. NfL is predominantly expressed in large-caliber myelinated axons where it serves as a scaffolding protein, providing structural integrity to the axon. White matter lesions and other injuries to subcortical brain regions induce NfL release into CSF, and conditions that exhibit increased CSF NfL concentrations include dementias such as FTD, VaD, HIV-associated dementia and AD but also multiple sclerosis, stroke, traumatic brain injury (TBI) and neuroinfectious conditions [193-197]. NfL has been less studied than $A\beta_{1-42}$, T-tau and P-tau but has great potential for use in disease monitoring and prognosis in neurodegenerative conditions through its cross-disease biomarker properties, correlation to on-going neurodegeneration, and accessibility in being able to measure in serum and plasma as discussed below.

Emerging biomarkers and the pursuit of prospects

The amyloid cascade hypothesis, although not yet proven, might be considered the core model of the disease processes in AD, and the

biomarker triad of $A\beta_{1-42}$, T-tau and P-tau each reflect the main components of this model. However, recent studies in AD biomarkers highlight several other important pathological changes and the molecules that reflect them.

Neurogranin (Ng) is a protein involved in long term potentiation/depression of synapses, and can be used as a biomarker of synaptic loss and to predict rate of cognitive decline in AD [198, 199]. Portelius et al. has further shown that Ng can contribute to the diagnostic accuracy of the core AD biomarkers ($A\beta_{1-42}$, T-tau and P-tau) and increase the discrimination of AD and other neurodegenerative disorders [200].

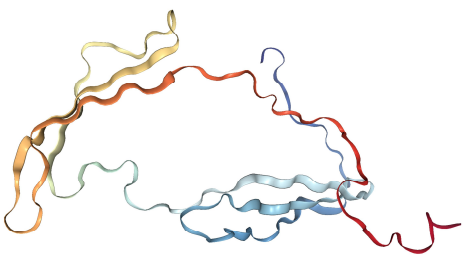
The physiological role of YKL-40 is unclear, but it is known as a marker of activated astrocytes and microglia, and to be upregulated in several conditions and disorders characterized by inflammation including, but not limited to, inflammatory bowel disease, rheumatoid arthritis, scleroderma, certain infections and cancers like melanoma and myeloid leukemia. It has also been suggested as a biomarker for neurodegeneration in traumatic brain injury, multiple sclerosis and AD [201-203]. Data suggest that YKL-40 levels are elevated in AD but also in FTD and prion disease but not vascular dementia and PD [204].

The platelet-derived growth factor receptor-β (sPDGFRβ) is abundant in brain capillary pericytes and envelops capillary blood vessels in the brain [205]. When measured in CSF, sPDGFRβ is closely correlated with blood-brain-barrier dysfunction and was recently shown

to be increased in individuals with incipient cognitive dysfunction in AD independent of other CSF biomarkers [206].

In short, additional biomarkers can help provide a deeper understanding of the pathological mechanisms involved in AD, more nuanced and dynamic characterizations of processes contributing to neurodegeneration and might help tailor treatments for individual patients in the future.

Pleiotrophin



In paper IV, the discovery of a new potential biomarker of AD is laid out. Using a novel strategy for hypothesis generation through analysis of mass spectrometry data applied in a large sample of patients (n = 120), a peptide from the protein pleiotrophin, $PTN_{151-166}$, was discovered as a new candidate biomarker of AD. Pleiotrophin is expressed in the CNS and PNS specifically during embryonic development, but also in non-neural tissues, including lung, kidney, gut and bone [207]. While previously not implicated in AD, pleiotrophin is abundantly expressed in the adult hippocampus and can be induced by ischemic insults or neuronal damage in the entorhinal cortex, areas of high interest in AD since tau pathology typically develops there early in the disease process [208-210].

PTN mainly exerts function by binding to the receptors heparan sulfate proteoglycan N-syndecan and chondroitin sulfate proteoglycan receptor-type protein tyrosine phosphatase ζ (PTPRZ), and evidence suggest that the C-terminal region of the protein, the peptide identified as a possible AD biomarker in paper IV, is vital to maintaining stable interactions with these receptors [211-214]. As previously mentioned, evidence has indicated that tau pathology might propagate in a prion-like fashion and more specifically by interactions with heparan sulfate proteoglycans [186, 187]. Binding of PTN to PTPRZ is thought to promote clustering and by that inhibiting its function [215]. A possible hypothesis for the role of $PTN_{151-166}$ in AD could thus be that PTN binding to heparan sulfate proteoglycans such as PTPRZ is hampered by some unknown post translational processing of PTN where its active C-terminal region, i.e. $PTN_{151-166}$, is shed, increasing the concentration of $PTN_{151-166}$ in CSF and facilitating axonal tau pathology spread through a disrupted inhibition of PTPRZ.

Another known receptor of PTN, LDL receptor-related protein (LRP), is also a major receptor of APP and apoE, and has been genetically linked to AD [216, 217]. Evidence suggest PTN and midkine, another highly homologous protein, both bind and activate LRP, possibly by formation of a receptor complex [218]. LRP has several different functions and many of them are important in relation to AD pathology. Neurons need cholesterol to function and import cholesterol by apoE via LRP receptors. It has been proposed that decreased LRP leads to intracellular cholesterol deficiency, and studies have shown increased Aβ production correlate with cholesterol reduction [219]. LRP

expression is reduced as a part of normal aging, providing a possible link to age related increase in Aβ build-up [220]. Further, LRP is involved in increasing Aβ production via processing of APP, but also clearance of Aβ by endocytosis of complexes formed by Aβ, apoE and lactoferrin [221]. LRP, as well as PTN, can be found deposited in amyloid plaques in AD brains [222-224].

Further studies are needed to detail the true meaning of the $PTN_{151-166}$ finding in paper IV, but the study stands on its own as a tantalizing hint at the potential fruitfulness of further hypothesis generating studies.

Plasma and blood biomarkers

Blood is a more accessible source of biomarker information than CSF. Medical care professionals in some countries oppose the invasiveness and time consuming nature of the lumbar puncture and might be more willing to perform the less intrusive and simple procedure of taking a blood sample, which would accelerate the implementation of biomarkers in clinical practice. Blood biomarkers might serve an important role as a screening tool at primary care units, mainly to exclude patients with memory complaints but no signs of biochemical AD pathology from referral. A blood biomarker with high sensitivity would be ideal for this purpose, even with low or modest specificity. Patients that test positive would be referred to a memory clinic for further and more costly investigations, including CSF, PET, MRI and neurocognitive examination.

Blood is in less close proximity to the CNS by virtue of the blood-brain-barrier, to different degrees hindering potential biomarkers from diffusing into the blood stream. New technological advances in recent years have yielded ultrasensitive measurement techniques able to detect the by orders of magnitude lower concentrations of brain-specific proteins in blood [225]. Plasma concentrations of NfL have been shown to correlate well with CSF concentrations and predict cognitive decline, and might be particularly suitable as a measure of longitudinal disease progression in clinical trials, but also as a tool in AD diagnostics where a receiver operating characteristics (ROC) area under the curve (AUC) of 0.87 against healthy controls have been measured in the ADNI cohort [226-230]. A study of blood NfL in ADAD showed increased NfL levels before symptom onset and a correlation of NfL levels and time to symptom onset [126].

Plasma tau correlates with higher CSF tau and lower CSF $A\beta_{1-42}$, and has shown strong associations with AD in meta-analysis with average tau levels 1.95 times increased vs healthy controls [180]. A study of plasma tau in the ADNI cohort confirmed this result but showed a significant overlap between normal aging and AD [231]. Yet another study of plasma tau found a correlation of tau and cognitive decline independent of CSF Aβ, suggesting a non-disease specific neurodegeneration measuring property of plasma tau [232].

ELISA studies of plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ have shown both biomarkers to be unaltered in AD compared to healthy controls, while a single-molecule array (Simoa) study have shown both markers to be decreased in AD, as opposed to in CSF where $A\beta_{1-42}$ concentrations are

low, but $A\beta_{1-40}$ normal [233]. However, while study results have been conflicting, recent studies have shown that very high performance in predicting brain amyloid-β burden can be achieved using plasma $A\beta_{1-42}/A\beta_{1-40}$ ratios measured using mass spectrometry [234].

Aims

The general aim of this PhD project was to study and expand the understanding of the properties of known CSF biomarkers of AD and neurodegeneration across a wide array of neurodegenerative diseases, including the most prevalent dementia disorders.

The specific aim of study I was to study the prevalence of AD-like pathology in dementias besides AD, and the dynamics of the CSF biomarkers $A\beta_{1-42}$, T-tau and P-tau in relation to clinical outcomes of disease severity across dementia disorders. We hypothesized that the most clear AD-like biomarker pattern would be found in AD, but that biomarker levels in other dementias also carry similarities to AD.

The specific aim of study II was to study the potential of CSF NfL as a biomarker of on-going axonal degeneration, and its association with clinical outcomes of survival and cognitive measures in the major dementia disorders. We hypothesized that CSF NfL concentrations

would be particularly high in diseases characterized by white matter loss and that CSF NfL concentrations would predict survival.

The specific aim of study III was to evaluate the performance of T-tau and the T-tau/P-tau ratio in diagnosis of CJD. We further aimed to study the longitudinal dynamics of the CSF T-tau concentrations in CJD and relations to survival. We hypothesized that CSF T-tau levels would distinguish CJD from important differential diagnoses, and that CSF T-tau would predict survival.

The specific aim of study IV was to develop and test the feasibility of a new strategy of analyzing LC MS/MS data to generate hypotheses for new biomarkers in AD. We hypothesized that the new strategy of data analysis would be fertile grounds for biomarker discovery.

Methods

The first three studies included in this thesis compile data from clinical routine measurements of CSF biomarkers. These measurements were obtained by enzyme-linked immunosorbent assays (ELISAs) in clinical routine at the neurochemistry laboratory at the Mölndal site of the Sahlgrenska university hospital, which serves the whole of Sweden in CSF biomarker measurements. Paper IV explores the peptidome in AD in relation to MCI and healthy controls using mass spectrometry. The following chapter will provide a background to ELISA and mass spectrometry as the main techniques for biomarker measurements used in the production of the studies of this thesis.

ELISA

ELISA is a common technique used in both clinical and research settings for analyzing ligands, most commonly proteins, in liquids. It was developed in the 1970s and uses antibodies directed at the ligand to be

measured [235]. There are several types of ELISA tests where analytes and antibodies are used in different ways. In sandwich ELISA, which was used for all samples in the studies in this thesis, samples are introduced to a surface, usually the bottom of a well out of an array of wells on a plate, pre-coated with capture antibodies (figure 7). A second antibody directed at the ligand and conjugated with an enzyme, typically horseradish peroxidase, is added. The final step is to add a substance containing the enzyme's substrate. When horseradish peroxidase is used, this leads to a detectable and quantifiable change in color where enzyme-carrying antibodies are bound to ligands in turn bound to the antibody-coated surface. Other enzyme/substrate combinations may be used that employ other mechanisms for quantification such as spectrophotometry, where transmission of specific wavelengths of light is measured [236].

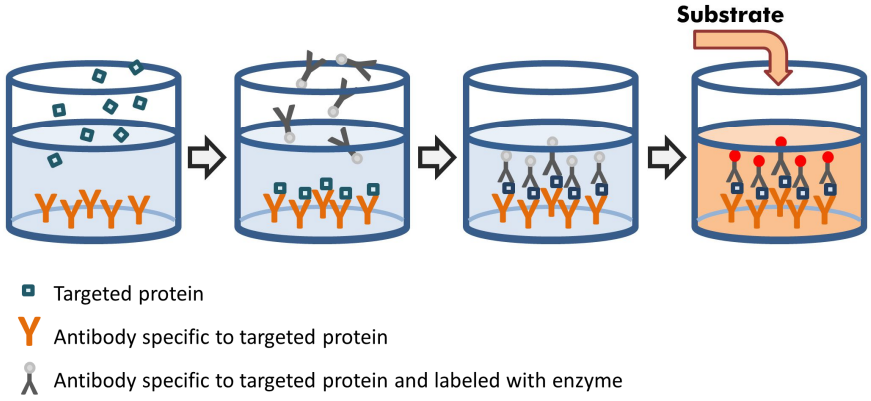


Figure 7. The principle of sandwich ELISA. The targeted protein binds to antibodies attached to the well. Enzyme labeled antibodies are then added, and bind to the targeted protein. The enzyme substrate is subsequently added and the amount of color change is recorded.

Mass spectrometry

Mass spectrometry (MS) is a technique to measure molecular mass that has found extensive use in biology and biomedicine for analysis of a broad range of biomolecules. In MS, the analyte molecules are transferred to the gas phase and ionized, after which electric and/or magnetic fields are used to separate them according to their mass-to-charge ratio (m/z). The first steps on the road leading up to modern mass spectrometry trace all the way back to the late 19th century, and several key advances in the development of the techniques have yielded Nobel prizes to their inventors [237-239]. Today, MS is used widely in many different fields to study physical, chemical or biological properties of molecules. It is used by governments and regulatory services to secure the cleanliness of air, the purity of water, quality of food and absence of contamination in medical agents [240]. Athletic oversight committees use MS in their monitoring of illegal substance use in athletes [28]. Miniaturized mass spectrometers have even been sent to Mars, Venus, Jupiter and Saturn on board the Viking, Pioneer, Galileo and Cassini landers for the purpose of analyzing planetary atmospheres and soil [241, 242].

The spectrometry process is divided into three key stages: Ionization, analysis and detection. These three stages can individually be accomplished in many different ways, and many different instrument designs exist to suit the analytical task and the properties of the analyte molecules. MS was long inapplicable to the analysis of polypeptides; the energy required to transfer these high-mass molecules to the gas phase

and ionize them led to their decomposition, prohibiting their mass analysis. That was changed by two major scientific breakthroughs in the field in the 1980s. The first was the discovery of matrix-assisted laser desorption/ionization (MALDI), an ionization technique where the crystallized analyte is mixed together with a matrix of relatively light-weight organic compound that strongly absorbs UV or IR light. The crystals are then irradiated by a pulsed laser (figure 8). The matrix molecules absorb the major part of the energy, leading to desorption of a portion of the sample, bringing along the analyte molecules to the gas phase while simultaneously protecting them from decomposition. The produced analyte ions are then transferred into the mass analyzer and subsequently to the detector [243, 244].

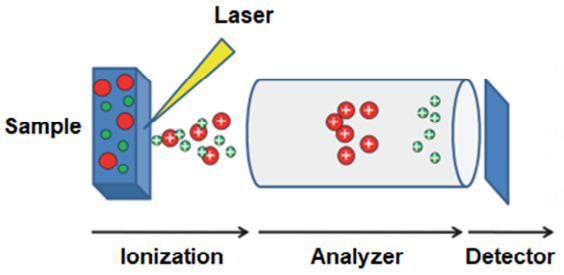


Figure 8. MALDI principle, courtesy of Carson Szot, Antony Croxatto, Guy Prod'hom, Gilbert Greub under CC BY-SA 4.0

The second breakthrough in biological MS was the invention of the electrospray injection (ESI), which was first described in the literature in by Yamashita and Fenn in 1984 [245]. Fenn was awarded the Nobel prize in 2002 for his contributions [237]. In ESI the liquid containing the analyte in aqueous/organic solvent is ejected through a thin needle positioned in front of the MS inlet. High voltage (2000-4000 V) applied between the needle and the MS inlet causes the electrospray process to occur, resulting in an aerosol of charged droplets being infused into the mass spectrometer. The solvent evaporates due to heat

being applied, sometimes assisted by a nitrogen gas flow, leaving the analyte bare, carrying the charges each containing droplet confined. Using MALDI or ESI large molecules, like proteins and peptides, could now be studied. In this thesis, hybrid quadrupole Orbitrap mass spectrometers (Q-Exactive and Tribrid Fusion from Thermo Scientific) were used for ESI-MS in the fourth paper.

Orbitrap

The Orbitrap mass analyzer was introduced in the early 21st century by Makarov et al. [246]. It provides unparalleled performance in resolution, mass accuracy, through-put and dynamic range and have set the standard for high-resolution mass spectrometers in proteomics [247]. The Orbitrap exploits the oscillational behavior of ions around a central electrode in an electrostatic field to obtain precise m/z values (figure 9). The behavior is reminiscent of planetary bodies getting trapped in gravitational wells. Ions injected into the Orbitrap oscillate coherently along the central electrode with a frequency that is proportional to the m/z of the ion. The oscillating ions induce an image current that is picked up by the outer

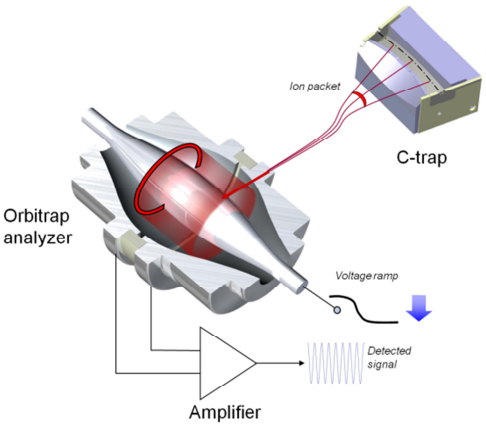


Figure 9. Schematic representation of an Orbitrap mass analyzer. Reprinted with permission from Thermo Scientific, copyright 2019.

electrodes. The detected wave signal is transformed using Fourier transformation from the time domain into the frequency domain, and after calibration into m/z scale. This analyzer affords very high, i.e. sub-ppm, mass accuracy [248].

Tandem mass spectrometry

Another key concept in proteomic MS is tandem mass spectrometry, also known as MS/MS or MS^2. While the general principle for MS is to measure the mass-to-charge ratio (m/z) of intact ions, tandem mass spectrometry involves a second isolation step of a selected ion and subsequent fragmentation of this ion. The m/z of the produced fragments is then measured, providing means for obtaining structural information on the analytes. The atomic bonds between amino acids in proteins and peptides vary in stability depending on the properties of the bound amino acids and the nearby chemical environment, resulting in partially predictable fragmentation characteristics [249]. Thus, the information obtained in a tandem mass spectrum can be used in several different ways. Databases exist, such as Mascot (Matrix Science) or SeQuest (Thermo), that facilitate the commonly used method of precursor ion fingerprinting. These databases list known precursor ion fingerprints, i.e. molecular weights and fragment patterns with corresponding source protein identity information. Detected m/z and fragment patterns are run against these databases to find their identities. Although efficient and well established, these databases cannot deliver

full coverage and identify all fragment spectra. However, peptides that are not recognized in database searches can still be identified. By exploiting the predictability of how amino acid sequences fragment in combination with the known molecular weights of the 20 amino acids coded for in the human genome, and possible fragment peptide weights calculated *in silico*, the peptide sequence of the fragmented peptide can be calculated. This process is called de-novo-sequencing and can be aided by software such as Peaks (Bioinformatics solutions Inc.). In short, de-novo-sequencing is carried out by measuring the distance (in m/z) between peaks in the MS^2 spectra, and matching the distances against amino acid weights, step by step constructing so called sequence tags and gradually working against revealing the identity of the peptide being sequenced [250].

Labeling techniques

MS spectra can only be used to reach relative quantifications of abundances of peptides in a sample. This prohibits evaluation of most biomarkers as they typically need to be assessed in relation to reference ranges and cut-offs, most commonly derived from examinations on patients vs healthy controls. Stable isotope labelling can be used to nominally quantify the concentration of a specific compound in a solution. A peptide of interest is selected and artificially synthesized with the addition of a heavy isotope label. A known quantity of the heavy isotope peptide is then added to the sample before MS^2 analysis. The

ratio of the surface area under the heavy and the light (or endogenous) isotope peak can then be used to quantify the concentration of the light compound in the sample.

In isobaric labeling by tandem mass tags (TMT), chemical labels designed to be identical in structure and molecular weight but vary in the distribution of heavy isotopes are introduced [251]. The labels react with primary amines in the samples to be analyzed, which can then be pooled together and analyzed in one run. When exposed to fragmentation energies the labels shed reporter ions that are unique in m/z, revealing the relative abundances of labeled peptides in the pooled samples. The isobaric labelling serves two purposes: it enables multiplexing, i.e. running analysis of several samples in one LC-MS run, and it improves the accuracy and robustness of quantitative MS by introducing a reference substance to relate intensities of measured ions to. In 10-plex TMT labeling, that was used in paper IV of this thesis, 10 samples are pre-prepared individually with one of the 10 TMT reagents, and subsequently pooled together before the LC-MS run.

Shotgun proteomics

Protocols for preparing protein samples for MS analysis vary widely depending on experimental goals and analytical methods to be applied. In hypothesis free experimentation, where the aim is to explore and map out a proteome as exhaustively as possible, shotgun proteomics is a common strategy. In shotgun proteomics proteins and peptides in

complex samples are first digested using a protease, most commonly trypsin, and the resulting peptides are then separated by liquid chromatography [252, 253]. By trypsination large proteins are truncated into smaller molecules that are easier to separate, ionize and fragment successfully. This approach allows for a wide scope of analysis, high through-put and good sensitivity.

Registries

Svedem — The Swedish dementia registry

Svedem is a national registry that was started 2007. It collects data on patients with dementia diagnoses in Sweden by collecting reports filled out be the physicians following the patients. All memory clinics and 77% of all primary care units in Sweden are connected to the Svedem network. Information on diagnosis, date of diagnosis, clinical characteristics of each patient, cognitive assessments and prescribed medications are recorded. [254]

The Swedish mortality registry

The Swedish mortality registry is a national registry managed by the Swedish national board of health and welfare. It provides data for the official statistical reports on rates and causes of death of Swedish

citizens. It was started in 1961 and is updated yearly [255]. All deceased citizens are registered with personal information and information on date, time, place and cause of death coded according to ICD. A total of 60 variables are recorded for each death.

Statistics

Where distributions of quantitative measures were significantly skewed, non-parametric methods or log-transformed data was used throughout the papers in this thesis. Group differences of averages, medians and categorical parameters were analyzed by ANCOVA, median regression, chi-square analysis, Mann-Whitney U and Kruskal-Wallis analysis. Linear and multiple regression models were fitted for association testing between continuous variables, and log-transformations were applied where appropriate. Association testing was also carried out using Spearman rank correlations and t-tests.

ROC analysis was used to evaluate biomarker profiles and diagnostic performance, including sensitivity, specificity and predictive values. Survival analysis was conducted using Cox regression.

In paper I clustering of data was performed using the SPSS TwoStep algorithm, a variant of K-means clustering, where a preceding step determines to optimal number (k) of clusters to put into a K-means clustering run.

Statistical analysis was carried out in SPSS (IBM, Armonk, New York) and Stata (StataCorp, College Station, TX).

Ethics

Clinical data on patients gathered from Svedem was used in paper I-III of this thesis. All patients in Svedem were informed about their participation in the registry, the potential use of their submitted data for research, and had the right to decline participation. Biomarker data was fetched from the clinical routine lab database at the Sahlgrenska university hospital, Sweden. Paper I-III of this thesis was approved by the regional ethical committee at the University of Gothenburg (dnr: 752-12).

In paper IV, CSF and clinical data from 120 patients from the Amsterdam Dementia Cohort was used for biomarker discovery. All subjects gave written consent for usage of their samples and clinical data for research purposes, and the study was approved by the local Medical Ethics Committee at VU University Medical Center, Amsterdam. Biomarker validation was performed in CSF samples assembled from the BioFINDER study at Skåne University hospital, Sweden. The study was approved by the Regional Ethics Committee in Lund, Sweden, and the patients and/or their relatives gave their informed consent for research.

Results

Paper I — The core CSF AD biomarkers in the dementia spectrum

Paper I explores the core CSF biomarkers of AD, T-tau, P-tau and $A\beta_{1-42}$ in all of the most prevalent dementia diagnoses. While the biomarkers in this study reflect different neuropathological aspects of AD, there is known to be a large overlap of these pathologies as well as clinical phenotypes in other dementias. By cross-referencing the lab database at the Sahlgrenska university hospital with Svedem, an in this context unparalleled amount of study subjects could be garnered (n = 5676).

We found, as expected, the most clear AD-like biomarker pattern in patients with clinically diagnosed AD. However, large shares of

patients with other clinical dementia diagnoses also exhibited a biomarker pattern indicating possible concomitant AD pathology. Pathologic $A\beta_{1-42}$ concentrations were detected in more than 50% of VaD, DLB and PDD patients. This is consistent with previous findings, including post-mortem neuropathological examinations, and further demonstrates evidence of the widespread prevalence of AD-like disease processes in other dementia disorders [256, 257]. Evidence of tau-pathology was less widespread than that of Aβ-pathology but still 45% of VaD, 44% of FTD, 32% of DLB and 29% of PDD patients had pathological levels of either T-tau or P-tau. This could indicate tau-pathology, but could also be attributed to tau-leakage due to general neurodegeneration or normal variability in CSF tau concentrations.

Using cluster analysis, we were able to identify a natural classification of patients with regards to their AD biomarker CSF concentrations. Nearly half of the patients sorted into a cluster characterized by pathological AD biomarkers, indicating ongoing AD pathology. This cluster was dominated by the clinically diagnosed AD groups, while the other cluster contained a majority of the other dementias, corroborating the connection between the hallmark AD biomarker profile and the clinical phenotype of AD.

The large number of study subjects in this study provided enough power to detect small variations in cognitive performance in relation to biomarker concentrations, an aspect previously relatively unexplored. In late onset AD (LAD), but not in the other diagnoses, negative trends of lower MMSE scores were correlated to higher CSF

concentrations of both T-tau and P-tau, and also lower concentrations of CSF $A\beta_{1-42}$.

Paper II — CSF NfL and clinical outcomes in dementia

Neurofilament light has been described as a biomarker of general neurodegeneration, specifically reflecting damage to white matter structures, rich in myelinated axons where NfL is abundant [258]. Paper II aimed to test this characterization by comparing CSF NfL concentrations in the most common dementia disorders. As in study I, clinical routine measurements of NfL from the Sahlgrenska University hospital was collected and clinical information on all subjects was brought in from Svedem. However, in this study mortality information from the Swedish mortality registry was also linked in. The resulting dataset contained 3356 individuals in 10 different diagnostic groups (early onset AD [EAD], LAD, FTD, DLB, VaD, PDD, mixed AD/vascular dementia, dementia not otherwise specified, other dementias and healthy controls), whereof 478 had a registered date of death.

The highest CSF NfL concentrations were found in FTD, VaD and mixed AD and vascular dementia. This is in keeping with the idea of NfL as a biomarker of white matter loss as these diseases all cause damage to regions of the brain rich in myelinated axons. EAD had low

concentrations in parity with healthy controls, while LAD patients had higher concentrations. The explanation for this could be that EAD patients are known to exhibit more clear AD pathology, while LAD patients often exhibit concomitant pathologies and vascular components.

In LAD and mixed AD and vascular dementia we could also identify correlations between high CSF NfL concentrations and disease progress with MMSE scores as a proxy, which also ties into the role of NfL as a correlate for ongoing neurodegeneration. The MMSE test is designed to specifically measure hippocampal function, which might explain the lack of correlation in non-AD-dementias. We could however detect a universal association of shorter survival time and higher NfL CSF concentrations. This was true in both patients with AD-like and non-AD-like biomarker patterns of $A\beta_{1-42}$, T-tau and P-tau. These properties solidify the role of CSF NfL as a biomarker of general rate of neurodegeneration, and not a biomarker reflecting a disease specific pathologic mechanism or process. It also highlights the suitability of CSF NfL as an outcome marker in clinical trials of drugs aiming to limit or stop neurodegeneration, not only in AD but across the dementia spectrum.

Paper III — CSF Tau in CJD

Creutzfeldt-Jakob disease is the most aggressive neurodegenerative disease known, with time of survival from diagnosis seldom surpassing a year. Despite the fast progression, it can often be hard to clinically diagnose CJD and post-mortem analysis of the brain is still the gold standard for a definitive diagnosis. CJD further stands out from the crowd of neurodegenerative diseases in that it is transmittable, and by that important to correctly identify. CSF T-tau concentrations have in previous studies been shown to be markedly elevated in CJD [259-261]. In study III we gathered clinical routine CSF T-tau and P-tau measurements from the lab database at the Sahlgrenska university hospital and brought in clinical and mortality data from the Swedish mortality registry. Information on 9765 individual patients was collected, including 93 with CJD.

We could confirm that CJD patients exhibit considerably higher concentrations of CSF T-tau, as the CJD patients in our cohort had both mean and median levels of T-tau at more than 10-15 times those of non-CJD patients. However, P-tau levels were not elevated in the CJD patients, corroborating the specificity of P-tau for AD pathology.

Using the T-tau concentrations and T-tau/P-tau ratios of the patients in the study cohort, ROC analysis could discriminate CJD patients from controls and patients with AD and other dementias with very high performance (AUCs ranged from 0.949 - 0.984). The ROC analysis was calculated using the first biomarker measurement for those of the included patients with consecutive measurements. When relating T-tau concentrations to time of survival in our cohort a clear trend of rapidly rising T-tau concentrations closer to date of death could be seen (figure 10). On further inspection, the same phenomenon could be seen in the sub-sample of patients in our cohort with repeated CSF T-tau measurements. In this group an exponential increase in T-tau concentrations could be observed as time to death diminished (figure 11). This trend could not be observed in patients with AD or other dementias where both T-tau and P-tau concentrations remained stable in relation to survival.

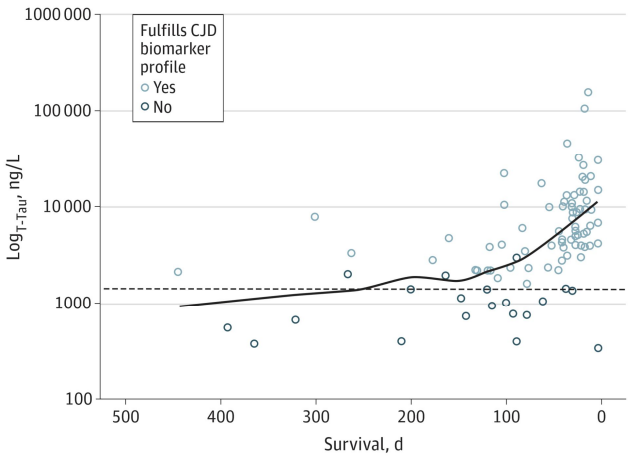


Figure 10. T-tau concentrations exponentially increase as date of death approaches.

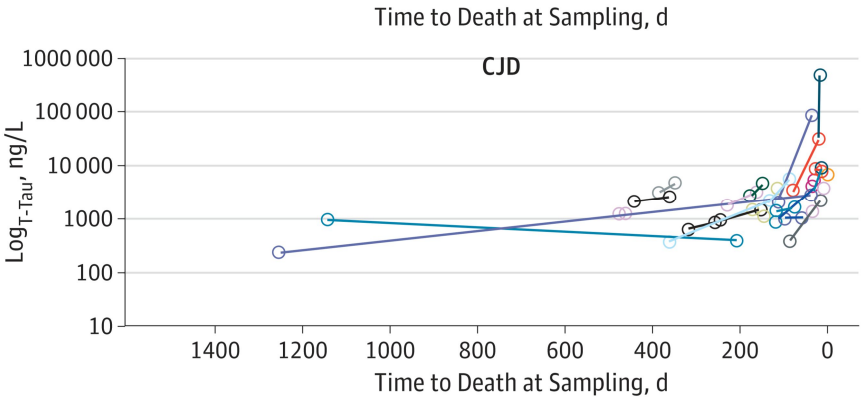


Figure 11. The longitudinal measurements of CSF T-tau in CJD reveal an exponential increase as date of death approaches.

This study demonstrates the diagnostic power of T-tau and the T-tau/P-tau ratio in CJD. It also highlights the violent nature of CJD through the unparalleled rise in T-tau concentrations as the disease progresses. This property has not been demonstrated through longitudinal data before and is most probably unique to CJD pathology, and might be utilized in the diagnostic process. A suspected but not verified case of CJD could or should be examined again to check for increased T-tau concentrations strengthening the diagnostic assessment.

Paper IV — Hypothesis generation with clustering in peptidomics and identification of $PTN_{151-166}$ as a biomarker of AD.

In paper IV we set aside prevailing paradigms in AD pathology theory, setting out to find new biomarker prospects and generate new hypotheses to hopefully further the field and deepen the understanding of AD pathology. To achieve this we developed a new way of analyzing the vast amount of data generated in TMT LC-MS/MS analysis of CSF samples. By applying this new strategy of analysis, we were able to mine valuable information from sections of data that would be discarded in conventional analysis work flows.

To organize and make sense of the massive data output from TMT LC-MS/MS analysis, the generated data, basically consisting of gigabytes of lists of detected peptides, their precursor m/z and the peptide spectrum produced at fragmentation, is matched against protein sequence databases of known peptides and accompanying fragment spectra. Identification is achieved by utilizing the combination of the precursor m/z and the fragment spectra as a unique fingerprint and key to identification [262]. These databases work well in identifying tryptic peptides, where about 90% of detected peptides can usually be identified.

However, the databases are far less complete when it comes to endogenous peptides. In an average endogenous peptide dataset about 20-30% of peptides are identified. Another limitation of trypsination is that the enzyme digestion discards valuable information. Trypsin cleaves peptide chains at lysine or arginine, thus proteins and peptides rich in those amino acids might get chopped up into parts too small for identification, permanently obscuring parts of the proteome [263]. Further, the proteome is accompanied by a peptidome, i.e. naturally occurring protein fragments that are part biologically inactive traces of degradation of proteins, but also contain bioactive species that interact with receptors, transmitting, modulating or counteracting responses [264, 265]. After trypsination, information on naturally occurring peptides and forms of proteins is partly lost, since it's not always possible to determine if a peptide is naturally present in the peptidome or the result of enzymatic cleavage by trypsin. The peptidome is important. Examples of important bioactive endogenous peptides include:

- **Substance P**, a neuropeptide that interacts with the neurokinin 1 receptor and mediates vasodilation, inflammation and pain [266].
- **Angiotensin I and II**, peptide hormones that are involved in vasoconstriction and increased blood pressure through the renin-angiotensin system [267].
- **Neuropeptide Y**, a neuropeptide with several functions that is active both in the CNS and the peripheral nervous system. It is considered stress-relieving, anxiolytic and neuroprotective [268].

There is also value in exploring the biologically inactive remains of protein degradation, as they might be traces and biomarkers of important upstream processes. A prominent example of this is $A\beta_{1-42}$ [269]. Exploration of the peptidome is prohibited by pre-analytical digestion as there is no way of discerning what peptides have been cut by artificial means or not.

In paper IV, we aimed to circumvent the limitations of trypsination and database searching and rearranged the work flow, skipping the database search and replacing it with a spectral clustering step. The spectral clustering uses an algorithm to sift through the data, clustering together spectra based on precursor m/z, charge state and fragment ion patterns [270]. The relative abundance of the peptides in the resulting clusters can then be mapped against individual patient samples by means of the TMT reporter ions. The resulting array of clusters can thus be used to identify potential biomarkers by quantifying the relative concentrations of the clustered peptide in healthy controls vs disease groups, and evaluating their performance in separating the groups of interest. When a biomarker candidate is identified, it is then a matter of manual labor to identify the peptide sequence. By this strategy we managed to tap into the unexplored realms of the endogenous peptidome in a discovery cohort consisting of 40 healthy controls, 40 MCI patients and 40 patients with AD from the Amsterdam Dementia Cohort [271].

The clustering algorithm generated a list of 220,869 clusters. The clusters where a quantifiable signal was detected for more than half of the patients in the study were selected and then subjected to individual

ROC analysis. The AUCs of each cluster were then used to rank all clusters according to their performance in separating the AD patients from the healthy controls in our cohort. The top 20 clusters are detailed in table 1.

Table 1. The top twenty clusters in the discovery set, ranked from their ability to separate patients with AD from cognitively normal controls using ROC analysis.

Column descriptions from left to right: Cluster # (identifier); precursor m/z; detected charge-state; the number of study subjects in which the cluster was quantified; the relative median difference in abundance between AD patients and cognitively healthy controls; calculated AUC (from ROC analysis); indication of successful identification of peptide sequence; the identified peptides' protein of origin where applicable.

Cluster #	m/z	Charge	Subjects (n)	Rel. diff. AD vs HC	AUC - HC vs AD	Peptide identified	Protein affiliation
7367	*794.115*	*5*	*119*	*215%***	*0.96**	-	
69078	*664.799*	*2*	*94*	*18%***	*0.92**	-	
32527	*777.426*	*3*	*68*	*13%***	*0.92**	-	
78065	*748.382*	*2*	*120*	*38%***	*0.91**	√	*Osteopontin*
4243	*696.457*	*5*	*59*	*103%**	*0.91**	-	
82223	*794.885*	*2*	*111*	*28%***	*0.90**	√	*Clusterin*
34935	*810.044*	*3*	*58*	*16%***	*0.9**	-	
9084	*836.597*	*5*	*86*	*47%***	*0.87**	-	
36033	*823.45*	*3*	*120*	*18%***	*0.87**	√	*ApoE*
61266	*1380.71*	*3*	*70*	*-20%***	*0.86**	-	
1889	*427.579*	*3*	*120*	*15%***	*0.86**	-	
33238	*787.612*	*3*	*69*	*19%***	*0.85**	-	
25327	*684.044*	*3*	*60*	*9%***	*0.85**	-	
13290	*840.651*	*4*	*111*	*17%***	*0.85**	√	*Secretogranin1*
10464	*786.901*	*4*	*59*	*22%***	*0.85*	-	
71623	*685.336*	*2*	*61*	*13%***	*0.83**	-	
12243	*911.104*	*5*	*70*	*-28%***	*0.82*	-	
87816	*848.511*	*2*	*69*	*16%***	*0.8**	-	
6405	*690.794*	*4*	*60*	*13%**	*0.79**	-	
53204	*1088.54*	*3*	*60*	*4%**	*0.79**	-	

*** Indicates statistical significance ($p < .001$)*

** Indicates statistical significance ($p < .05$)*

Cluster 7376 exhibited promising qualities as a biomarker candidate with an AUC of 0.96, i.e. a near perfect discrimination of AD

patients and controls, and a relative median abundance difference of 215% between the same groups. On further inspection the peptide revealed more interesting properties. The relative abundance of cluster 7376 in the MCI patients was at intermediate levels between the controls and the AD patients, further indicating a relation to AD pathology. Further, the MCI patients who at follow-up had progressed to AD (MCI-AD) had high abundances, reaching for the same as the AD patients, while the patients who remained at the MCI-stage at follow-up (MCI-S) had abundances comparable to the healthy controls (figure 12). This is a sought after feature in an AD biomarker as being able to distinguish the MCI-S from the MCI-AD patients is important but can be clinically challenging. Even further, the MCI patients who progressed to other dementia disorders exhibited low abundances of the cluster 7376 peptide, with a mean level 35% higher than the healthy controls and 50% lower than the MCI patients that progressed to AD. Although the number of patients in this group was very limited (n=4), this hints at a specificity for detecting AD pathology. Another positive finding when studying the details in the properties of

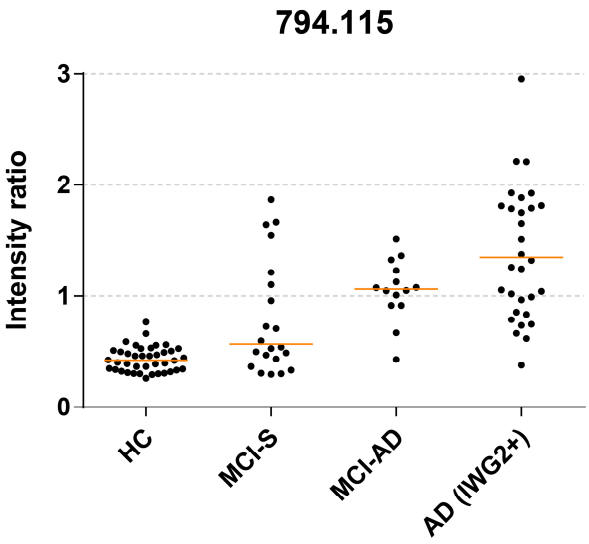


Figure 12. Scatter plot of the relative abundances of cluster 7376 in the sub groups of the study cohort.

cluster 7376 was that the few MCI-S patients who had high abundances of the cluster 7376 peptide tended to be amyloid positive, indicating a high risk of developing AD [272]. All in all, cluster 7376 exhibited ideal properties for an AD biomarker and was selected as the first candidate for further analysis.

The identity of the cluster 7376 peptide proved elusive. The sleuth-like process of deconvoluting the peptide sequence from a fragment pattern and the m/z, de novo sequencing, requires input in the form of an as rich fragment pattern as possible [273]. The original fragment pattern of cluster 7376 revealed little information, and when increasing the collision energy to crack more peptide bonds, the whole peptide seemed to obliterate, leaving no traces to aid the identification process. The key eventually turned out to be to switch fragmentation method. The Thermo Fusion mass spectrometer used in this study allows for not only the standard higher-collisional energy dissociation (HCD) fragmentation technique, but also electron transfer dissociation (ETD) fragmentation that is particularly well suited for fragmenting peptides with charge states >2 (the cluster 7376 peptide had a charge state of 5). The peptide sequence of cluster 7376 was revealed as AESKKKKKEGKKQEKM, and identified as amino acids 151-166 from the sequence of the protein pleiotrophin (PTN). PTN is described in the literature as being abundant in the hippocampus and entorhinal cortex, but with no specific link to AD [208, 209]. PTN is covered in detail in the CSF biomarkers chapter of this thesis.

After candidate selection and identification, PTN was validated in an independent secondary patient cohort, consisting of 15 healthy controls and 15 of each of AD, PD and PSP patients. A targeted Orbitrap parallel reaction monitoring (PRM) approach was used for analysis. The AD patients were verified as having a higher abundance of $PTN_{151-165}$ compared to controls, and the specificity for AD-pathology was further corroborated as the PD and PSP patients were indistinguishable from the healthy controls (figure 13).

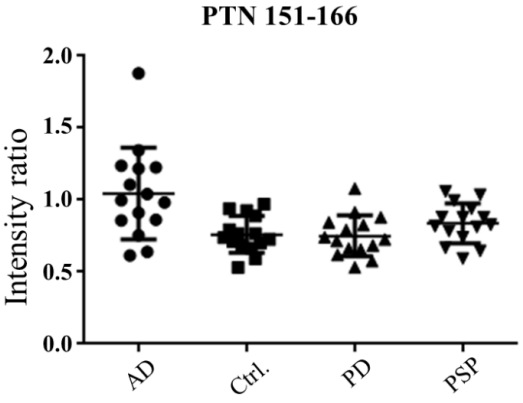


Figure 13. Scatter plot of the relative abundances of PTN151-166 in the validation cohort-

This study serves as a proof-of-concept of the utility of the novel spectral clustering work-flow as a hypothesis generating machine. It should be noted that the clustering algorithm used in this study was not specifically designed for this task and could likely be improved to better performance. It should further be noted that out of the twenty top biomarker candidates that emerged in the discovery cohort, only four were readily identifiable. Several tantalizing candidates still remain to be processed. For instance, cluster 4243 had an AUC of .91 and a relative abundance median difference of 103%. The identity of this peptide still remains to be discovered. The developed spectral clustering work-flow has yet to be applied to one large cohort, but is universally applicable to MS/MS data and has tremendous potential to reveal further secrets in

other disease groups, fluids (plasma? saliva? urine?) and in variations of pre analytical processing of samples and mass spectrometer settings.

$PTN_{151-166}$ is a promising biomarker candidate. The PRM method used in the validation cohort is not ideal for systematic $PTN_{151-166}$ assessment, but the development of a targeted assay has not yet succeeded. The unusual nature of $PTN_{151-166}$ in terms of extreme hydrophobicity and charge to mass ratio has proven hard to overcome hurdles in the method development. However, a targeted method will likely improve the diagnostic performance of $PTN_{151-166}$. When a targeted method is finalized, the doors are open for further studies to proceed in characterizing the relation and specificity of $PTN_{151-166}$ to AD-pathology, its potential in AD staging, and its relation to other biomarkers of AD and neurodegeneration. Hopefully, apart from providing clues to the inner workings of AD pathology, $PTN_{151-166}$ can add another tool to the AD biomarker toolbox complementary to $A\beta_{1-42}$, T-tau, P-tau, Ng and NfL. Potential functions of $PTN_{151-166}$ that need to be examined and that $PTN_{151-166}$ might add include earlier, more specific and more reliable diagnostics, sub classification of AD pathology or dependable assessment of rate of on-going pathology.

Discussion

The growing threat of dementia to global health has motivated extraordinary efforts to be put into research to understand the many facets of neurodegeneration and to find effective treatments. Despite these efforts several key issues remain to be resolved and no disease modifying treatments have been found. CSF biomarkers have several different applications in dementia research and can in several different ways be used to forward the field. The papers included in this thesis highlight several of them.

The results in paper I validate the value of the core AD biomarkers, $A\beta_{1-42}$, T-tau and P-tau, in discriminating AD from other dementias. It also demonstrates the lack of clear clinical and pathological syndromes in dementia in the large amount of overlap and spread in biomarker concentrations between the clinical diagnoses, indicating and corroborating what many previous studies have shown, i.e. that presence of concomitant AD-like pathology is common in other dementias. The inverse is also true in many cases. It might even in some settings be more

appropriate to consider dementia as a spectrum of clinical phenotypes exhibiting symptoms of degeneration stemming from a set of pathological concomitant and often related processes. There is an important difference in the clinical phenotype and the neuropathological correlate. The complex relationships between clinical presentations and immunohistochemical classifications in FTLD and FTD demonstrate this. FTD patients often suffer from frontotemporal tau or TDP-43 pathology, but TDP-43 aggregates can also be seen in MND, although in different anatomical regions. FTD is in itself an array of similar disorders with different clinical presentations dependent on varying anatomical focal points of neurodegeneration and varying degrees of influences of tau and TDP-43 aggregation. Tau pathology is also present in AD, but in different anatomical regions of the brain, and with different influence of tau isoforms. And further, TDP-43 pathology is present in as many as 40% of AD patients, but in the hippocampus and the entorhinal cortex, as opposed to in the frontal regions in FTD. Aβ plaque accumulation is a hallmark feature of AD but can also be seen in abundance in DLB, a disorder that is recognized neuropathologically by a build-up of α-synuclein containing Lewy bodies in the cortex and substantia nigra. And inversely, Lewy bodies are found in more than half of the brains of AD patients' post-mortem. Further, DLB doesn't differ from PDD in neuropathological terms, but are only distinguished in clinical presentations where parkinsonian symptoms precede dementia in PDD while the order is reversed in DLB. Effects of vascular pathology is also very common in the elderly and might in addition to the mentioned disease processes further color the clinical presentation and affect the neurodegenerative processes. A drug trial might thus benefit from

assessing not only signs of the particular disease that it is aimed to treat, but also amounts of concomitant neuropathological processes that might influence the clinical phenotype and outcome measures of the trial. Treatments could be considered to target pathological processes rather that dementia syndromes. Awareness of the intertwined nature of the pathological processes in dementia should also be taken into account in studies aiming to explore the underlying causes of neurodegenerative diseases. Well-characterized biomarkers could help identify and quantify influences of different pathological processes in a patient and to tailor future treatments based on that information.

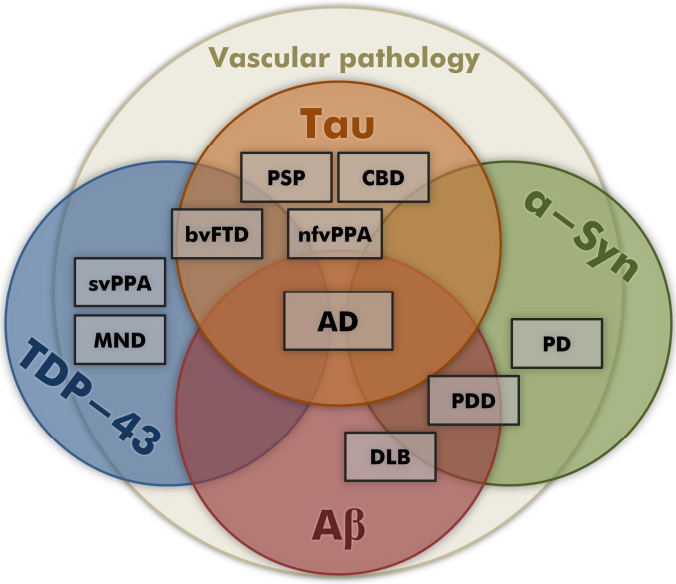


Figure 14. Venn diagram of related pathologies in Dementia.

Well characterized biomarkers play important roles in drug trials, where a prerequisite to produce dependable results is to enroll well suited subjects. There are several challenges in this task. Timing is of the essence as the neurodegenerative aspects of AD and other dementia

disorders lead to neuronal damage that is likely irreversible, making it urgent to stop disease processes before damage sufficient to render clinical symptoms have occurred. CSF biomarkers can help identify subjects in the subclinical stages of disease that are more likely to be eligible for treatment. Having pathological concentrations of the biomarkers covered in paper I, i.e. CSF $A\beta_{1-42}$, T-tau or P-tau, while at the MCI stage has been shown to be associated with a heightened risk of developing AD [274]. CSF NfL concentrations have also been shown to predict a more rapid decline from MCI at baseline into dementia [275]. CSF concentrations of PTN, the candidate biomarker identified in paper IV, were markedly elevated in AD, but also in MCI patients that on follow-up progressed to AD and in MCI patients that on follow-up had not yet progressed but were Aβ-positive, i.e. likely to progress to AD at a later point in time [276]. These properties would be ideal to help identify MCI patients likely to progress to AD, but need to be validated in further studies.

Another reason for the importance of timely administration of treatment could be that hindering an upstream event might be necessary to be able to stop the disease progression in dementia, again highlighting the need for early biomarkers. In CJD, prions propagate their destructive properties from cell to cell in an ever multiplying fashion. Neurodegeneration escalates at an exponential rate as demonstrated in paper III by the marked increases in T-tau as the affected patient approach death. A single unfortunate event in the misfolding of a PrP-protein might be sufficient to spark this process, but a single misfolded PrP-protein left behind after a nearly complete PrP^{Sc}-eradication by a

fictional future drug might also re-ignite it. As previously discussed, evidence suggests that tau and Aβ-pathology might also propagate in a prion-like fashion. This might be one of the reasons drug trials in AD in humans have thus far been futile.

Another important aspect of successful drug trials and feature of well-researched biomarkers is to properly assess the effects of the given treatment. In dementia, stopping neurodegenerative processes is a core focus and means to measure the dynamics of these processes is needed to set up a primary outcome. In paper II, CSF NfL was shown to be increased in several dementias and to be correlated to cognitive performance and survival time. Several other studies have also provided evidence for CSF NfL as a measure of on-going neurodegeneration, particularly in subcortical regions of the brain [258]. These properties make NfL a suitable primary outcome measure in drug trials in neurodegenerative disorders, including AD, vascular dementia and FTD. In paper III, the CSF T-tau concentrations in CJD patients were observed to increase with disease burden and in relation to survival. T-tau could thus be a suitable candidate marker for the monitoring of disease modifying treatments in CJD.

The lack of positive results in AD drug trials indicates that the amyloid cascade hypothesis has limitations. The BACE1-inhibitor Verubecestat was developed by Merck and showed promising results in phase 1/2 studies with CSF Aβ concentrations in treated patients reduced by as much as 90 %, and no serious adverse effects. However, the following phase 2/3 EPOCH study had to be aborted in February of 2018 when it was discovered in an interim analysis that treated patients

performed worse than the placebo group in CDR-SB and ADAS-Cog13 [277]. Similar results came of the ELAN/Wyeth active vaccine trial where plaque removal was found at autopsy, despite continued clinical cognitive decline [278]. It might be that some unknown event or series of events lead up to the evolution of a self-replicating disease process, that can withstand targeting by, for instance, BACE1-inhibitors by no longer being dependent on $A\beta_{1-42}$ shedding to advance. Another explanation for the failure of Aβ-targeting drugs could be that Aβ-deposition is not the culprit in AD pathogenesis but merely a side-effect of other processes that cause neurodegeneration, or that Aβ is a physiological response to some other unknown pathological process [279]. Transgenic mice engineered to produce excess amounts of Aβ not generated from APP form Aβ plaques but exhibit no cognitive decline [280]. The Arctic familial mutation in *APP* lead to ADAD through more aggregation prone Aβ but does not show amyloid on PET, although diffuse plaques are present histopathologically, pointing to other forms of Aβ such as oligomers being important for the pathological processes in AD [281]. It has been pointed out that $A\beta_{1-42}$ production always generate a complementary APP-product, the APP intracellular domain (AICD). Being intracellular, the AICD is better situated to instigate cell damage than Aβ plaques [282]. The AICD has, however, not yet been thoroughly investigated. Aβ, or some form of Aβ, might even be a protective agent, which would explain why the Verubecestat treated patients had a faster rate of progression than the placebo group. In any case, this highlights the need to further detail the pathological mechanisms in AD and dementia in general.

To deepen the understanding of the molecular processes leading up to the pathologies present in dementia, innovative and explorative studies are needed. Efficient hypothesis generation and testing, as demonstrated in paper IV of this thesis, might be an important tool to uncover missing links, unravel the complex biochemical pathways in dementia and to guide further studies into uncharted territory. In paper IV, a vast amount of peptides were found and tested for their properties as biomarkers of AD. It should be noted that only five of those promising biomarkers were sequenced and one chosen for further processing and validation. 15 more biomarkers that all separated AD from controls with an AUC of >0.75 and with p-values < .05 were left untouched. We recognize the problem of multiple testing in studies like this, but through further validation the false positives would readily be discovered. Further effort put into automatization and fine-tuning of the clustering, as well as the selection and identification processes would likely maximize output and limit the amount of manual labor needed to run further studies applying the spectral clustering work-flow described in paper IV.

The neurodegeneration biomarker toolbox

The results of paper I-III of this thesis demonstrate the power of carefully mapping out the properties of biomarkers across and between disorders and stages of disease. By rigorous characterization of biomarkers by the many research groups in the field, a biomarker toolbox has been created, containing gear to address a plethora of important questions that arise in relation to neurodegeneration in both

the clinical and research settings. $A\beta_{1-42}$, T-tau and P-tau, can be used to identify AD pathology even at very early and preclinical stages of disease, providing insights into the pathological processes underlying the disease (figure 14). Being able to reliably identify the most common dementia disorder and discriminate it from important differential diagnoses aids the diagnostic process of dementia. CSF $A\beta_{1-42}$, t-tau and p-tau might further be used to assess the efficacy of potential treatments of AD.

As demonstrated in paper III, T-tau, but not P-tau, is also a biomarker of CJD where even higher concentrations than in AD can be seen. A pattern of ever increasing concentrations is also a hallmark CJD sign, not seen in other diseases.

NfL can be used to assess rate of ongoing subcortical neurodegeneration across several dementias, and might be considered a biomarker of general neurodegeneration. It is also associated with survival, in that higher concentrations are linked to shorter life expectancy in AD, but also other dementias. Particularly high CSF NfL concentrations are seen in FTD that engage the frontal cortex, which is rich in myelinated axons, and can be used to strengthen the case for FTD in differential diagnostic inquiries.

$PTN_{151-166}$, the new biomarker prospect identified in paper IV, is naturally a lot less well characterized than the other biomarkers described in this thesis. However, it has showed promising properties so far. The MCI patients in the discovery set had higher levels of $PTN_{151-166}$ than the healthy controls, and at follow-up, the MCI patients with higher concentrations at baseline had more often progressed to AD than those

with low concentrations. As previously discussed, this is a central feature of any AD biomarker. Further studies are needed to more carefully establish at what stage of disease concentrations of $PTN_{151-166}$ might start to change, and to relate these changes to detectable changes in other biomarkers and cognition, i.e. to fit it into the Jack-curve (figure 15). Further studies are also needed to identify the pathological significance of $PTN_{151-166}$ to AD pathology, and its relation to disease processes in other diseases.

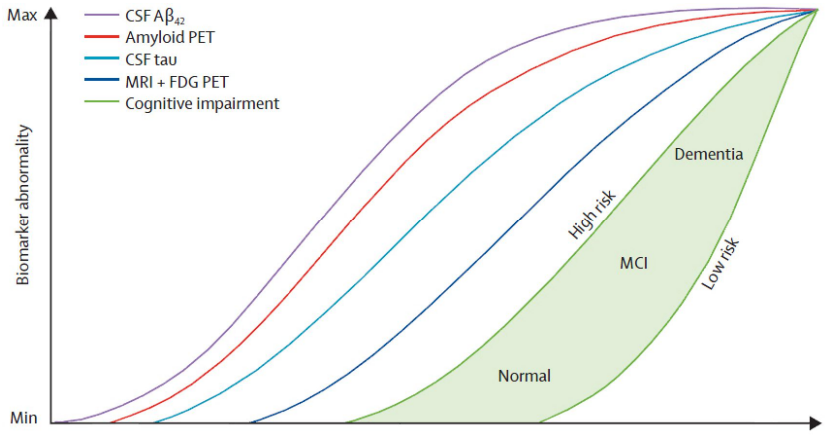


Figure 15. The Jack-curve. A popular model of the order of events in AD. Image courtesy of Clifford Jack and Lancet Neurology.

These are only the CSF biomarkers covered in this thesis. Many other imaging and fluid biomarkers exist that all add utility to the biomarker toolbox. However, and as previously discussed, the need to further expand this toolbox is still high. No cure for any dementia yet exists, and proper equipment to tackle the task of finding one is in demand.

Concluding remarks and outlook

The findings presented in the studies of this thesis demonstrate the value of CSF biomarkers from several aspects. The diagnostic value of CSF biomarkers is shown in paper I, III and IV where AD and CJD were discriminated from healthy controls and important differential diagnoses with high accuracy. The prognostic value of CSF biomarkers were displayed in paper I, II, III and IV where clinical outcomes measured by conversion from MCI to AD, cognitive test scoring and time of survival where predicted. The investigative value of a CSF biomarker was exhibited in paper IV, where a peptide was shown to have a previously unknown association with AD pathology, leaving further investigation into the implications of this relationship to be addressed by further studies.

The hunt for disease modifying treatments of neurodegenerative diseases is on. While no clinical trials have been fruitful in recent years, many are sure still to come. All major experimental drugs tested in these clinical trials have been developed in models of ADAD, i.e. not in SAD, the most prominent and common type of the disease, and not the one that these drugs have been subjected to treat. This design flaw will

probably have to be remedied in future trials. Biomarkers will continue to aid these trials in patient recruitment and assessment of effectiveness. Hopefully, successful trials will soon yield effective treatments, and when that goal is reached, biomarkers will likely be needed to continuously identify patients eligible for treatment. Future studies into blood biomarkers will likely widen the scope of use of biomarkers in neurodegeneration.

Some tools are still missing in the biomarker toolbox. No effective biomarkers of PD or PDD exist today. A way of assessing α-synuclein pathology would aid a difficult diagnostic process, which is today based on the clinical features of the patient, and would benefit patients, doctors and researchers alike. The same would be true of a biomarker of TDP-43 pathology in FTD, and different types of tau pathology in tauopathies.

The continued failure of drugs targeting the usual suspects as stated by the amyloid cascade hypothesis emphasizes the value in keeping an open mind to revising the model and to find new drug targets. Explorative studies like the one in paper IV might aid this process. Most certainly, there are still secrets to unveil that will shed light onto the inner workings of the pathologic processes in AD.

Acknowledgements

Thank you, Henrik Zetterberg, for being my main supervisor. You are a true inspiration and, for me, one of the biggest enigmas in AD. How can such an unmeasurable quantity of competence, positivity, humor and pure likability be fitted into one man? Further studies are needed to reveal the underlying causes of this syndrome, that if harnessed would surely solve many of the world's problems.

Thank you, Niklas Mattsson, for being my co-supervisor and for meeting me at a seminar at the BF2 course and mistakenly believing my knowledge in statistics was far greater than in reality, and thank you for not outing me when discovering otherwise. Thank you for all your efforts into correcting my many errors in manuscript writing and data analysis, and by that (sometimes painfully) hammering out the skillset I proudly have acquired during my time as a PhD student.

Thank you, Kaj Blennow, the phenomenal scientist and the patriarch of the lab, for being my co-supervisor. Thank you for letting me be part of the well-oiled machinery that is the internationally renowned Blennow lab for a while, and thank you for lending an ear to my uninitiated questions on countless occasions over these years.

Thank you, Johan Gobom, for being my mirror image in fascination of the unknown, nerdy, funny and generally cool, including, but not limited to, astronomy, physics, engrish, sci-fi, bad design, the singularity etc., etc.. Thank you for letting me participate in your exploration of the endogenous human peptidome and probably the most novel-worthy and exciting period of my professional career, the summer of 2014, when we applied clustering algorithms to huge amounts of unexplored MS/MS data, instilling a sense of "I see wonderful things" on many work days.

Thank you to all my co-authors from the papers included in this thesis, as well as those that were not included.

Thank you to my family. Thank you, Ellen, the love of my life, for supporting me through all the ups and downs over the years. Whatever our souls are made of, yours and mine are the same. Thank you little ones, Iris, Vera and Edith. I still have a hard time fathoming your mere existence and how lucky I am to be forever outnumbered by you. Thank you, mom, for giving me a much needed kick in the butt to actually start my university studies all those years ago. I'll never forget the proud smile on your face when you told me that you had googled my name to look for my e-mail address and all these impressive looking and incomprehensible scientific articles appeared. I miss you every day. Thank you, dad, for your generosity and genuine kindness, and for promoting my interest in computers as a child, which has paid off so many times and led to me getting into both programming and science. Thank you, Sara and Cecilia, for being my sisters and my best friends at

the same time. I would be a whole different, and much worse, person without you.

Thank you, Kerstin and Jonas, for being my second parents, for being almost as big fans of my kids as I am, and for providing much needed psychological counseling and support over the years.

Thank you to all my friends. I can't believe there's so many of you despite my general weirdness. Thank you for being there for me! A special thanks to Calle and Henrik N for being my go-to sources of advice and support regarding relationships, parenting, and life in general. Thank you Henrik R and Erik W for providing an outlet for my nerdiest sides, while also being the funniest people I know. And thank you Olle for helping get through medical school, but also Braid, Trine, Limbo, The witness etc. at the same time.

Thank you Hlin, Simon, Karl and Christoffer, my co-PhD-students, for all great discussions over the years, both science- and GoT-related (but mostly GoT-related).

Thank you to all the wonderfully distinct characters at the lab. Thank you Gunnar and Ann, who are sharing a soul mate-ship that I feel somewhat part of by sharing Gunnar's taste in music and Ann's taste in TV series and all things nerdy. Thank you Celia, Erik P, Ulf, Staffan, Bob and Rahil for helping me out in various ways throughout my PhD studies. Thank you Marianne Wall, one very intelligent and very cool lady, who turned my prejudice of the Excel skills of women over 50 on end several times over.

References

1. *Global, regional, and national age-sex-specific mortality and life expectancy, 1950-2017: a systematic analysis for the Global Burden of Disease Study 2017.* Lancet, 2018. **392**(10159): p. 1684-1735.
2. Preston, S.H., *The changing relation between mortality and level of economic development. Population Studies, Vol. 29, No. 2, July 1975.* Int J Epidemiol, 2007. **36**(3): p. 484-90.
3. Gakidou, E., et al., *Increased educational attainment and its effect on child mortality in 175 countries between 1970 and 2009: a systematic analysis.* Lancet, 2010. **376**(9745): p. 959-74.
4. Larson, E.B., K. Yaffe, and K.M. Langa, *New Insights into the Dementia Epidemic.* N Engl J Med, 2013. **369**(24): p. 2275-7.
5. Prince, M., et al., *Recent global trends in the prevalence and incidence of dementia, and survival with dementia.* Alzheimers Res Ther, 2016. **8**(1): p. 23.
6. Richly, P., et al., *Are medical doctors in Latin America prepared to deal with the dementia epidemic?* Int Psychogeriatr, 2018: p. 1-2.
7. Sathianathan, R. and S.J. Kantipudi, *The dementia epidemic: Impact, prevention, and challenges for India.* Indian J Psychiatry, 2018. **60**(2): p. 165-167.
8. Association, A.P., *Diagnostic and Statistical Manual of Mental Disorders (DSM–5).* 2013.
9. Kukull, W.A., et al., *Dementia and Alzheimer disease incidence: a prospective cohort study.* Arch Neurol, 2002. **59**(11): p. 1737-46.
10. Qiu, C., et al., *Twenty-year changes in dementia occurrence suggest decreasing incidence in central Stockholm, Sweden.* Neurology, 2013. **80**(20): p. 1888-94.
11. Matthews, F.E., et al., *A two-decade comparison of prevalence of dementia in individuals aged 65 years and older from three geographical areas of England: results of the Cognitive Function and Ageing Study I and II.* Lancet, 2013. **382**(9902): p. 1405-12.
12. Schrijvers, E.M., et al., *Is dementia incidence declining?: Trends in dementia incidence since 1990 in the Rotterdam Study.* Neurology, 2012. **78**(19): p. 1456-63.
13. Hickman, R.A., A. Faustin, and T. Wisniewski, *Alzheimer Disease and Its Growing Epidemic: Risk Factors, Biomarkers, and the Urgent Need for Therapeutics.* Neurol Clin, 2016. **34**(4): p. 941-953.

14. *2017 Alzheimer's disease facts and figures.* Alzheimer's & Dementia: The Journal of the Alzheimer's Association, 2017. **13**(4): p. 325-373.
15. Alzheimer, A., H. Forstl, and R. Levy, *On certain peculiar diseases of old age.* Hist Psychiatry, 1991. **2**(5 Pt 1): p. 71-101.
16. Alzheimer, A., *A Characteristic Disease of the Cerebral Cortex: Meeting of South-West Germany Psychiatrists Held in Tubingen on November 3rd and 4th, 1906.* 1907.
17. Burns, A. and S. Iliffe, *Alzheimer's disease.* Bmj, 2009. **338**: p. b158.
18. Mayeux, R. and Y. Stern, *Epidemiology of Alzheimer disease.* Cold Spring Harb Perspect Med, 2012. **2**(8): p. 1-18.
19. Masters, C.L., et al., *Amyloid plaque core protein in Alzheimer disease and Down syndrome.* Proc Natl Acad Sci U S A, 1985. **82**(12): p. 4245-4249.
20. Grundke-Iqbal, I., et al., *Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology.* Proc Natl Acad Sci U S A, 1986. **83**(13): p. 4913-7.
21. Hardy, J.A. and G.A. Higgins, *Alzheimer's disease: the amyloid cascade hypothesis.* Science, 1992. **256**(5054): p. 184-5.
22. Glenner, G.G. and C.W. Wong, *Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein.* Biochem Biophys Res Commun, 1984. **120**(3): p. 885-90.
23. De Strooper, B., *Lessons from a failed gamma-secretase Alzheimer trial.* Cell, 2014. **159**(4): p. 721-6.
24. Mangialasche, F., et al., *Alzheimer's disease: clinical trials and drug development.* The Lancet Neurology, 2010. **9**(7): p. 702-716.
25. Citron, M., *Alzheimer's disease: strategies for disease modification.* Nat Rev Drug Discov, 2010. **9**(5): p. 387-398.
26. Stewart, K.L. and S.E. Radford, *Amyloid plaques beyond Aβ: a survey of the diverse modulators of amyloid aggregation.* Biophys Rev, 2017. **9**(4): p. 405-419.
27. Armstrong, R.A., *A critical analysis of the 'amyloid cascade hypothesis'.* Folia Neuropathol, 2014. **52**(3): p. 211-25.
28. Thevis, M., T. Kuuranne, and H. Geyer, *Annual banned-substance review: Analytical approaches in human sports drug testing.* Drug Test Anal, 2018.
29. Shea, Y.F., et al., *A systematic review of familial Alzheimer's disease: Differences in presentation of clinical features among three mutated genes and potential ethnic differences.* J Formos Med Assoc, 2016. **115**(2): p. 67-75.

30. Games, D., et al., *Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein.* Nature, 1995. **373**(6514): p. 523-7.
31. Kepp, K.P., *Ten Challenges of the Amyloid Hypothesis of Alzheimer's Disease.* J Alzheimers Dis, 2017. **55**(2): p. 447-457.
32. Price, J.L., et al., *Neuropathology of nondemented aging: presumptive evidence for preclinical Alzheimer disease.* Neurobiol Aging, 2009. **30**(7): p. 1026-36.
33. Rodrigue, K.M., et al., *beta-Amyloid burden in healthy aging: regional distribution and cognitive consequences.* Neurology, 2012. **78**(6): p. 387-95.
34. Jack, C.R., Jr., et al., *Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade.* Lancet Neurol, 2010. **9**(1): p. 119-28.
35. Nelson, P.T., et al., *Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature.* J Neuropathol Exp Neurol, 2012. **71**(5): p. 362-81.
36. Mehta, D., et al., *Why do trials for Alzheimer's disease drugs keep failing? A discontinued drug perspective for 2010-2015.* Expert Opin Investig Drugs, 2017. **26**(6): p. 735-739.
37. Sevigny, J., et al., *The antibody aducanumab reduces Abeta plaques in Alzheimer's disease.* Nature, 2016. **537**(7618): p. 50-6.
38. Cummings, J., et al., *Alzheimer's disease drug development pipeline: 2018.* Alzheimers Dement (N Y), 2018. **4**: p. 195-214.
39. Salloway, S., et al., *Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease.* N Engl J Med, 2014. **370**(4): p. 322-33.
40. Scheltens, P., et al., *Biomarker data from scarlet road: A global phase 3 study of gantenerumab in patients with prodromal Alzheimer's disease.* Alzheimer's & Dementia: The Journal of the Alzheimer's Association, 2015. **11**(7): p. P331.
41. Alzforum.org, *Aducanumab Overview.* 2019.
42. Bouras, C., et al., *Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of elderly patients: a quantitative evaluation of a one-year autopsy population from a geriatric hospital.* Cereb Cortex, 1994. **4**(2): p. 138-50.
43. Braak, H., et al., *Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years.* J Neuropathol Exp Neurol, 2011. **70**(11): p. 960-9.
44. Schmitz, C., et al., *Hippocampal neuron loss exceeds amyloid plaque load in a transgenic mouse model of Alzheimer's disease.* Am J Pathol, 2004. **164**(4): p. 1495-502.

45. Mucke, L. and D.J. Selkoe, *Neurotoxicity of amyloid β-protein: synaptic and network dysfunction.* Cold Spring Harb Perspect Med, 2012. **2**(7): p. a006338.
46. Jellinger, K.A. and C. Bancher, *Senile dementia with tangles (tangle predominant form of senile dementia).* Brain Pathol, 1998. **8**(2): p. 367-76.
47. Kamp, J.A., et al., *Amyloid beta in hereditary cerebral hemorrhage with amyloidosis-Dutch type.* Rev Neurosci, 2014. **25**(5): p. 641-51.
48. Drummond, E. and T. Wisniewski, *Alzheimer's disease: experimental models and reality.* Acta Neuropathol, 2017. **133**(2): p. 155-175.
49. VALESKI, J.E., et al., *DIFFERENTIATION OF BULLOUS PEMPHIGOID FROM EPIDERMOLYSIS BULLOSA ACQUISITA ON FROZEN SKIN BIOPSIES.* International Journal of Dermatology, 1992. **31**(1): p. 37-41.
50. Di Paolo, G. and T.-W. Kim, *Linking lipids to Alzheimer's disease: cholesterol and beyond.* Nature Reviews Neuroscience, 2011. **12**: p. 284.
51. Kim, J., J.M. Basak, and D.M. Holtzman, *The role of apolipoprotein E in Alzheimer's disease.* Neuron, 2009. **63**(3): p. 287-303.
52. Wolozin, B., et al., *Simvastatin is associated with a reduced incidence of dementia and Parkinson's disease.* BMC Med, 2007. **5**: p. 20.
53. Rocca, W.A., et al., *Sex and gender differences in the causes of dementia: a narrative review.* Maturitas, 2014. **79**(2): p. 196-201.
54. Lingwood, D. and K. Simons, *Lipid rafts as a membrane-organizing principle.* Science, 2010. **327**(5961): p. 46-50.
55. Ball, M.J., et al., *Intracerebral propagation of Alzheimer's disease: strengthening evidence of a herpes simplex virus etiology.* Alzheimers Dement, 2013. **9**(2): p. 169-75.
56. Alvarez, G., et al., *Herpes simplex virus type 1 induces nuclear accumulation of hyperphosphorylated tau in neuronal cells.* J Neurosci Res, 2012. **90**(5): p. 1020-9.
57. Dominy, S.S., et al., *Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors.* Science Advances, 2019. **5**(1): p. eaau3333.
58. World Health Organization., *The ICD-10 classification of mental and behavioural disorders : diagnostic criteria for research*. 1993, Geneva: World Health Organization. xiii, 248 p.
59. McKhann, G., et al., *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, 1984. **34**(7): p. 939-44.
60. Dubois, B., et al., *Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria.* Alzheimers Dement, 2016. **12**(3): p. 292-323.
61. Dubois, B., et al., *Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria.* Lancet Neurol, 2014. **13**(6): p. 614-629.
62. Sorbi, S., et al., *EFNS-ENS Guidelines on the diagnosis and management of disorders associated with dementia.* Eur J Neurol, 2012. **19**(9): p. 1159-79.
63. Simonsen, A.H., et al., *Recommendations for CSF AD biomarkers in the diagnostic evaluation of dementia.* Alzheimers Dement, 2017. **13**(3): p. 274-284.
64. Montine, T.J., et al., *National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach.* Acta Neuropathol, 2012. **123**(1): p. 1-11.
65. Hamilton, R.L., *Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using alpha-synuclein immunohistochemistry.* Brain Pathol, 2000. **10**(3): p. 378-84.
66. Jellinger, K.A. and J. Attems, *Prevalence of dementia disorders in the oldest-old: an autopsy study.* Acta Neuropathol, 2010. **119**(4): p. 421-33.
67. Goedert, M., *Tau protein and neurodegeneration.* Semin Cell Dev Biol, 2004. **15**(1): p. 45-9.
68. Buee, L., et al., *Tau protein isoforms, phosphorylation and role in neurodegenerative disorders.* Brain Res Brain Res Rev, 2000. **33**(1): p. 95-130.
69. Jellinger, K.A. and A.D. Korczyn, *Are dementia with Lewy bodies and Parkinson's disease dementia the same disease?* BMC Med, 2018. **16**(1): p. 34.
70. Briggs, R., S.P. Kennelly, and D. O'Neill, *Drug treatments in Alzheimer's disease.* Clin Med (Lond), 2016. **16**(3): p. 247-53.
71. Birks, J. and R.J. Harvey, *Donepezil for dementia due to Alzheimer's disease.* Cochrane Database Syst Rev, 2006(1): p. Cd001190.
72. Olin, J. and L. Schneider, *Galantamine for Alzheimer's disease.* Cochrane Database Syst Rev, 2002(3): p. Cd001747.
73. Kishi, T., et al., *Memantine for Alzheimer's Disease: An Updated Systematic Review and Meta-analysis.* J Alzheimers Dis, 2017. **60**(2): p. 401-425.

74. Fleischhacker, W.W., A. Buchgeher, and H. Schubert, *Memantine in the treatment of senile dementia of the Alzheimer type.* Prog Neuropsychopharmacol Biol Psychiatry, 1986. **10**(1): p. 87-93.
75. Matsunaga, S., T. Kishi, and N. Iwata, *Memantine monotherapy for Alzheimer's disease: a systematic review and meta-analysis.* PLoS One, 2015. **10**(4): p. e0123289.
76. Iadecola, C., *The pathobiology of vascular dementia.* Neuron, 2013. **80**(4): p. 844-66.
77. O'Brien, J.T. and A. Thomas, *Vascular dementia.* Lancet, 2015. **386**(10004): p. 1698-706.
78. Korczyn, A.D., V. Vakhapova, and L.T. Grinberg, *Vascular dementia.* J Neurol Sci, 2012. **322**(1-2): p. 2-10.
79. Custodio, N., et al., *Mixed dementia: A review of the evidence.* Dement Neuropsychol, 2017. **11**(4): p. 364-370.
80. Jellinger, K.A. and J. Attems, *Neuropathological evaluation of mixed dementia.* J Neurol Sci, 2007. **257**(1-2): p. 80-7.
81. Skoog, I., et al., *Decreasing prevalence of dementia in 85-year olds examined 22 years apart: the influence of education and stroke.* Sci Rep, 2017. **7**(1): p. 6136.
82. Goldman, J.S., et al., *Comparison of family histories in FTLD subtypes and related tauopathies.* Neurology, 2005. **65**(11): p. 1817-9.
83. Snowden, J.S., D. Neary, and D.M. Mann, *Frontotemporal dementia.* Br J Psychiatry, 2002. **180**: p. 140-3.
84. Olney, N.T., S. Spina, and B.L. Miller, *Frontotemporal Dementia.* Neurol Clin, 2017. **35**(2): p. 339-374.
85. Perri, R., et al., *Alzheimer's disease and frontal variant of frontotemporal dementia-- a very brief battery for cognitive and behavioural distinction.* J Neurol, 2005. **252**(10): p. 1238-44.
86. Rosen, H.J., et al., *Utility of clinical criteria in differentiating frontotemporal lobar degeneration (FTLD) from AD.* Neurology, 2002. **58**(11): p. 1608-15.
87. Desmarais, P., et al., *Social inappropriateness in neurodegenerative disorders.* Int Psychogeriatr, 2018. **30**(2): p. 197-207.
88. Gorno-Tempini, M.L., et al., *Classification of primary progressive aphasia and its variants.* Neurology, 2011. **76**(11): p. 1006-14.
89. Young, J.J., et al., *Frontotemporal dementia: latest evidence and clinical implications.* Ther Adv Psychopharmacol, 2018. **8**(1): p. 33-48.
90. Mackenzie, I.R., et al., *Novel types of frontotemporal lobar degeneration: beyond tau and TDP-43.* J Mol Neurosci, 2011. **45**(3): p. 402-8.

91. Forrest, S.L., et al., *Retiring the term FTDP-17 as MAPT mutations are genetic forms of sporadic frontotemporal tauopathies.* Brain, 2018. **141**(2): p. 521-534.
92. Andersen, P.M., *ALS and FTD: two sides of the same coin?* Lancet Neurol, 2013. **12**(10): p. 937-8.
93. Lillo, P. and J.R. Hodges, *Frontotemporal dementia and motor neurone disease: overlapping clinic-pathological disorders.* J Clin Neurosci, 2009. **16**(9): p. 1131-5.
94. Kwiatkowski, T.J., Jr., et al., *Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis.* Science, 2009. **323**(5918): p. 1205-8.
95. Mackenzie, I.R., R. Rademakers, and M. Neumann, *TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia.* Lancet Neurol, 2010. **9**(10): p. 995-1007.
96. Tsai, R.M. and A.L. Boxer, *Treatment of frontotemporal dementia.* Curr Treat Options Neurol, 2014. **16**(11): p. 319.
97. Bott, N.T., et al., *Frontotemporal dementia: diagnosis, deficits and management.* Neurodegener Dis Manag, 2014. **4**(6): p. 439-54.
98. Pezzoli, S., et al., *Structural and Functional Neuroimaging of Visual Hallucinations in Lewy Body Disease: A Systematic Literature Review.* Brain Sci, 2017. **7**(7).
99. St Louis, E.K., A.R. Boeve, and B.F. Boeve, *REM Sleep Behavior Disorder in Parkinson's Disease and Other Synucleinopathies.* Mov Disord, 2017. **32**(5): p. 645-658.
100. Jurek, L., et al., *Behavioral and psychological symptoms in Lewy body disease: a review.* Geriatr Psychol Neuropsychiatr Vieil, 2018. **16**(1): p. 87-95.
101. Almeida, L., et al., *Depressive Symptoms are Frequent in Atypical Parkinsonian Disorders.* Mov Disord Clin Pract, 2017. **4**(2): p. 191-197.
102. McKeith, I.G. and U.P. Mosimann, *Dementia with Lewy bodies and Parkinson's disease.* Parkinsonism Relat Disord, 2004. **10 Suppl 1**: p. S15-8.
103. Gomperts, S.N., *Lewy Body Dementias: Dementia With Lewy Bodies and Parkinson Disease Dementia.* Continuum (Minneap Minn), 2016. **22**(2 Dementia): p. 435-63.
104. Boot, B.P., et al., *Treatment of dementia with lewy bodies.* Curr Treat Options Neurol, 2013. **15**(6): p. 738-64.
105. Johnson, R.T. and C.J. Gibbs, Jr., *Creutzfeldt-Jakob disease and related transmissible spongiform encephalopathies.* N Engl J Med, 1998. **339**(27): p. 1994-2004.
106. Prusiner, S.B., *The Nobel Prize in Physiology or Medicine.* 1997.

107. Brown, P., et al., *Creutzfeldt-Jakob disease in France: II. Clinical characteristics of 124 consecutive verified cases during the decade 1968--1977.* Ann Neurol, 1979. **6**(5): p. 430-7.
108. Khan, Z. and P.C. Bollu, *Insomnia, Fatal Familial*, in *StatPearls*. 2018, StatPearls Publishing

StatPearls Publishing LLC.: Treasure Island (FL).
109. Zigas, V. and D.C. Gajdusek, *Kuru: clinical study of a new syndrome resembling paralysis agitans in natives of the Eastern Highlands of Australian New Guinea.* Med J Aust, 1957. **44**(21): p. 745-54.
110. Liberski, P.P., *Gerstmann-Straussler-Scheinker disease.* Adv Exp Med Biol, 2012. **724**: p. 128-37.
111. Lee, J., et al., *Prion diseases as transmissible zoonotic diseases.* Osong Public Health Res Perspect, 2013. **4**(1): p. 57-66.
112. Hamaguchi, T., et al., *The risk of iatrogenic Creutzfeldt-Jakob disease through medical and surgical procedures.* Neuropathology, 2009. **29**(5): p. 625-31.
113. Kupfer, L., W. Hinrichs, and M.H. Groschup, *Prion protein misfolding.* Curr Mol Med, 2009. **9**(7): p. 826-35.
114. Vazquez-Fernandez, E., et al., *The Structure of Mammalian Prions and Their Aggregates.* Int Rev Cell Mol Biol, 2017. **329**: p. 277-301.
115. Takada, L.T., et al., *Prion disease.* Handb Clin Neurol, 2018. **148**: p. 441-464.
116. Castle, A.R. and A.C. Gill, *Physiological Functions of the Cellular Prion Protein.* Front Mol Biosci, 2017. **4**: p. 19.
117. Whitehouse, I.J., et al., *Prion protein is decreased in Alzheimer's brain and inversely correlates with BACE1 activity, amyloid-beta levels and Braak stage.* PLoS One, 2013. **8**(4): p. e59554.
118. Iba, M., et al., *Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer's-like tauopathy.* J Neurosci, 2013. **33**(3): p. 1024-37.
119. Morales, R., et al., *De novo induction of amyloid-β deposition in vivo.* Molecular Psychiatry, 2011. **17**: p. 1347.
120. Lannfelt, L., et al., *Perspectives on future Alzheimer therapies: amyloid-beta protofibrils - a new target for immunotherapy with BAN2401 in Alzheimer's disease.* Alzheimers Res Ther, 2014. **6**(2): p. 16.
121. Alzforum.org, *BAN2401 Overview.* 2019.
122. Biogen, E.C., Ltd., *Eisai and Biogen Announce Detailed Results of Phase II Clinical Study of BAN2401 in Early Alzheimer's Disease at*

Alzheimer's Association International Conference (AAIC) 2018. 2018.
123. Bateman, R.J., et al., *Clinical and biomarker changes in dominantly inherited Alzheimer's disease.* N Engl J Med, 2012. **367**(9): p. 795-804.
124. Fagan, A.M., et al., *Longitudinal change in CSF biomarkers in autosomal-dominant Alzheimer's disease.* Sci Transl Med, 2014. **6**(226): p. 226ra30.
125. Preische, O., et al., *Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease.* Nat Med, 2019.
126. Weston, P.S.J., et al., *Serum neurofilament light in familial Alzheimer disease: A marker of early neurodegeneration.* Neurology, 2017. **89**(21): p. 2167-2175.
127. Risacher, S.L. and A.J. Saykin, *Neuroimaging Biomarkers of Neurodegenerative Diseases and Dementia.* Semin Neurol, 2013. **33**(4): p. 386-416.
128. Bonifacio, G. and G. Zamboni, *Brain imaging in dementia.* Postgraduate Medical Journal, 2016. **92**(1088): p. 333-340.
129. Johnson, K.A., et al., *Brain Imaging in Alzheimer Disease.* Cold Spring Harb Perspect Med, 2012. **2**(4).
130. Rubi, S., et al., *Concordance between brain (18)F-FDG PET and cerebrospinal fluid biomarkers in diagnosing Alzheimer's disease.* Rev Esp Med Nucl Imagen Mol, 2018. **37**(1): p. 3-8.
131. Zwan, M., et al., *Concordance between cerebrospinal fluid biomarkers and [11C]PIB PET in a memory clinic cohort.* J Alzheimers Dis, 2014. **41**(3): p. 801-7.
132. Pannee, J., et al., *Reference measurement procedure for CSF amyloid beta (Abeta)1-42 and the CSF Abeta1-42 /Abeta1-40 ratio - a cross-validation study against amyloid PET.* J Neurochem, 2016. **139**(4): p. 651-658.
133. Wright, B.L.C., J.T.F. Lai, and A.J. Sinclair, *Cerebrospinal fluid and lumbar puncture: a practical review.* Journal of Neurology, 2012. **259**(8): p. 1530-1545.
134. Wright, B.L., J.T. Lai, and A.J. Sinclair, *Cerebrospinal fluid and lumbar puncture: a practical review.* J Neurol, 2012. **259**(8): p. 1530-45.
135. Blennow, K., et al., *Cerebrospinal fluid and plasma biomarkers in Alzheimer disease.* Nat Rev Neurol, 2010. **6**(3): p. 131-44.
136. Maddalena, A., et al., *Biochemical diagnosis of Alzheimer disease by measuring the cerebrospinal fluid ratio of phosphorylated tau*

protein to beta-amyloid peptide42. Arch Neurol, 2003. **60**(9): p. 1202-6.
137. de Jong, D., et al., *Cerebrospinal fluid amyloid beta42/phosphorylated tau ratio discriminates between Alzheimer's disease and vascular dementia.* J Gerontol A Biol Sci Med Sci, 2006. **61**(7): p. 755-8.
138. Hansson, O., et al., *Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study.* Lancet Neurol, 2006. **5**(3): p. 228-34.
139. Lewczuk, P., et al., *Amyloid-beta 42/40 cerebrospinal fluid concentration ratio in the diagnostics of Alzheimer's disease: validation of two novel assays.* J Alzheimers Dis, 2015. **43**(1): p. 183-91.
140. Schoonenboom, N.S., et al., *Cerebrospinal fluid markers for differential dementia diagnosis in a large memory clinic cohort.* Neurology, 2012. **78**(1): p. 47-54.
141. Rosen, C., et al., *Fluid biomarkers in Alzheimer's disease - current concepts.* Mol Neurodegener, 2013. **8**: p. 20.
142. Zekry, D., J.J. Hauw, and G. Gold, *Mixed dementia: epidemiology, diagnosis, and treatment.* J Am Geriatr Soc, 2002. **50**(8): p. 1431-8.
143. Forman, M.S., et al., *Frontotemporal dementia: clinicopathological correlations.* Ann Neurol, 2006. **59**(6): p. 952-62.
144. Kertesz, A., et al., *The evolution and pathology of frontotemporal dementia.* Brain, 2005. **128**(Pt 9): p. 1996-2005.
145. Mawuenyega, K.G., et al., *Decreased clearance of CNS beta-amyloid in Alzheimer's disease.* Science, 2010. **330**(6012): p. 1774.
146. Ring, S., et al., *The secreted beta-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice.* J Neurosci, 2007. **27**(29): p. 7817-26.
147. Rosenberg, R.N., et al., *Genomics of Alzheimer Disease: A Review.* JAMA Neurol, 2016. **73**(7): p. 867-74.
148. Willem, M., S. Lammich, and C. Haass, *Function, regulation and therapeutic properties of beta-secretase (BACE1).* Semin Cell Dev Biol, 2009. **20**(2): p. 175-82.
149. Moustafa, A.A., et al., *Genetic underpinnings in Alzheimer's disease - a review.* Rev Neurosci, 2018. **29**(1): p. 21-38.
150. Citron, M., et al., *Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice.* Nat Med, 1997. **3**(1): p. 67-72.

151. Arber, C., et al., *Familial Alzheimer's disease patient-derived neurons reveal distinct mutation-specific effects on amyloid beta.* Mol Psychiatry, 2019.
152. Strozyk, D., et al., *CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study.* Neurology, 2003. **60**(4): p. 652-6.
153. Jansen, W.J., et al., *Association of Cerebral Amyloid-beta Aggregation With Cognitive Functioning in Persons Without Dementia.* JAMA Psychiatry, 2018. **75**(1): p. 84-95.
154. Pawlowski, M., S.G. Meuth, and T. Duning, *Cerebrospinal Fluid Biomarkers in Alzheimer's Disease-From Brain Starch to Bench and Bedside.* Diagnostics (Basel), 2017. **7**(3).
155. Blennow, K., *Cerebrospinal fluid protein biomarkers for Alzheimer's disease.* NeuroRx, 2004. **1**(2): p. 213-25.
156. Iwatsubo, T., et al., *Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43).* Neuron, 1994. **13**(1): p. 45-53.
157. Miller, D.L., et al., *Peptide compositions of the cerebrovascular and senile plaque core amyloid deposits of Alzheimer's disease.* Arch Biochem Biophys, 1993. **301**(1): p. 41-52.
158. Motter, R., et al., *Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease.* Ann Neurol, 1995. **38**(4): p. 643-8.
159. Vanderstichele, H., et al., *Development of a Specific Diagnostic Test for Measurement of β-Amyloid (1-42) [βA4(l-42)] in CSF*, in *Progress in Alzheimer's and Parkinson's Diseases*, A. Fisher, I. Hanin, and M. Yoshida, Editors. 1998, Springer US: Boston, MA. p. 773-778.
160. Bousiges, O. and F. Blanc, *Diagnostic value of cerebro-spinal fluid biomarkers in dementia with lewy bodies.* Clin Chim Acta, 2019. **490**: p. 222-228.
161. Gomperts, S.N., et al., *Brain amyloid and cognition in Lewy body diseases.* Mov Disord, 2012. **27**(8): p. 965-73.
162. Sakono, M. and T. Zako, *Amyloid oligomers: formation and toxicity of Abeta oligomers.* Febs j, 2010. **277**(6): p. 1348-58.
163. Lambert, M.P., et al., *Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins.* Proc Natl Acad Sci U S A, 1998. **95**(11): p. 6448-53.
164. Walsh, D.M., et al., *Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo.* Nature, 2002. **416**(6880): p. 535-9.

165. Nilsberth, C., et al., *The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation.* Nat Neurosci, 2001. **4**(9): p. 887-93.
166. Kalimo, H., et al., *The Arctic AβPP mutation leads to Alzheimer's disease pathology with highly variable topographic deposition of differentially truncated Aβ.* Acta Neuropathol Commun, 2013. **1**: p. 60.
167. Walker, L.C. and M. Jucker, *Neurodegenerative diseases: expanding the prion concept.* Annu Rev Neurosci, 2015. **38**: p. 87-103.
168. Meyer-Luehmann, M., et al., *Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host.* Science, 2006. **313**(5794): p. 1781-4.
169. Rangel, A., et al., *Distinct patterns of spread of prion infection in brains of mice expressing anchorless or anchored forms of prion protein.* Acta Neuropathol Commun, 2014. **2**: p. 8.
170. Braak, H. and E. Braak, *Staging of Alzheimer's disease-related neurofibrillary changes.* Neurobiol Aging, 1995. **16**(3): p. 271-8; discussion 278-84.
171. Alonso, A.D., et al., *Abnormal phosphorylation of tau and the mechanism of Alzheimer neurofibrillary degeneration: sequestration of microtubule-associated proteins 1 and 2 and the disassembly of microtubules by the abnormal tau.* Proc Natl Acad Sci U S A, 1997. **94**(1): p. 298-303.
172. Domise, M., et al., *AMP-activated protein kinase modulates tau phosphorylation and tau pathology in vivo.* Sci Rep, 2016. **6**: p. 26758.
173. Sanabria-Castro, A., I. Alvarado-Echeverria, and C. Monge-Bonilla, *Molecular Pathogenesis of Alzheimer's Disease: An Update.* Ann Neurosci, 2017. **24**(1): p. 46-54.
174. Kovacs, G.G., *Molecular Pathological Classification of Neurodegenerative Diseases: Turning towards Precision Medicine.* Int J Mol Sci, 2016. **17**(2).
175. Rapoport, M., et al., *Tau is essential to beta -amyloid-induced neurotoxicity.* Proc Natl Acad Sci U S A, 2002. **99**(9): p. 6364-9.
176. Zempel, H., et al., *Aβ Oligomers Cause Localized Ca²⁺ Elevation, Missorting of Endogenous Tau into Dendrites, Tau Phosphorylation, and Destruction of Microtubules and Spines.* The Journal of Neuroscience, 2010. **30**(36): p. 11938-11950.
177. Rocher, A.B., et al., *Structural and functional changes in tau mutant mice neurons are not linked to the presence of NFTs.* Exp Neurol, 2010. **223**(2): p. 385-93.

178. Santacruz, K., et al., *Tau suppression in a neurodegenerative mouse model improves memory function.* Science, 2005. **309**(5733): p. 476-81.
179. Sato, C., et al., *Tau Kinetics in Neurons and the Human Central Nervous System.* Neuron, 2018. **97**(6): p. 1284-1298.e7.
180. 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): p. 673-684.
181. Zetterberg, H., *Review: Tau in biofluids - relation to pathology, imaging and clinical features.* Neuropathol Appl Neurobiol, 2017. **43**(3): p. 194-199.
182. Kaerst, L., et al., *Using cerebrospinal fluid marker profiles in clinical diagnosis of dementia with Lewy bodies, Parkinson's disease, and Alzheimer's disease.* J Alzheimers Dis, 2014. **38**(1): p. 63-73.
183. Ermann, N., et al., *CSF nonphosphorylated Tau as a biomarker for the discrimination of AD from CJD.* Ann Clin Transl Neurol, 2018. **5**(7): p. 883-887.
184. Schraen-Maschke, S., et al., *Tau as a biomarker of neurodegenerative diseases.* Biomark Med, 2008. **2**(4): p. 363-84.
185. Mudher, A., et al., *What is the evidence that tau pathology spreads through prion-like propagation?* Acta Neuropathol Commun, 2017. **5**(1): p. 99.
186. Falcon, B., et al., *Conformation determines the seeding potencies of native and recombinant Tau aggregates.* J Biol Chem, 2015. **290**(2): p. 1049-65.
187. Holmes, B.B., et al., *Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds.* Proc Natl Acad Sci U S A, 2013. **110**(33): p. E3138-47.
188. Seeley, W.W., et al., *Neurodegenerative diseases target large-scale human brain networks.* Neuron, 2009. **62**(1): p. 42-52.
189. Zhou, J., et al., *Predicting regional neurodegeneration from the healthy brain functional connectome.* Neuron, 2012. **73**(6): p. 1216-27.
190. Frost, B., R.L. Jacks, and M.I. Diamond, *Propagation of tau misfolding from the outside to the inside of a cell.* J Biol Chem, 2009. **284**(19): p. 12845-52.
191. Liu, L., et al., *Trans-synaptic spread of tau pathology in vivo.* PLoS One, 2012. **7**(2): p. e31302.
192. Guo, J.L. and V.M. Lee, *Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles.* J Biol Chem, 2011. **286**(17): p. 15317-31.

193. Schlaepfer, W.W. and R.G. Lynch, *Immunofluorescence studies of neurofilaments in the rat and human peripheral and central nervous system.* J Cell Biol, 1977. **74**(1): p. 241-50.
194. Zetterberg, H., et al., *Neurochemical aftermath of amateur boxing.* Arch Neurol, 2006. **63**(9): p. 1277-80.
195. Lycke, J.N., et al., *Neurofilament protein in cerebrospinal fluid: a potential marker of activity in multiple sclerosis.* J Neurol Neurosurg Psychiatry, 1998. **64**(3): p. 402-4.
196. Zanier, E.R., et al., *Neurofilament light chain levels in ventricular cerebrospinal fluid after acute aneurysmal subarachnoid haemorrhage.* J Neurol Neurosurg Psychiatry, 2011. **82**(2): p. 157-9.
197. Zetterberg, H., *Neurofilament Light: A Dynamic Cross-Disease Fluid Biomarker for Neurodegeneration.* Neuron, 2016. **91**(1): p. 1-3.
198. Kvartsberg, H., et al., *Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease.* Alzheimers Dement, 2015. **11**(10): p. 1180-90.
199. Thorsell, A., et al., *Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease.* Brain Res, 2010. **1362**: p. 13-22.
200. Portelius, E., et al., *Cerebrospinal fluid neurogranin concentration in neurodegeneration: relation to clinical phenotypes and neuropathology.* Acta Neuropathol, 2018. **136**(3): p. 363-376.
201. Ober, C. and G.L. Chupp, *The chitinase and chitinase-like proteins: a review of genetic and functional studies in asthma and immune-mediated diseases.* Curr Opin Allergy Clin Immunol, 2009. **9**(5): p. 401-8.
202. Bonneh-Barkay, D., et al., *YKL-40, a marker of simian immunodeficiency virus encephalitis, modulates the biological activity of basic fibroblast growth factor.* Am J Pathol, 2008. **173**(1): p. 130-43.
203. Bonneh-Barkay, D., et al., *In vivo CHI3L1 (YKL-40) expression in astrocytes in acute and chronic neurological diseases.* J Neuroinflammation, 2010. **7**: p. 34.
204. Llorens, F., et al., *YKL-40 in the brain and cerebrospinal fluid of neurodegenerative dementias.* Mol Neurodegener, 2017. **12**(1): p. 83.
205. Zetterberg, H. and J.M. Schott, *Biomarkers for Alzheimer's disease beyond amyloid and tau.* Nat Med, 2019. **25**(2): p. 201-203.
206. Nation, D.A., et al., *Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction.* Nat Med, 2019. **25**(2): p. 270-276.

207. Silos-Santiago, I., et al., *Localization of pleiotrophin and its mRNA in subpopulations of neurons and their corresponding axonal tracts suggests important roles in neural-glial interactions during development and in maturity.* J Neurobiol, 1996. **31**(3): p. 283-96.
208. Wanaka, A., S.L. Carroll, and J. Milbrandt, *Developmentally regulated expression of pleiotrophin, a novel heparin binding growth factor, in the nervous system of the rat.* Brain Res Dev Brain Res, 1993. **72**(1): p. 133-44.
209. Gonzalez-Castillo, C., et al., *The absence of pleiotrophin modulates gene expression in the hippocampus in vivo and in cerebellar granule cells in vitro.* Mol Cell Neurosci, 2016. **75**: p. 113-21.
210. Yeh, H.J., et al., *Upregulation of pleiotrophin gene expression in developing microvasculature, macrophages, and astrocytes after acute ischemic brain injury.* J Neurosci, 1998. **18**(10): p. 3699-707.
211. Maeda, N., et al., *6B4 proteoglycan/phosphacan, an extracellular variant of receptor-like protein-tyrosine phosphatase zeta/RPTPbeta, binds pleiotrophin/heparin-binding growth-associated molecule (HB-GAM).* J Biol Chem, 1996. **271**(35): p. 21446-52.
212. Meng, K., et al., *Pleiotrophin signals increased tyrosine phosphorylation of beta beta-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase beta/zeta.* Proc Natl Acad Sci U S A, 2000. **97**(6): p. 2603-8.
213. Ryan, E., D. Shen, and X. Wang, *Structural studies reveal an important role for the pleiotrophin C-terminus in mediating interactions with chondroitin sulfate.* Febs j, 2016. **283**(8): p. 1488-503.
214. Bernard-Pierrot, I., et al., *The lysine-rich C-terminal tail of heparin affin regulatory peptide is required for mitogenic and tumor formation activities.* J Biol Chem, 2001. **276**(15): p. 12228-34.
215. Senis, Y.A. and A.J. Barr, *Targeting Receptor-Type Protein Tyrosine Phosphatases with Biotherapeutics: Is Outside-in Better than Inside-Out?* Molecules, 2018. **23**(3): p. 569.
216. Ulery, P.G. and D.K. Strickland, *LRP in Alzheimer's disease: friend or foe?* J Clin Invest, 2000. **106**(9): p. 1077-9.
217. Lambert, J.C., et al., *Association at LRP gene locus with sporadic late-onset Alzheimer's disease.* Lancet, 1998. **351**(9118): p. 1787-8.
218. Herradón, G. and C. Pérez-García, *Targeting midkine and pleiotrophin signalling pathways in addiction and neurodegenerative disorders: recent progress and perspectives.* Br J Pharmacol, 2014. **171**(4): p. 837-48.

219. Liu, Q., et al., *Amyloid precursor protein regulates brain apolipoprotein E and cholesterol metabolism through lipoprotein receptor LRP1.* Neuron, 2007. **56**(1): p. 66-78.
220. Shibata, M., et al., *Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier.* J Clin Invest, 2000. **106**(12): p. 1489-99.
221. Kadomatsu, K. and T. Muramatsu, *Midkine and pleiotrophin in neural development and cancer.* Cancer Lett, 2004. **204**(2): p. 127-43.
222. Wisniewski, T., et al., *HB-GAM is a cytokine present in Alzheimer's and Down's syndrome lesions.* Neuroreport, 1996. **7**(2): p. 667-71.
223. Namba, Y., et al., *Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease.* Brain Res, 1991. **541**(1): p. 163-6.
224. Arelin, K., et al., *LRP and senile plaques in Alzheimer's disease: colocalization with apolipoprotein E and with activated astrocytes.* Brain Res Mol Brain Res, 2002. **104**(1): p. 38-46.
225. Blennow, K., *A Review of Fluid Biomarkers for Alzheimer's Disease: Moving from CSF to Blood.* Neurol Ther, 2017. **6**(Suppl 1): p. 15-24.
226. Khalil, M., et al., *Neurofilaments as biomarkers in neurological disorders.* Nat Rev Neurol, 2018. **14**(10): p. 577-589.
227. Gisslen, M., et al., *Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study.* EBioMedicine, 2016. **3**: p. 135-140.
228. Rissin, D.M., et al., *Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations.* Nat Biotechnol, 2010. **28**(6): p. 595-9.
229. Shahim, P., et al., *Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports.* Neurology, 2017. **88**(19): p. 1788-1794.
230. Mattsson, N., et al., *Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease.* JAMA Neurol, 2017. **74**(5): p. 557-566.
231. Mattsson, N., et al., *Plasma tau in Alzheimer disease.* Neurology, 2016. **87**(17): p. 1827-1835.
232. Mielke, M.M., et al., *Association of Plasma Total Tau Level With Cognitive Decline and Risk of Mild Cognitive Impairment or Dementia in the Mayo Clinic Study on Aging.* JAMA Neurol, 2017. **74**(9): p. 1073-1080.

233. Janelidze, S., et al., *Plasma beta-amyloid in Alzheimer's disease and vascular disease.* Sci Rep, 2016. **6**: p. 26801.
234. Nakamura, A., et al., *High performance plasma amyloid-beta biomarkers for Alzheimer's disease.* Nature, 2018. **554**(7691): p. 249-254.
235. Weiland, G., *[The enzyme-linked immunosorbent assay (ELISA)--a new serodiagnostic method for the detection of parasitic infections (author's transl)].* MMW Munch Med Wochenschr, 1978. **120**(44): p. 1457-60.
236. Aydin, S., *A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA.* Peptides, 2015. **72**: p. 4-15.
237. Fenn, J.B., *Electrospray wings for molecular elephants (Nobel lecture).* Angew Chem Int Ed Engl, 2003. **42**(33): p. 3871-94.
238. Yoshida, T., *["Process of the research and development received Nobel Prize in Chemistry 2002"].* Rinsho Byori, 2004. **52**(1): p. 35-41.
239. Foundation, T.N., *The Nobel Prize in Physics 1989.* 1989.
240. Mittal, M., et al., *ICP-MS: Analytical Method for Identification and Detection of Elemental Impurities.* Curr Drug Discov Technol, 2017. **14**(2): p. 106-120.
241. Palmer, P.T. and T.F. Limero, *Mass spectrometry in the U.S. space program: past, present, and future.* J Am Soc Mass Spectrom, 2001. **12**(6): p. 656-75.
242. Navarro-Gonzalez, R., et al., *The limitations on organic detection in Mars-like soils by thermal volatilization-gas chromatography-MS and their implications for the Viking results.* Proc Natl Acad Sci U S A, 2006. **103**(44): p. 16089-94.
243. Hillenkamp, F. and M. Karas, *Mass spectrometry of peptides and proteins by matrix-assisted ultraviolet laser desorption/ionization.* Methods Enzymol, 1990. **193**: p. 280-95.
244. Hillenkamp, F., et al., *Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers.* Anal Chem, 1991. **63**(24): p. 1193a-1203a.
245. Yamashita, M. and J.B. Fenn, *Electrospray ion source. Another variation on the free-jet theme.* The Journal of Physical Chemistry, 1984. **88**(20): p. 4451-4459.
246. Makarov, A., *Electrostatic axially harmonic orbital trapping: a high-performance technique of mass analysis.* Anal Chem, 2000. **72**(6): p. 1156-62.

247. Eliuk, S. and A. Makarov, *Evolution of Orbitrap Mass Spectrometry Instrumentation.* Annu Rev Anal Chem (Palo Alto Calif), 2015. **8**: p. 61-80.
248. Olsen, J.V., et al., *Parts per million mass accuracy on an Orbitrap mass spectrometer via lock mass injection into a C-trap.* Mol Cell Proteomics, 2005. **4**(12): p. 2010-21.
249. Steen, H. and M. Mann, *The ABC's (and XYZ's) of peptide sequencing.* Nat Rev Mol Cell Biol, 2004. **5**(9): p. 699-711.
250. Seidler, J., et al., *De novo sequencing of peptides by MS/MS.* Proteomics, 2010. **10**(4): p. 634-49.
251. Dayon, L. and J.C. Sanchez, *Relative protein quantification by MS/MS using the tandem mass tag technology.* Methods Mol Biol, 2012. **893**: p. 115-27.
252. McDonald, W.H. and J.R. Yates, 3rd, *Shotgun proteomics and biomarker discovery.* Dis Markers, 2002. **18**(2): p. 99-105.
253. Zhang, Y., et al., *Protein analysis by shotgun/bottom-up proteomics.* Chem Rev, 2013. **113**(4): p. 2343-94.
254. Svedem. *Svenska demensregistret - Årsrapport 2016*. 2016; Available from: www.ucr.uu.se/svedem/om-svedem/arsrapporter/svedem-arsrapport-2016/viewdocument.
255. Socialstyrelsen. *Dödsorsaksregistret* 2018; Available from: www.socialstyrelsen.se/register/dodsorsaksregistret.
256. Tsuboi, Y., H. Uchikado, and D.W. Dickson, *Neuropathology of Parkinson's disease dementia and dementia with Lewy bodies with reference to striatal pathology.* Parkinsonism Relat Disord, 2007. **13 Suppl 3**: p. S221-4.
257. Bibl, M., et al., *CSF amyloid-beta-peptides in Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease dementia.* Brain, 2006. **129**(Pt 5): p. 1177-87.
258. Sjogren, M., et al., *Neurofilament protein in cerebrospinal fluid: a marker of white matter changes.* J Neurosci Res, 2001. **66**(3): p. 510-6.
259. Blennow, K., A. Johansson, and H. Zetterberg, *Diagnostic value of 14-3-3beta immunoblot and T-tau/P-tau ratio in clinically suspected Creutzfeldt-Jakob disease.* Int J Mol Med, 2005. **16**(6): p. 1147-9.
260. Skinningsrud, A., et al., *Cerebrospinal fluid markers in Creutzfeldt-Jakob disease.* Cerebrospinal Fluid Res, 2008. **5**: p. 14.
261. Bahl, J.M., et al., *The diagnostic efficiency of biomarkers in sporadic Creutzfeldt-Jakob disease compared to Alzheimer's disease.* Neurobiol Aging, 2009. **30**(11): p. 1834-41.
262. Eng, J.K., A.L. McCormack, and J.R. Yates, *An approach to correlate tandem mass spectral data of peptides with amino acid*

sequences in a protein database. J Am Soc Mass Spectrom, 1994. **5**(11): p. 976-89.
263. Tsiatsiani, L. and A.J. Heck, *Proteomics beyond trypsin.* Febs j, 2015. **282**(14): p. 2612-26.
264. Hallberg, M., *Neuropeptides: metabolism to bioactive fragments and the pharmacology of their receptors.* Med Res Rev, 2015. **35**(3): p. 464-519.
265. Romanova, E.V., S.E. Dowd, and J.V. Sweedler, *Quantitation of endogenous peptides using mass spectrometry based methods.* Curr Opin Chem Biol, 2013. **17**(5): p. 801-8.
266. Mashaghi, A., et al., *Neuropeptide substance P and the immune response.* Cell Mol Life Sci, 2016. **73**(22): p. 4249-4264.
267. Patel, S., et al., *Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies.* Biomed Pharmacother, 2017. **94**: p. 317-325.
268. Reichmann, F. and P. Holzer, *Neuropeptide Y: A stressful review.* Neuropeptides, 2016. **55**: p. 99-109.
269. Rosa, M.I., et al., *Accuracy of cerebrospinal fluid Abeta(1-42) for Alzheimer's disease diagnosis: a systematic review and meta-analysis.* J Alzheimers Dis, 2014. **40**(2): p. 443-54.
270. Wan, K.X., I. Vidavsky, and M.L. Gross, *Comparing similar spectra: from similarity index to spectral contrast angle.* J Am Soc Mass Spectrom, 2002. **13**(1): p. 85-8.
271. van der Flier, W.M., et al., *Optimizing patient care and research: the Amsterdam Dementia Cohort.* J Alzheimers Dis, 2014. **41**(1): p. 313-27.
272. Blennow, K. and H. Hampel, *CSF markers for incipient Alzheimer's disease.* The Lancet Neurology, 2003. **2**(10): p. 605-613.
273. Ma, B., et al., *PEAKS: powerful software for peptide de novo sequencing by tandem mass spectrometry.* Rapid Communications in Mass Spectrometry, 2003. **17**(20): p. 2337-2342.
274. Mattsson, N., et al., *CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment.* JAMA, 2009. **302**(4): p. 385-93.
275. Zetterberg, H., et al., *Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression.* JAMA Neurol, 2016. **73**(1): p. 60-7.
276. Hansson, O., et al., *Evaluation of plasma Abeta(40) and Abeta(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment.* Neurobiol Aging, 2010. **31**(3): p. 357-67.
277. Alzforum.org, *Therapeutics - Verubecestat.* 2019.

278. Holmes, C., et al., *Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial.* Lancet, 2008. **372**(9634): p. 216-23.
279. Gouras, G.K., T.T. Olsson, and O. Hansson, *β-Amyloid peptides and amyloid plaques in Alzheimer's disease.* Neurotherapeutics, 2015. **12**(1): p. 3-11.
280. Kim, J., et al., *Normal cognition in transgenic BRI2-Abeta mice.* Mol Neurodegener, 2013. **8**: p. 15.
281. Scholl, M., et al., *Low PiB PET retention in presence of pathologic CSF biomarkers in Arctic APP mutation carriers.* Neurology, 2012. **79**(3): p. 229-36.
282. Bukhari, H., et al., *Small things matter: Implications of APP intracellular domain AICD nuclear signaling in the progression and pathogenesis of Alzheimer's disease.* Prog Neurobiol, 2017. **156**: p. 189-213.